CENTRAL VENOUS CATHETER INFECTION

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submitted for the degree of Doctor of Philosophy

in the

European Institute of Health and Medical Sciences

University of Surrey

1996

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ABSTRACT

Infection is the most frequent life threatening complication of central venous catheterisation. This thesis consists of four studies examining risk factors for catheter-related infection, and their modification.

Prevention of infection is hindered by a lack of agreement over the predominant route of infection. Following a retrospective study which highlighted at-risk groups of patients with a triple-lumen catheter, or receiving TPN, a prospective study used sampling and culture methods to detect intra- and extraluminal catheter colonisation, and developed a classification method to discriminate between isolates from different sources. Tip colonisation was demonstrated by both routes, including simultaneously in a single catheter, or by different routes in successive catheters in a single patient. The risk of extraluminal colonisation was increased by extended use of semi-permeable dressings and increasing duration of catheterisation. Hub colonisation was related to the total number of disconnections to the catheter/administration system and though less frequent than insertion site colonisation, was more frequently linked to tip colonisation. A high frequency of hub colonisation was observed in catheters used for TPN. Whilst overall, an equal frequency of extra- and intraluminal sources of tip colonisation were observed, all cases of bacteraemia had an intraluminal source, even in the presence of skin or extraluminal colonisation. Implementation of in-line IV filters prevented intraluminal colonisation where present throughout the duration of catheterisation. Examination of bacterial growth in IV electrolyte solutions by impedance measurement demonstrated an increased risk when small amounts of nutrient containing drugs such as insulin or heparin were added. Growth of E. faecalis was superior to S. epidermidis, and unexpectedly equivalent to that of K. pneumoniae.
ACKNOWLEDGEMENTS

I would like to acknowledge the many people without whose support this thesis would not have been possible. In particular, I would like to thank my supervisors, Professor R. Crow currently of the University of Surrey, and Dr. A. B. Mulhall and Professor S. G. McLaren, formerly of the University of Surrey, for their advice and encouragement. I would also like to thank Dr. R. R. Marples and the staff of the Staphylococcal, Streptococcal and Antibiotic Reference Laboratories, Colindale, for their help in the classification of organisms.

Additionally I wish to acknowledge the co-operation and help received from the laboratory, medical, and nursing staff and patients of the Intensive Care Unit, Central Treatment Room, Theatres and Surgical wards of the study hospital during data collection, and to express my thanks to Dr. N. S. Cumberland for his microbiological advice during the write-up of the thesis.

Finally, I would like to thank my husband for his patience and practical support.

This study was part funded by the South West Thames Regional Health Authority Locally Organised Research Scheme.
DECLARATION

I declare that this thesis is my own composition and that I have conducted the research reported. Due acknowledgements are given to the people who assisted with this work, with details of their contribution.
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CHAPTER ONE: REVIEW OF THE LITERATURE

A) THE PROBLEM OF CENTRAL VENOUS CATHETER INFECTION

A central venous catheter is an intravenous cannula inserted into the superior vena cava primarily to monitor central venous pressure or to administer medications, fluid or total parenteral nutrition, or to obtain blood samples, frequently in patients who are critically ill. The type of catheter and site of insertion influence the rate of infection. Central venous catheterisation approaches are illustrated in Figure 1.1.

Specialist catheters are available for, for example, pulmonary artery pressure monitoring, cardiac pacing, haemodialysis, and long-term total parenteral nutrition (TPN) or chemotherapy. The presence of these catheters predisposes the patient to nosocomial infection by damaging the normal epithelial and mucosal barriers to infection, supporting the growth of micro-organisms and providing a reservoir for their dissemination, often in a site protected from host defence mechanisms (Stamm 1978). Infection, in the form of septicaemia, is the most frequent life-threatening complication of central venous catheterisation with reported rates in the range of 3-10% (Maki 1991). The mortality rate varies with the organism involved, rising to 81% with fungal septicaemia (Bozzetti 1985).

In a large European multicentre study of Surgical patients, Nystrom et al. (1983) found an incidence of bacteraemia of 0.15% in patients without an intravascular cannula, 0.69% in patients with a peripheral cannula, and 5.9% in patients with a central venous cannula. From recently published studies, the risk of septicaemia is estimated to be 0.2% for peripheral cannulae, 1% for arterial catheters, 1% for pulmonary artery catheters, 3% for multi-lumen catheters and 10% for haemodialysis catheters (Maki 1991).

In the UK it is estimated that 200,000 central venous catheters are inserted annually. A 39% increase in the number of reports of catheter-associated bacteraemia was observed by the Public Health Laboratory Service Communicable Disease Surveillance Centre between 1989 and 1991 (Elliott 1993). In the United States, it is estimated that 50,000 patients develop catheter-related septicaemia per year of which 90% are associated with central venous catheters (Maki 1990a), with a 3.8-14 fold increase in mortality and a hospital stay increased by an average of 2-3 weeks.
FIGURE 1.1: CENTRAL VENOUS CATHETERISATION APPROACHES

1. SUBCLAVIAN PUNCTURE
2. BRACHIOPHALIC PUNCTURE
3. INTERNAL JUGULAR PUNCTURE
4. INTERNAL JUGULAR PUNCTURE
In a 2 year analysis of bacteraemia in a District General Hospital, 37 patients developed catheter-related sepsis of whom 6 patients died, with the infection “playing a leading role” in three and contributing to death in three others. One further patient developed vertebral osteomyelitis (Waghorn 1994). Intravascular catheter-related bacteraemia in the critically ill has been associated with an attributable mortality of 28% (Smith et al. 1991). The cost of an episode of catheter-related sepsis was estimated at in excess of $6000 in 1988 (Maki et al. 1988). Device-related sepsis has been proposed to be the least frequently recognised nosocomial infection with the true incidence considerably underestimated in most centres because the catheter is not suspected and thus not cultured (Maki 1991).

B) PATHOGENESIS OF CATHETER COLONISATION

Foreign body infections have been observed to derive from a complex interaction between the microsurface of the foreign body, the host, and the pathogen (Mermel and Maki 1994) as shown in the following examples.

THE CATHETER

Teflon and polyurethane catheters appear more resistant to colonisation by coagulase-negative staphylococci than polyethylene, polyvinyl chloride or silicone (Mermel and Maki 1994). In contrast, S. aureus and Candida sp. adhere better to polyvinyl chloride than to Teflon (Sheth et al. 1983). The physico-chemical properties of the catheter surface influence bacterial attachment in vitro (Jansen and Peters 1991). In addition, components of the catheter material may impair polymorphonuclear leukocyte activity as demonstrated by Lopez-Lopez et al. (1990).

The initial stage of bacterial attachment has been demonstrated to occur in areas of surface irregularity in vitro (Locci et al. 1981) and in vivo (Cheesbrough 1984). Irregular catheter surfaces have also been associated with increased thrombogenicity (Hecker 1981). Thrombogenicity is related to stiffness and to catheter material. In an animal model, silicone catheters caused less clot and fibrin deposition than polyvinylchloride, with polyurethane the most thrombogenic (Borow and
A thrombus may develop within hours of catheter insertion, and provide a nidus for infection by entrapping bacteria (Elliott et al. 1984). This may be exacerbated by the infusion of hypertonic fluids, or those containing particulate matter, which irritate the vascular intima (Henderson 1988). Continuous low dose heparin infusion has been demonstrated to reduce catheter colonisation (Bailey 1979).

**THE HOST**

*In vivo*, the catheter surface quickly becomes coated by plasma and tissue proteins such as albumin, fibrinogen, fibronectin and collagen (Mermel and Maki 1994). The *in vitro* effects of the different catheter materials on adhesion are reduced in experiments performed with pre-coated catheters (Maki 1991). *In vivo*, albumin has been demonstrated to inhibit adherence of staphylococci whilst it is promoted by fibronectin (Maki 1991).

Following insertion, a fibrin sheath is formed by deposit of platelets and fibrin along the catheter in a tube-like fashion forming a fibrin sleeve, not adherent to the catheter or the vein, extending over time towards and beyond the catheter tip (Wickham et al. 1992). It is unclear whether this hinders bacterial adherence or provides a nidus for infection (Henderson 1988).

**THE PATHOGEN**

Wide variation in adherence between bacterial strains within *S. epidermidis* has been noted *in vitro*, with slime producing strains more adherent (Kristinsson 1989). Adherence of *S. epidermidis* appears to be facilitated by protease-sensitive adhesins (Mermel and Maki 1994). In addition, exoenzymes may be influential, for example *S. aureus* and *C. albicans* produce coagulase which promotes thrombogenesis. Attraction of the organisms to the catheter surface may also be influenced by chemical properties of the organism such as electrical charge and hydrophobicity (Elliott 1988).

It has been demonstrated by electron microscopy that bacteria growing in natural and pathological ecosystems are surrounded by a glycocalyx in which cells divide within an exopolysaccharide matrix forming microcolonies of identical cells. These adhere to the catheter surface, expand and eventually coalesce to form a biofilm.
The biofilm mode of growth protects the organisms from phagocytosis (Costerton et al. 1985) and from antibiotic therapy that would be bactericidal to planktonic cells (Nickel et al. 1985). Growth can occur in the absence of a nutrient source by utilisation of catheter components, with demonstrable catheter surface erosion in vitro (Norwood 1991).

**CATHETER COLONISATION**

In *in vitro* studies utilising electron microscopy, coagulase-negative staphylococci are seen to adhere to areas of catheter surface irregularities within 30 minutes of exposure. By 60 minutes, microcolonies are visible, with heavy colonisation, first in single then in multiple layers, apparent within 6-12 hours. After 12 hours, extracellular slime is produced, eventually covering the organisms completely (Peters et al. 1982). Passerini et al. (1987) found biofilms on all 20 pulmonary artery catheters, *in situ* an average 2.6 days, and evident within 24 hours of insertion. Raad et al. (1993), from examination of 359 silicone catheters, suggest that colonisation may be a universal event for all indwelling catheters, although only 11% gave a positive culture result. Factors mediating initial attachment of bacteria to the catheter may be different to those facilitating persistence and multiplication, and from those influencing the development of infection from colonisation (Goldman 1990).

In summary, whilst the catheter as an inanimate object may not be infected in the strictest sense, evidence suggests that bacteria live and multiply on, and derive nutrients from, the catheter surface (Norwood 1991), causing local effects such as endothelial damage, and releasing toxins (e.g. endotoxin) and free bacterial cells into the bloodstream (Costerton et al. 1985).

**C) ROUTE OF CATHETER-RELATED INFECTION**

Infections involving invasive medical devices are proposed to be the most preventable of nosocomial infections, often with well defined routes of infection and known methods of prevention (Stamm 1978).
There are four theoretical routes of catheter-related infection:
1) contamination of the catheter during the insertion procedure
2) via contaminated infusate or migration via intraluminal surfaces
3) through the subcutaneous catheter tract or migration via the external catheter surface, or
4) colonisation via blood-borne organisms from a distant site (Kruse and Shah 1993).

Micro-organisms may therefore be introduced on insertion or transported via the bloodstream from an infective focus. However, the majority of infections are historically proposed to arise from colonisation of the catheter entry site with subsequent migration to the tip (Maki and McCormack 1987). The microbial flora at the insertion site is influenced by skin preparation, type of dressing and frequency of dressing changes (Maki 1986). The extraluminal route of infection is illustrated in Figure 1.2.

FIGURE 1.2: THE EXTRALUMINAL ROUTE OF INFECTION (MAKI 1986)
More recently there has been an increased awareness of infections arising intraluminally via the catheter hub due to manipulations of the administration system (Sitges-Serra et al. 1985). Suggested infection control measures include frequent change of administration sets (Williams 1986), sealing junctions with antiseptic gauze (Linares et al. 1986), heating of metallic hubs (Holm and Wretlind 1975) and use of full aseptic technique for each manipulation (Payne-James et al. 1989). The intraluminal route of infection is illustrated in Figure 1.3.
Once the catheter has been inserted, care of the site and the administration system is primarily the responsibility of the nurse. Unfortunately review of the relevant literature on care of the patient with a central venous catheter shows it to be confusing with no established standard of care. Many types of nursing practice are proposed which are often contradictory. For example, a variety of antimicrobial preparations and dressings have been advocated (Schwartz-Fulton et al. 1981) with suggested intervals for dressing change ranging from 1-5 days (Schwart-Fulton et al. 1981, Daschner and Frank 1987, Jarrard et al. 1980). The majority of studies in this area have attempted to change practice and demonstrate a change in the rate of infection. Precise patient groups and other areas of care of the insertion site/administration system are often not defined. Furthermore even controlled studies, e.g. of dressing type (in particular the use of semi-permeable dressings) have produced conflicting results, (Young et al. 1988, Colny et al. 1989) due to the influence of many confounding variables such as type of catheter and duration of catheterisation.

There is also controversy over which of the main routes of infection, intraluminal or extraluminal, is the more frequent or important (Maki et al. 1988). Comparison of individual research papers is difficult due to a lack of randomised controlled trials with similar patient groups, methods and definitions. For example, patients in a hospital wide study with single lumen catheters are generally less severely ill than patients with triple-lumen catheters. The delivery of total parenteral nutrition provides an additional confounding variable.

Even where randomised trials are available they are often difficult to compare due to differences in the culture techniques employed or in the definition of infection. As discussed below, diagnosis of catheter-related sepsis may be made at three levels; clinical, qualitative bacteriological, and semiquantitative / quantitative bacteriological (Bozzetti 1985) of which one, all three or any combination of the three may be reported. Semiquantitative, roll-plate culture alone may miss infections arising via the intraluminal route (Cleri et al. 1980).

In addition to catheter-related septicaemia, authors have taken catheter-related "sepsis", catheter infection or colonisation as an end point. Where organisms isolated from different sources have been compared to determine the route of tip colonisation or bacteraemia, the validity of the results is dependent on the level of typing as discussed
below, rendering the majority of early studies examining the source of catheter-related infection invalid.

Many studies have cultured the insertion site and catheter tip and isolated organisms of the same species which they have presumed to be identical. The likelihood of this being the case is dependent on the level of identification and typing employed which is in turn related to the age of the study. There is also the possibility that the catheter tip may have been contaminated on removal and thus the discriminatory ability of the culture method is important. In addition, studies have demonstrated the presence of the same organism on both site and hub (Di Cicco et al. 1989, Fan et al. 1988), thus hub culture is required to exclude the intraluminal route.

As outlined above, the variable results in studies aiming to determine the predominant route of catheter-related infection are due to differences in the following:

- definition of infection
- culture methods
- level of classification of organisms
- patient/catheterisation variables
- clinical practice in maintenance of the catheter
- catheter type
- use of catheter, particularly TPN administration.

These will be discussed in turn in the following sections:

1. DEFINITIONS/DIAGNOSIS OF CATHETER RELATED INFECTION

Infectious catheter complications may be local or systemic, and include:

**Focal/local infection**
- Cellulitis, thrombophlebitis, abscess formation
- Endocarditis

**Systemic infection**
- Metastatic infection e.g. arthritis, osteomyelitis, endophthalmitis,
  other organ infection
• Bacteraemia and septicaemia (Henderson 1988).

Recently, Arnow et al. (1993) documented that from 102 episodes of percutaneous catheter-related sepsis, complications occurred in 45%, with major complications in 32%; septic shock (12%), sustained sepsis (12%), suppurative thrombophlebitis (7%), metastatic infection (5%), and endocarditis (2%). One patient (of 94) died as a direct consequence of catheter-related infection.

**Focal/local site infection**

Has recently been defined as either:

- Isolation of a significant number of micro-organisms whether or not inflammation is present, or
- presence of purulent drainage, or
- a spreading erythema indicative of cellulitis in the absence of bloodstream infection. (Moro et al. 1994)

Clinical evidence may include erythema, thrombophlebitis, oedema or exudate formation. The patient may complain of irritation and/or pain at the insertion site, and a low grade pyrexia may be present (Elliott et al. 1994).

**Systemic infection: Catheter related "sepsis"**

Systemic catheter related infection is much more difficult to define, and diagnosis may be performed at three levels:

- Clinical
- Qualitative bacteriological
- Semiquantitative/quantitative bacteriological (Bozzetti 1985).

**Clinical diagnosis**

The symptoms of catheter-associated septicaemia are indiscernible from septicaemia arising from any local source, however several features have been identified as indicating the catheter as a source (Maki 1991);
• presence of an intravascular catheter, particularly central venous
• no other obvious source
• an unexpected infection in a patient without underlying predisposition
• isolation of coagulase-negative staphylococci, Candida or other typical catheter organism on blood culture (Maki 1991)
• fever, usually with sudden onset or chills, which quickly resolves after withdrawal of the catheter (Bozzetti 1985)
• sepsis apparently refractory to appropriate antibiotic therapy
• microbiological results typical for infusion or environmental reservoir
• clustered infection by an infusion related organism (Henderson 1988).

Limitations of a clinical diagnosis are that catheter-related infection may be overlooked if pyrexia is mild, or if septicaemia is severe and fails to resolve quickly. Horowitz et al. (1990) observe that the requirement for a clinical improvement with defervescence after removal of the catheter may be too stringent in the Intensive Care setting, where multisystemic disease is common, and fevers may be multifactorial. A further underestimation may occur by the exclusion of infected catheters when a separate primary site is infected with the same organism as the catheter (Horowitz et al. 1990).

Qualitative bacteriological diagnosis

Bacteriological diagnosis may be made by tip culture, blood culture or a combination of the two. Skin site and catheter hub cultures may be of value in predicting infection without removal of the catheter.

Qualitative culture identifies those organisms present. Isolation of the same organism from peripheral blood and the catheter tip with no other source provides evidence of catheter-related septicaemia. This is limited by the reliance on staff alertness in taking blood cultures, with higher rates found in prospective studies than in retrospective studies (Bozzetti 1985). In addition, catheters may cause intermittent bacteraemia and it may be difficult to ensure that the peripheral blood culture was taken at an appropriate time (Sitges-Serra 1983). As bacteraemia has been shown to
occur approximately one hour before the development of rigors or pyrexia, three sets of blood cultures over a twenty four hour period have been proposed to detect intermittent bacteraemia (Washington 1986).

_Semiquantitative/quantitative bacteriological diagnosis_

Diagnosis relies on isolation of "significant" numbers of organisms in central versus peripheral blood cultures or on the catheter tip (see below). The number of organisms present is graded to represent contamination, colonisation or infection. "Contamination" is generally taken to indicate the presence in a specimen taken for culture, of organisms introduced during the taking of the specimen and not reflective of infection in the patient (Norwood et al. 1991).

Catheter colonisation has been defined as the presence of viable micro-organisms on the surface of an indwelling catheter in the absence of any clinical signs of infection and a blood culture negative for that organism (Kruse and Shah 1993). Catheter colonisation is recognised as a precursor to bacteraemia/septicaemia (Sitges-Serra and Linares 1988) and specified levels are termed catheter infection by some authors, (e.g. Norwood et al. 1991) or "significant" catheter colonisation, (Maki et al. 1977) and may indicate bacteraemia/septicaemia as outlined below. However, the value of a positive tip culture in predicting catheter related septicaemia is low (8.8-16%, Johnson and Oppenheim 1992).

Several studies have demonstrated that semiquantitative/quantitative surveillance cultures can predict infected catheters: Snydman et al. (1982) proposed surveillance site swabs were of value in predicting TPN patients at high risk of infection. McGeer and Righter (1987) found that in patients with clinical signs of infection, the probability of catheter-related infection with a negative Gram stain of the insertion site was 16-20%, rising to 72-80% with a positive Gram stain. By logistic regression analysis, Armstrong et al. (1990) found a relative risk of infection of 4.50 in TPN catheters with bacterial growth at the insertion site.

Salzman et al. (1992) cultured catheter hubs in a neonatal ICU three times weekly and found that 54% of episodes of bacteraemia were preceded by colonisation of the hub. Flowers et al. (1989), in ICU patients, found a positive predictive value of
tip colonisation of 45.5% with a positive hub culture and 60% with a positive site culture. Other authors have reported that a combination of both cultures provides the best sensitivity in diagnosing infection without removing the catheter: Fan et al. (1988) found daily hub and twice weekly skin cultures gave a combined sensitivity for bacteraemia of 79.3%, with a positive predictive value of 44.2% and negative of 93.3%. With clinical signs of infection, Cerenado et al. (1990) found combined skin and hub cultures gave a sensitivity, for a positive tip culture, of 97.1%, with a positive predictive value of 34% and negative of 99.4%. Combined surveillance cultures thus appear to be of value, particularly in excluding the catheter as a source of infection in patients with clinical symptoms.

II) CULTURE METHODS

TIP CULTURE METHODS

Qualitative Tip Culture

Qualitative tip culture refers to culture by broth immersion and incubation only. Although possessing a high sensitivity, contamination of the catheter with low numbers of organisms on removal will give a false positive result. False positive rates of 20-50% have been recorded (Hampton and Sherertz 1988). The number of organisms obtained is dependent on growth rates, medium, incubation time and temperature, and does not reflect clinical significance.

Semiquantitative Tip Culture

This method was devised by Maki et al. (1977) to differentiate infection from contamination: Whilst exerting downward pressure with flamed forceps, the external surface of the catheter tip was rolled or smeared across a blood agar plate at least four times: In a study of mainly peripheral cannulae, >15 colony forming units (cfu) correlated best with clinical significance, identifying all cases of catheter related septicaemia, and 64% cases of local site infection, and was chosen to signify significant catheter colonisation. Most catheters associated with septicaemia had confluent growth. Whilst Gil et al. (1989) confirmed this cut-off point for triple-lumen central venous catheters, Collignon et al. (1986), in an 18 month study of central lines in ICU determined that a level of >5 cfu gave a more sensitive result
(92%, specificity 83%). Whilst the negative predictive value of a negative result for catheter related septicaemia was 99.8%, the positive predictive value was 8.8% where all tips were routinely cultured. This is in agreement with the findings of Linares et al. (1985) where >7cfu was associated with bacteraemia. In contrast, Rello et al (1991) found that a cut off point of 25cfu detected catheter-related bacteraemia with a 100% sensitivity (in catheters removed because of suspected infection). There is thus a lack of consensus over the determination of the level of significant growth, and whilst all catheters associated with bacteraemia may be significantly colonised, the positive predictive value of bacteraemia with a significantly colonised tip is low. A major limitation of this method of external surface culture is that it may fail to detect intraluminal catheter colonisation.

**Quantitative Tip Culture**

Quantitative tip culture was devised by Cleri et al. (1980). Tips were immersed in broth and flushed, and the number of colony forming units per tip was estimated by serial dilution prior to incubation. This allows detection of organisms in the catheter lumen, comparison of numbers in mixed infections and an assessment of clinical significance. All catheters associated with bacteraemia gave >1000cfu. Less than 1000 represented colonisation or contamination. This method is more sensitive than semiquantitative culture but again the rate of false positive results was high at 48%. The method was validated for catheters used for TPN by Pettigrew et al. (1985) who found that catheters with >1000 cfu had a ten fold higher probability of being associated with "sepsis". The method was simplified by Brun-Buisson et al. (1987) who agitated the tip in 1ml sterile water and plated a 10μl volume. Again >1000cfu appeared most highly correlated with bacteraemia, however Kristinsson et al. (1989) proposed that for central venous catheters, 100cfu was more sensitive an indicator than 1000cfu.

Linares et al. (1985) adapted the method to examine intraluminal colonisation in TPN catheters by flushing the tip lumen with broth and counting colonies by serial dilution: Catheters associated with bacteraemia were found to have >1000cfu in this study, but >100cfu in later studies by Cheesbrough et al. (1986) and Kristinsson et al. (1989).
BLOOD CULTURE METHODS

Blood cultures are generally performed in the UK by qualitative methods (Elliott 1994). By a quantitative pour plate method, Wing et al. (1979) found blood taken via a central line associated with septicaemia gave 400x the number of organisms cultured from peripheral blood. In a larger study Weightman et al. (1988) found at least a ten fold difference in catheter versus peripheral blood cultures in patients with bacteraemia.

*Catheter-related bacteraemia* has been defined as isolation of the same organism from catheter and peripheral blood in the absence of clinical symptoms with no other likely source. In *catheter-related septicaemia* (CRS) clinical features such as pyrexia>38°C, rigors, hypotension and oliguria are present (Moro et al. 1994). With the need for appropriate blood and tip cultures, this strict microbiological definition will miss many cases of CRS and studies using it will tend to under-estimate the problem (Henderson 1990).

In practice, a diagnosis is generally made by a combination of clinical judgement and microbiological results where available. The following definitions are proposed by Raad (1994):

*Definite catheter related sepsis*: Primary septicaemia caused by any organism (or unknown) with clinical or quantitative microbiological evidence implicating the catheter as the source of sepsis as follows:

- pus at the insertion site with the same organism isolated from the pus and the bloodstream
- clinical sepsis that is refractory to antibiotics but resolves after catheter removal
- positive (significant) quantitative/semiquantitative catheter culture with the same organism isolated from catheter and peripheral blood.
- differential quantitative blood cultures with a ten-fold or greater colony count of organisms isolated from blood cultures drawn through a central catheter compared to simultaneous peripheral venous culture.
Whilst catheter-related septicaemia may be diagnosed by exclusion as a bloodstream infection by a skin organism, (e.g. coagulase-negative staphylococci, Micrococci, Bacillus, Corynebacterium and Propionibacterium species), in a patient with a catheter, and clinical manifestations of sepsis with no apparent alternative source, Raad (1994) suggests that this is better termed "primary nosocomial bloodstream infection", as defined by the Centers for Disease Control, Atlanta, or probable catheter related septicaemia. Many other definitions have been used in published studies examining the aetiology of catheter-related infection as illustrated in Table 1.1 with many differing combinations of culture methods as shown in Table 1.6, page 46.

TABLE 1.1: EXAMPLES OF DEFINITIONS OF CATHETER RELATED INFECTION

<table>
<thead>
<tr>
<th>AUTHOR</th>
<th>TERMINOLOGY</th>
<th>DEFINITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maki et al. 1977, Snydman et al. 1982, Armstrong et al. 1990</td>
<td>catheter infection/catheter-related infection</td>
<td>&gt;15 colonies on semi-quantitative culture</td>
</tr>
<tr>
<td>Cheesbrough et al. 1986</td>
<td>Probable sepsis</td>
<td>fever &gt; 38.5°C for &gt;2 hours and accompanied by rigors</td>
</tr>
<tr>
<td></td>
<td>Possible sepsis</td>
<td>fever &gt; 37°C for 24 hours without significant systemic upset</td>
</tr>
<tr>
<td></td>
<td>Microbiologically confirmed sepsis</td>
<td>possible or probable sepsis with peripheral blood isolate same as CVC tip.</td>
</tr>
<tr>
<td>Kristinsson et al. 1989</td>
<td>Catheter sepsis</td>
<td>same organism from peripheral and central blood</td>
</tr>
<tr>
<td></td>
<td>Probable infected</td>
<td>infection likely but other possible source</td>
</tr>
<tr>
<td></td>
<td>Probably not infected</td>
<td>catheter source unlikely but cant be excluded</td>
</tr>
<tr>
<td>Bozzetti et al. 1991</td>
<td>clinical sepsis</td>
<td>prompt disappearance of fever after removal of CVC</td>
</tr>
<tr>
<td></td>
<td>microbiological sepsis</td>
<td>same organism on CVC tip and in peripheral blood</td>
</tr>
</tbody>
</table>

III) IDENTIFICATION AND CHARACTERISATION OF CLINICAL ISOLATES.

Many of the above definitions of catheter-related infection rely on the isolation of the same organism from two separate sites. The accuracy of this assumption is determined by the level of discrimination of the characterisation system. As the majority of isolates are skin flora, predominately coagulase-negative staphylococci (CNS), methods for typing CNS will be discussed.
The skin as a source of nosocomial infection

The normal flora of the skin contains a variety of micro-organisms which are able to cause disease if the normal protective defence mechanisms are breached. In addition, the skin has become one of the most important reservoirs of nosocomial pathogens in the hospital (Maki 1986). Each individual has approximately 1.5 square metres of skin which is both open to contamination from many environmental sources, and itself contaminating the environment by the continual shedding of squames of which about 10% carry bacteria (Noble 1986).

The major components of the normal skin flora are the coagulase-negative staphylococci, predominately Staphylococcus epidermis (Gallis 1984). Staphylococci are non-motile, Gram-positive, catalase positive facultatively anaerobic cocci, usually arranged in irregular clusters (Oeding 1983). Their primary habitat is mammalian skin (Kloos and Schliefer 1981) where they play a useful role in metabolising products of the skin and associated glands, and may prevent colonisation by more pathogenic microorganisms (Baird-Parker 1990).

Between 1908 and 1957 only two species were recognised in the first to seventh editions of Bergey's manual (1957); Staphylococcus aureus which produced a coagulase, and Staphylococcus epidermidis which did not. Since then a further 25 species and 7 subspecies have been classified as shown in Appendix, and it is expected that more will be discovered (Kloos 1990).

Six species groups have been determined by DNA-DNA hybridisation, supported by phenotypic analysis (Kloos 1990) of which 4 are coagulase-negative:

1. *S. epidermidis* species group, including *S. capitis*, *S. caprae*, *S. haemolyticus*, *S. hominis*, *S. saccharolyticus* and *S. warneri*
2. *S. saprophyticus* species group, including *S. cohnii* and *S. xylosus*
3. *S. simulans* species group, including *S. carnosus*
4. *S. sciuri* species group, including *S. lentus*.

In studies by Schliefer and Kloos (1975) the percentage of individuals carrying the various staphylococcal species on the skin was found to be:
S. epidermidis 100%
S. hominis 100%
S. haemolyticus 78%
S. saprophyticus 70%
S. capitis 65%
S. warneri 52%
S. xylosus 42%
S. cohnii 35%
S. simulans 12%

Of these, S. epidermidis, S. hominis, S. simulans and S. capitis appear to be host specific for human skin (Kloos and Schliefer 1981). Samples from nares and axillae contained fewer species than samples from head, legs or arms reflecting differences in relative resistance to factors such as light, moisture and body secretions (Kloos 1986). For example, S. epidermidis is dominant on the head and thorax, and S. hominis on the arms and legs which are drier with less sebum (Noble 1990).

The coagulase-negative Staphylococci have historically been regarded as saprophytes with little pathogenic potential (Sewell et al. 1982), however, under appropriate conditions, they can produce serious, even fatal, disease. Recognition of the importance of S. epidermidis in particular as a pathogen has evolved over the last twenty years, paralleling advances in medical technology (Cowry 1983), and it is now recognised as a major cause of infection of intravenous cannulae (Peters et al. 1982), peritoneal dialysis catheters (Rudin et al. 1980), central nervous system shunts (Schoenbaum et al. 1975), prosthetic heart valves (Karchmar et al. 1983), vascular grafts (Liekweg 1977), orthopaedic implants (Fitzgerald et al. 1977) and certain post-operative wounds (Newsom 1986).

In addition, the Staphylococci have an ability to develop resistance to a wide range of antibiotic agents in the hospital environment (Christensen et al. 1982), and their ubiquity make their containment unlikely (Weinstein et al. 1982). Furthermore, analysis of resistance determining plasmids of coagulase-negative staphylococci and S. aureus suggests ongoing interspecific transfer (Cohen 1982, Naidoo 1984). Thus the coagulase-negative staphylococci, which are a common component of normal flora are being...
increasingly recognised as resistant hospital acquired pathogens in compromised patients, and may serve as an endemic reservoir for more virulent organisms.

**Taxonomy and Classification of the Staphylococci**

Rosenbach in 1884, was the first person to isolate and study pure cultures of staphylococci which had been recognised as a cause of pyogenic abscesses a year earlier. He noted that two differently pigmented colonies were produced by the organism, and he named them *Staphylococcus pyogenes aureus* (golden colonies) and *Staphylococcus pyogenes albus* (white colonies).

In 1891, the name *Staphylococcus epidermidis albus* was proposed for a non-virulent form of *S. pyogenes albus* (Watankunakorn 1970).

In 1900, Staphylococci were divided into two subgenera, such that golden pigmented strains became *Aureococcus aureus* and white became *Albococcus epidermidis* (Kocur and Mortensen 1967). The genus Staphylococcus was reintroduced in the seventh edition of Bergey's manual in 1957, being distinguished from the genus Micrococcus by the ability to produce acid anaerobically from glucose. *S. aureus* and *S. epidermidis* were differentiated by the ability to produce acid anaerobically from mannitol and to produce a coagulase. Only one coagulase-negative species was recognised.

Their heterogeneity was discovered in 1959, with the advent of numerical taxonomy (Hill 1959), and in 1963, Baird-Parker subdivided the staphylococci into six subgroups, S1-SV1 on the basis of some simple biochemical tests, including production of acid anaerobically from sugars such as arabinose, lactose, maltose and mannitol, and the production of coagulase, phosphatase and acetoin. Micrococci were divided into eight subgroups M1-8 (Baird Parker 1963, 1965).

Later studies of deoxyribonucleic acid base composition showed that in fact only subgroups M7 and M8 represented the genus Micrococcus, the guanine-cytosine content being 30-40 moles%, with that of the genus Staphylococcus being 66-75 moles% (Oeding 1983). Baird-Parker revised his classification such that subgroup

S1 became *S. aureus*

S11-V1 became *S. epidermidis* biotypes 1-4, and...
M1-4 became *S. saprophyticus* biotypes 1-4.

In 1965, he stated that most of these biotypes deserved the rank of species (Baird-Parker 1965).

In 1975, Schliefer and Kloos redefined *S. epidermidis* and *S. saprophyticus* as single biotypes, and distinguished between nine species of coagulase-negative staphylococci, all present on human skin, on the basis of morphological, physiological and biochemical characteristics, including antibiotic susceptibility and cell wall composition, and confirmed by DNA-DNA hybridisation studies (Kloos and Wolfshohl 1982). Additional species have subsequently been characterised by the Schleifer and Kloos system.

**Characterisation of clinical isolates**

Initially, standard laboratory procedure was to identify all coagulase-negative staphylococci as *S. albus* (*epidermidis*) and to identify any coagulase-positive strains as pathogenic - *S. aureus* (Archer et al. 1984). Since the observation that coagulase-negative staphylococci are often themselves the cause of disease, it has been suggested that it is now no longer acceptable for the clinical laboratory to limit its diagnosis to "coagulase-negative staphylococci" (Christensen et al. 1983), although this remains the procedure in many hospitals. Oeding and Digranes (1977) classified 108 coagulase-negative staphylococci and found that "a considerable number" of CNS isolated from clinical specimens belonging to species other than *S. epidermidis* or *S. saprophyticus* were capable of causing infection and should be identified in the diagnostic laboratory.

There are many advantages of more detailed analysis. Identification to at least species level increases our knowledge of the relative pathogenicity of the various species, which in turn aids in the evaluation of whether a clinical isolate is of aetiological significance or merely a contaminant. The universal presence of coagulase-negative staphylococci as normal flora, and therefore their common presence as contaminants of routine cultures make diagnosis of true infection difficult. If the clinical or research laboratory is able to determine whether repeated or multiple isolates from a patient are identical, that is to say likely to have a common origin, the reisolation of the same strain indicates a persistent and probably pathogenic involvement.
To date, investigations of the bacteriology of the coagulase-negative staphylococci have taken two different approaches: Taxonomists seek to group organisms into species or classes with common properties whereas medical microbiologists attempt to detect minor differences between strains of clonally related organisms, where a strain is a population of identical cells descended from a common ancestor (Kloos 1990). Whereas CNS are universally found on human skin and many strains of each species can be found on any individual, infections and epidemics are generally caused by a single strain, the characteristics of which must be demonstrated, and contrasted with those of other strains (Marples 1986).

Thus for epidemiological purposes, for example confirmation of an epidemic strain in the presence of sporadic infections, the level of characterisation of strains must be so discriminatory that a reference laboratory is frequently necessary (Parisi et al 1985). Characteristics for strain identification should be variable within the species but exhibit good stability within a strain. Biotyping and phage typing techniques have been devised for *S. epidermidis*, the most common and most frequently pathogenic isolate, to attempt to trace specific isolates from the environment, but as an epidemiological tool these have proved inadequate (Willet 1984). Information such as the length of carriage of *S. epidermidis* by hospital personnel, the modes of transmission of the organism within the hospital and whether patients are infected endogenously by their own normal flora or exogenously via hospital-acquired strains is currently unknown (Kloos 1981).

In the following sections, the techniques currently available for the epidemiological typing of coagulase-negative staphylococci will be discussed.

**Biological profiles**

Typing of micro-organisms is used to determine their genetic relatedness and thus their likelihood of originating from a single source. Biotyping is based on various properties of the organism such as morphology, metabolism and growth. As it is only possible to test a small range of the properties of the organism, tests are generally chosen to provide Genus and species identification and are designed for taxonomic rather than epidemiological purposes.

Since the late 1960's, Baird-Parkers classification schemes have become widely established. Although these are not able to identify most individual species, they are able
to identify the more medically important species *S. aureus* (S. subgroup I), *S. epidermidis* (S. subgroup II, later *S. epidermidis* biotype I) and *S. saprophyticus* (M subgroup 3, later *S. saprophyticus* biotype III), with an accuracy of 70% (Parisi and Hamary 1984).

Although Schleifer and Kloos' classification has superseded that of Baird-Parker, the routine laboratory generally does not have the capacity to use such a complicated taxonomic scheme for the identification of clinical isolates. This has led to the development of simplified methods in the form of commercially available kits for species identification, such as API-Staph, a rapid mini-system. This was developed by Brun *et al.* (1978) who tested 269 strains of coagulase-negative staphylococci by a full Kloos-Schleifer reference method and a mini-system. Seventy-seven substrates were tested of which nineteen (plus one control) were retained and placed in wells in a strip of inert plastic to form API-Staph. Reproducibility on testing the same inocula on the same day and different inocula on different days ranged from 91-100%. Agreement with the reference method was 100% for type strains, and 97.9% for hospital acquired strains. Baldellon and Megraud (1985) found that of 212 isolates, 89.1% were identified by API-Staph compared to 94.3% by conventional (Kloos-Schleifer) methods.

Fermentation and oxidation tests are those most commonly used in identification systems. Fermentation reactions test the ability of an organism to metabolise carbohydrates in the absence of atmospheric oxygen, or in oxidation, with oxygen as the final electron acceptor, demonstrated by pH indicators in the test media. Assimilation, degradation and hydrolysis tests may also be employed.

**Antibiogram**

An antibiogram is based on determination of the pattern of susceptibility or resistance of an organism to a panel of antibiotics. Antibiotic sensitivity testing is carried out routinely in the clinical laboratory and thus has the advantage of being readily available, standardised, reproducible and relatively inexpensive. An unusual sensitivity and resistance pattern can be used as a marker for the detection of similar strains. For example, the presence of methicillin resistance has been used to monitor the frequency and spread of multiply antibiotic resistant organisms (Archer and Tenebaum 1981). Unfortunately the now widespread occurrence of multiply resistant strains of coagulase-
negative staphylococci (Atkinson and Lorian 1984) has reduced the usefulness of this method in epidemiological studies. *S. epidermidis* appears to be the most resistant of the Gram-positive cocci, showing increasing resistance to almost all antimicrobial agents (Atkinson and Lorian).

*Bacteriophage typing*

Bacteriophages (phages) are bacterial viruses. Virulent viruses can attach to and enter a susceptible bacterium and after a period of multiplication cause lysis of the cell. All staphylococci contain phages, to which they themselves are immune, but which will demonstrate lytic activity against those other strains possessing different restriction-modification systems. For phage typing, sets of these are chosen which can best differentiate between sets of isolates. Phage typing has been used to study the epidemiology of infection with *S. aureus* for more than fifty years (Parker 1983). Coagulase-negative staphylococci however are rarely lysed by phages isolated from *S. aureus* and appear to be more resistant to bacteriophages such that considerably more concentrated phage suspensions are necessary. The isolation of a number of different phages from coagulase-negative staphylococci has enabled the development of several possible typing systems for *S. epidermidis* (Verhoef et al. 1972, Pulverer et al. 1976) although as yet a standard typing set for the coagulase-negative staphylococci has not been established, and a large proportion of strains are untypable (Pulverer et al. 1975). Furthermore, in one survey approximately one third of strains that were lysed by any one phage were lysed by six or more phages, many by greater than ten. Although not all of these "long patterns" were identical, differences between them were not consistent enough to enable subdivision into distinguishable patterns. In addition, these long patterns appear to be proportionately more common among strains of *S. epidermidis* (biotype I). Moreover, unlike with *S. aureus*, phage patterns within biotype I do not appear to identify strains with any special pathogenic properties (Dean et al. 1973).

The sensitivity of the method is therefore related to the number of different phage "types" which can be distinguished and the numbers of clinical isolates falling within a given category. Further difficulties include those of standardisation, reproducibility, lack of availability and the labour intensive nature of the testing which is generally only performed in reference laboratories. De-Saxe et al. (1981) evaluated
the CNS phage typing systems of 12 laboratories in Europe and America for typeability, reproducibility and discrimination, and found that none was satisfactory as an epidemiological tool.

Additional research is necessary to identify new phages active against strains which are at present resistant to lysis, and to recognise an international set of phages which can be readily used by a routine laboratory.

**Serological typing**

Serological typing relies on the presence or absence of strain (type)-specific antigens in the cell wall or on the surface of a micro-organism (flagellar, capsular or envelope).

These were identified for *S. aureus* in 1939 by Cowan who divided isolates of *S. aureus* into three serological groups using slide agglutination. Type-specific *S. aureus* agglutinogens do not occur in the coagulase-negative staphylococci (Oeding 1967) with the exception of *S. hyicus* (Devrieuse and Oeding 1975). Nevertheless, agglutinogens specific for *S. epidermidis* and *S. saprophyticus* have been demonstrated (Assen and Oeding 1971) and it is proposed that each of the nine species of coagulase-negative staphylococci commonly found on human skin possess characteristic agglutinogens (Pillet and Orta 1979), although no serological typing system is as yet available.

**Plasmid analysis**

Amongst the Gram-positive bacteria, the staphylococci have been the most extensively studied with regard to plasmid content and structure (Poston and Naidoo 1983). Plasmids are self-replicating pieces of genetic material consisting of covalently closed circular double stranded DNA, which is stably inherited in an extrachromosomal state. They are widely distributed throughout the Prokaryotes, varying in size from less than 1x10^6 daltons to greater than 200x10^6 daltons. They can be responsible for various properties of an organism such as antibiotic and heavy metal resistance, production of antibiotics, enterotoxins and haemolysins, and sugar fermentations (Old and Primrose 1985). Under most natural conditions they are not essential to the organism.

Plasmid analysis has been used by epidemiologists primarily to explain the occurrence of unusual or linked antibiotic resistance patterns (Parisi and Hamary 1984).
Several techniques may be employed, the most simple of which are the observance of spontaneous or artificially induced loss of antimicrobial resistance or other recognisable characteristic by the organism, or the experimental transfer of a characteristic from a donor strain to a recipient strain not possessing it.

A more recent technique is the determination of the total plasmid content (profile) of an isolate; a crude lysate of the organism is electrophoresed through agarose such that bands of plasmid DNA are separated according to molecular weight. Two isolates are assumed to be the same if they contain multiple plasmid bands of identical size. It is preferable that the organisms in question contain many plasmids, the possibility of their containing plasmids of identical size by chance alone decreasing as the number of plasmids they contain increases.

In 1980, 342 strains of staphylococci representing thirteen species were screened by Kloos et al. (1980) for plasmid composition. Small plasmids were common in most of the species and moderately large plasmids were common in *S. epidermidis* and *S. saprophyticus* species groups.

Plasmid profiles have since been used for *S. aureus*, in addition to conventional techniques in detailed epidemiological studies (Collins et al. 1984) and as a method of typing non-phage typable isolates (Dowsett et al. 1984). Paresi and Hecht (1980) used plasmid profiles in conjunction with biotyping, antibiograms and phage typing in a study of *S. epidermidis*.

In 1982, Archer et al. examined lysates of individual colonies from skin to study the heterogeneity of plasmid profiles of normal flora, and showed great diversity of profiles from different patients, different sites on the same patient and the same site on the same patient at different sampling times. Of clinical isolates, the plasmid profile of *S. epidermidis* from an individual patient was never identical to that of isolates from another patient. In contrast, in well documented infections, identical plasmid profiles were found in all sequential or paired isolates from each patient. They concluded that analysis of plasmid profiles should be "both sensitive and specific" for differentiating *S. epidermidis* isolates. The ability to distinguish between two isolates varies directly with the number of bands present and the difference in molecular size between bands.

This method is however, technically complex and labour intensive and generally limited to research and reference laboratories. Standardisation is difficult due to the
minute and invisible quantities utilised and may lead to low reproducibility; variations in the extraction method may convert one molecular form of the plasmid to another, and variations in the conditions of electrophoresis will alter the final profile (Pfeller and Herwaldt 1988). Furthermore, plasmid content is itself unstable, and existing plasmids may undergo a molecular rearrangement or deletion. Plasmids may also be lost due to antibiotic or environmental pressure or spontaneously. Parisi and Hecht (1980) found that strains found to be identical could subsequently differ by up to 3 plasmids.

Methods of examining chromosomal DNA are not yet available.

IV) PATIENT/CATHETERISATION VARIABLES

These are predominately intrinsic variables which reflect the patient's susceptibility to infection:

Sex/age

The function of the immune system deteriorates with age. From the SENIC study, Hooton et al. (1981) observed certain factors such as age and sex were highly associated with occurrence of nosocomial infection when analysed separately, although were less important when analysed jointly with other factors such as underlying disease.

In relation to central line infection, the skin of elderly patients loses elasticity and fat, becoming drier and thinner which contributes to an increased risk of skin colonisation (Moro et al. 1994). The risk of skin colonisation has been demonstrated to be higher amongst males probably due to the presence of body hair which facilitates the multiplication of organisms (Moro et al.).

Line number

Moro et al. (1994) found that the probability of developing a catheter-related infection was seven times higher for the second period of catheterisation. This was postulated to be due to the longer hospital stay and more severe clinical condition. In contrast, Richet et al. (1990) found no significant difference between the 1st, 2nd 3rd or greater than three catheters.
Days between admission and insertion

For nosocomial infections overall, Freeman and McGowan (1981) noted that the day-specific incidence rose from near zero at day one to a maximum between weeks 4-7 then gradually decreasing toward zero. The risk in the second week of hospitalisation was 3.9 times that in the first week. For central venous catheters, in a proportional hazards model, Armstrong et al. (1986) found the interval between admission and insertion to be significant, with a risk of 1.3-fold if the patient had been in hospital 50 days. Ena et al. (1992) estimated a 2.6-fold increase in the risk of catheter-related infection when the length of hospitalisation was longer than 14 days. Whilst Lucas et al. (1992) found the number of hospitalised days to be a significant predictor of infection, Rose et al. (1988) found no difference in prior hospital stay. Bueno Cavanillas et al. (1991) found that stay length was independently associated with risk of nosocomial infection, but in addition, increasing stay length potentiates the effect of other risk factors such as age and severity of illness.

Severity of illness and underlying disease

Gross et al. (1988) found that the number of pre-existing diseases excluding the reason for admission (comorbidities) correlated directly with the development of nosocomial infection in ICU patients; rates for 0, 1, 2, 3, 4 and 5 comorbidities were 0%, 7%, 3%, 9%, 24% and 33% respectively. Britt et al. (1978) determined that the severity of underlying disease at the time of admission in medical patients paralleled the risk of nosocomial infection: the infection rate was 2.1% in those with nonfatal disease, 9.6% in those with ultimately fatal disease and 23.6% in those expected to die within that admission. Severe underlying disease has been demonstrated to increase the risk of septicaemia (Scheckler 1977).

V) INFLUENCE OF CLINICAL PRACTICE IN MODIFYING THE RISK OF INFECTION

These are extrinsic variables relating to the choice of, and insertion of the catheter, and the subsequent maintenance of the catheter, insertion site and
administration system. Differences in practice within these areas may decrease or increase the risk of infection:

Catheter material

The effect of catheter material on bacterial adherence and catheter thrombogenicity was discussed on page 3. Typically, Welch et al. (1974) demonstrated CRS rates of 1.25% for silastic catheters compared to the stiffer and more thrombogenic polyethylene 9.5% and polyvinyl chloride 18.9%.

Asepsis at insertion/emergency insertion of catheters

Central catheters inserted under emergency conditions are more likely to become infected due to breaks in aseptic technique (Gobsell 1994). Clayton et al. (1985) found no advantage in using an aseptic technique in internal jugular vein cannulae in patients undergoing open heart surgery, but 91% were removed within 72 hours, with no patient in either group developing CRS. Raad et al. (1994) found that maximal sterile barrier precautions (sterile gowns, gloves, large drape, non-sterile mask and cap) as opposed to sterile gloves and small drapes significantly reduced the risk of septicaemia in long-term non-tunneled catheters. In pulmonary artery catheters, maximal barrier precautions were associated with a 2-fold lower risk of infection (Mermel et al. 1991).

Skill of inserter/number of attempts

The incidence of infection correlates directly with the number of attempts at insertion (Sitzmann 1985) and inversely with the experience of the operator (Armstrong et al. 1986).

Subcutaneous tunnelling of catheters

Von Meyenfeldt et al. (1980) found no difference in the sepsis rate when PVC catheters were tunneled. Mitchell et al. (1982) found that the introduction of a tunneled silicone catheter significantly reduced the infection rate of TPN catheters, however tunnelling of PVC catheters did not reduce the infection rate. They concluded that silicone catheters were best for central access, and that tunnelling
facilitates their nursing care. Keohane et al. (1983) found catheter sepsis in 13 of 47 (28%) untunnelled catheters and 6 of 52 (11.5%) tunnelled catheters (p<0.05), however, halfway through the study a nutrition nurse to care for the lines was appointed. There was no significant difference in sepsis rates between tunnelled and untunnelled catheters after the arrival of the nutrition nurse, with an overall rate of 4%. They concluded that rigorous aseptic nursing care is the most significant factor in the reduction of TPN catheter sepsis but tunnelling can reduce the sepsis rate when nursing care is suboptimal.

Guidewire exchange

The use of a guidewire to place a new catheter over a pre-existing one is controversial (Hilton et al. 1988). Guidewire exchange may help prevent infection by removing significant numbers of externally and internally adherent bacteria prior to developing a critical mass (Norwood et al. 1991), however, the difficulty in maintaining sterility when exchanging catheters over a guidewire has been observed (Graeve et al 1981). Bozzetti et al. (1983) found that the sepsis rate decreased when catheters were exchanged weekly over a guidewire. However, in randomised trials, Powell et al. (1988)(TPN) found no benefit in routine guidewire exchanges every three days, whilst Cobb et al. (1992) found a 2-fold increased risk of septicaemia. Hilton et al. (1988) found guidewire replacement was associated with a trend toward increased infection. Newsome et al. (1984) found equal rates of positive tip culture between guidewire exchange and insertion at a new site, as did Pettigrew et al. (1985) in lines changed for suspected sepsis. Whilst the findings of Armstrong et al. (1986) (using TPN catheters) are in agreement with the above, it was noted that catheters replacing previously infected lines nearly always became infected. The authors recommended that guidewire exchange should not be used if catheter infection was suspected. Transfer of Klebsiella sp. during guidewire exchange resulted in subsequent sepsis in one study (Pettigrew et al. 1985).

In an animal model, Lam et al. (1989) demonstrated that the exchange of a CVC over a guidewire carried a high risk of reinfecting the new CVC and of showering the lung with small septic emboli.
Site of insertion and duration of catheterisation

Collignon et al. (1988) found that the incidence of colonisation at the subclavian site was significantly lower than jugular or femoral sites (p<0.01). In a retrospective study of TPN patients, Kemp et al. (1994) found colonisation rates of triple lumen catheters of 5% subclavian, 17% internal jugular and 36% femoral (p<0.01). In a multicentre study of ICU patients in 8 hospitals involving 503 catheters, Richet et al. (1990) found the jugular as opposed to subclavian insertion site to be significantly associated with a positive tip culture, which was proposed to be due to the presence of hair, contamination with oropharyngeal secretions, insertion technique and the fact that jugular site dressings are often loose. The incidence of infection has been demonstrated to increase with increasing duration of catheterisation. Collignon et al. (1988) examined 780 catheters to determine the association between different insertion sites, duration of catheter insertion and catheter-related sepsis: The incidence of colonisation at the subclavian site was significantly lower than jugular or femoral sites (p<0.01). Microbial colonisation of the catheters increased with duration but at a rate dependent on the site of insertion of the catheter. The rate of colonisation of subclavian and femoral catheters increased linearly but with a lower rate of increase at the subclavian site. This was proposed to be due to the fact that the site is easier to sterilise during the insertion procedure, easier to maintain, and there is less movement of the catheter. Colonisation of jugular catheters appeared to increase steeply on the 3rd and 4th days. The average length of insertion of “sterile” versus colonised catheters was 8.0 vs 5.2 at the subclavian site, 8.5 vs 5.6 femoral and 5.1 vs 3.7 internal jugular. Bacteraemia occurred in approximately 10% of all colonised catheters, independent of site and duration. In 220 catheters in ICU patients, Gil et al. (1989) found that the incidence of sepsis rose from 1.5 to 10% when the duration of catheterisation exceeded 6 days.

Use of the central line

Breaks in the sterile closed system of the catheter and fluid administration system allow the potential for microbial contamination of the catheter. This is influenced by the type of fluid infused, degree of usage of the catheter, number of connections in the system, degree of asepsis when opening the system, and any
interventions aimed at reducing contamination such as routine giving set changes and the use of in-line filters (Maki 1973).

Sepsis due to contaminated IV infusions was first recognised in 1953 by O'Hare et al. and Michaels and Reubner. Four major outbreaks of septicaemia due to faulty manufacture were recognised between 1970-1973 in the USA (Maki et al. 1973) and in Britain (Philips et al. 1972). This intrinsic contamination is now rare, however fluids may be contaminated extrinsically during administration - The in-use contamination of IV infusions has been comprehensively reviewed by Holmes and Allwood (1979). Since the introduction of plastic containers, rates of 1-25% have been reported, usually with low numbers of Gram-positive cocci, through airborne contamination (particularly with vented or vacuumed glass bottles) and touch contamination during manipulation (Holmes and Allwood 1976). The entry port of plastic bags is protected from external touch contamination, but the spike remains vulnerable, and as well as accidental touch contamination, airborne skin scales are electrostatically attracted onto the spike/connector needle (Holmes and Allwood 1977). Contamination may subsequently occur from addition of drugs to the infusion container, injection of bolus drugs, introduction of stopcocks or manometers, change of infusion containers and administration sets, obtaining blood samples, accidental puncture and influx of unfiltered air (Maki et al. 1973). Using in-line filters, diffusion of organisms backwards, from the tip into the infusion system has not been demonstrated (Wilmore et al. 1969). Letcher et al. (1972), removing 365 plastic infusion bags and attached administration sets after being set up under in-use conditions by the nursing staff, found, by filtration, that 4.9% of bags and 5.5% sets were contaminated. Although a few showed heavy growth, the majority had a single colony of CNS or other skin flora introduced by touch contamination. D'arcy and Woodside (1973) sampled 101 IV fluid containers after the infusion was completed, of which 61 had contained additives: Bacterial growth was found in 55.7% of containers with additives compared to 12.5% without. From 582 finished glass bottles, Stjernstrom, Gunnarsson and Wilkner (1978) found 6.4% bottles with additives and 2.1% without to be contaminated (<10cfu/ml) with 10.2% amino-acid solutions contaminated compared to 3.2% of 10% dextrose solutions.
Several trials of different intervals of administration set change have documented little change in the frequency of sets detected with growth in infusion fluid within the administration set: Josephson et al. (1985), examining 197 peripheral cannulae, and 16 central venous catheters, (not used for blood products, blood sampling, TPN or pressure monitoring) compared a 48 hour change (mean 1.8 days) with no change (mean 4.3 days). One infusion in each group were contaminated, both with <2 colonies/ml, (1 S. epidermidis, 1 α-haemolytic streptococci.). The type of fluid infused, or number of additives to, or disconnections of the system was not noted. Similar results were found by Buxton et al. (1979) and Band and Maki (1979) comparing 24 vs 48 hour, and 24 vs 48 vs 72 hour changes respectively, in predominately peripheral cannulae, culturing fluid in the system via the injection port. The overall contamination rates were 2% and 0.6% by this method.

In Intensive Care Unit patients, Gorbea et al. (1984) assigned patients to have an in-line burette changed at 24 or 48 hours. Contaminated fluid was obtained from 2% of 24 hour and 4% of 48 hour burettes. The number of punctures per burette was unfortunately the same in both groups, thus the 24 hour group had twice the rate of manipulations. Snydman et al. (1987) compared 48 vs 72 hour burette change in an ICU; again there was no difference in number of punctures with an average of only 1.7 punctures in 72 hours. Fluid contamination rates of 5.0 vs 4.4% were observed.

Regarding hub and/or tip colonisation, in a controlled randomized trial of 52 TPN patients, Sitges-Serra et al. (1985) compared line changes at 48 hours with 96 hours; there were no significant differences in the rate of hub or tip colonisation in either group. Jakobsen et al. (1986) randomized 387 peripheral cannulae to 24 vs 48 vs 72 vs 96 vs 120 hour change of administration set, and cultured the distal lumen of the administration set with cotton wool: Overall, 25% of administration sets were contaminated by this method which may give a more realistic reflection of the levels of contamination observed than culture of infusion fluid. The rate of both (i.e. paired) positive infusion systems and cannulae cultures increased from 12.5% at 24 hours to 25% at 120 hours. Only 4 patients in the study developed septicaemia, and statistically this was not correlated with intraluminal contamination, however patients with fever were found to have significantly higher rates of intraluminal contamination of the administration system.
**Additional devices in the administration system**

The addition of devices such as CVP manometers has been demonstrated to increase the rate of contamination (Hoshal 1972). Contamination rates of three-way taps/stopcocks of up to 48% have previously been reported (Walrath et al. 1979) and it is recommended that they are avoided where possible.

The use of in-line filters with intravenous administration systems has been advocated to protect patients from the clinical consequences of air, particle, microorganism and endotoxin contamination (Marshall and Lloyd 1988), pump-related pressure complications (Gill 1984), and to extend the life of the administration set thus reducing cost and saving nursing time (Ashworth 1990). High levels of particulate matter are found intrinsically in IV fluids and particularly small volume medications such as reconstituted antibiotics (Backhouse et al. 1987). In addition, Walpot et al. (1989) found particles of glass, rubber and plastic in IV fluid during administration which he demonstrated (in ICU patients at autopsy) were deposited in the capillary bed, particularly in the lungs, causing endothelial damage and granuloma formation. He therefore recommended the use of terminal filtration in ICU patients. Infusion of particulate matter irritates the vascular intima (Henderson 1988), encouraging thrombus formation which may provide a nidus for bacterial growth (Elliott 1984). Use of in-line filtration on peripheral cannulae has been shown to reduce the incidence of phlebitis (Francome 1988). Use of filters has also been shown to produce cost savings of 35-38% in an ICU as administration sets need only be replaced every 96 hours (Spencer 1990, Cousins 1988, Ballard 1990, Stromberg and Wahlgren 1989) resulting in decreased time in preparation of administration systems and reduced drug wastage (Ashworth 1990).

Whilst the use of in-line filters is not generally recommended as an infection control measure (Spencer 1990, Gurevich 1989) it has been suggested that they may be of benefit in selected groups of high risk patients (Francombe 1988, Weinstein 1987). As yet, no studies have examined the effectiveness of a 0.2µm in-line filter, changed (as recommended) 96 hourly, in reducing tip colonisation of central venous catheters.

A reduction in the rate of infection has been demonstrated with measures aimed at reducing the number of disconnections of the system, such as a closed hub-administration set connection system rather than stopcocks and piggy-back infusions.
(Inoue et al. 1992), use of a catheter with an integral hub and improved junctional care (Stotter et al. 1987), and incorporating the hub in a povidone-iodine connection shield (Halpin et al. 1991).

Care of the insertion site

The type of antimicrobial agent used for skin cleansing, the dressing applied and the frequency of change affect the microbial flora of the insertion site and may reduce or increase the rate of infection (Maki 1982). Bjornson et al. (1982) demonstrated that a threshold level of skin colonisation (of 1000cfu) was required before catheter colonisation occurred.

Disinfection of the insertion site with a chemical antiseptic is one of the most important measures for prevention of intravascular device related infection (Maki et al. 1991). Lawrence and Lilly (1985) found 1% aqueous chlorhexidine superior (with significantly reduced numbers of bacteria) to “Savlodil” (0.015% chlorhexidine + cetrimide) or 0.5% alcoholic chlorhexidine after 4 days under occlusive dressings on the skin of volunteers. Holmstrom and Svensson (1987) found that disinfecting intact skin of the upper arm of ICU patients with 4% chlorhexidine with detergent maintained a low level of bacterial growth beneath a Tegaderm dressing after 5 days (mean 35 cfu). In a study by Ludlam et al. (1989) of CAPD catheters, cleansing of the insertion site with alcoholic chlorhexidine rather than aqueous povidone-iodine, was one of several new measures which reduced infection rate from 49 to 12%. Use of ointments at the insertion site has not been shown to have a significant benefit: Iodophor ointments are of little value (Prager and Silva 1984). Band and Maki (1979), in predominately peripheral cannulae, found a small benefit in reducing the rate of infection of a polymixin-neomycin-bacitracin ointment versus an iodophor ointment or none, but it appeared to increase the rate of colonisation with Candida. Antibiotic ointments may also select for antibiotic resistant strains (Zinner et al. 1969).

Colonisation can be effectively suppressed by effective site care (Maki and Ringer 1987). For example, Jarrard et al. (1980) found that daily dressings with povidone iodine, antibiotic ointment and gauze eliminated all colonisation of subclavian insertion sites, with no positive skin cultures in 242 patient days. Conversely, as early as 1983, a rise in local infections of IV cannula sites and
bacteraemia due to *S. aureus* was noted following the introduction of Tegaderm, which resolved following its discontinued use (Katich and Band 1985). Since then, several studies of transparent plastic dressings, now the most common type in use, have shown that a significant build up of cutaneous flora can occur, with an increased risk of catheter-related sepsis (Conly et al. 1989, Dickerson et al. 1989, Maki and Will 1990).

Under normal circumstances, pathogenic micro-organisms such as *Streptococcus pyogenes*, *S. aureus* and Gram-negative bacilli placed on the surface of intact skin die rapidly and do not become established as part of the resident flora. However, this may be altered by increasing the humidity of the skin above physiological levels, allowing survival of Gram-negative bacilli (Maki and Ringer 1987). Total occlusion of the skin e.g. with plastic tapes, causes the normal flora to proliferate, with an increase in viable count up to 50,000 fold, and an increase in the proportion of Gram-negative bacilli (Hutchison and Lawrence 1991). The presence of blood or moisture beneath the dressing has been shown to be a significant risk factor for infection (Maki and Ringer 1987). In comparing dressings applied to non-disinfected skin on the chest of volunteers, with 1.5 ml serum introduced underneath, bacterial counts 100-fold higher than with normal skin were obtained with Op-site, and 1000 fold with Tegaderm and Tegaderm plus. Counts of $10^6$ to $10^8$ cfu/ml. were obtained from serum remaining beneath Op-site and Tegaderm dressings (Richardson 1991). Cooper et al. (1989) demonstrated in an animal model that bacteria suspended in a fluid phase were passively carried from the insertion site by capillary action over the surface of the plastic catheter to the tip, and suggest that moisture may accumulate under plastic occlusive dressings to a larger extent than gauze dressings and so increase the likelihood that skin flora will enter into suspension with droplets of moisture. Once suspended, these bacteria may invade the tunnel and move along the catheter surface by capillary action. Powell et al. (1988) and Conly et al. (1989) demonstrated that the increased growth at the insertion site under transparent dressings was related to increased tip colonisation and septicaemia. A meta-analysis by Hoffman et al. (1992) examining 5 prospective randomised trials of gauze versus transparent dressings, (4 cases redressed at same interval, one 7 days Tegaderm versus 3x weekly gauze which whilst being not comparable and perhaps flawed the meta-
analysis, represents a common clinical practice), found a significantly increased risk of catheter colonisation. With pulmonary artery catheters, comparing dressing change every five days, Maki, Stolz and Wheeler (1991) found a mean log cfu of 1.3 with gauze (SD 1.5) versus 2.0 with Tegaderm (SD 1.8). Many types of transparent dressing and intervals of dressing change are in use, with differing risks of infection.

Use of specialist iv therapy/ nutrition teams or nurses

The importance of nursing/medical practice in the maintenance of the insertion site and administration system in modifying the infection rate is seen by the influence of specialist practitioners: In a prospective study of 200 catheters by Ryan et al. (1974), catheters maintained with strict aseptic technique had a sepsis rate of 3% as compared to 20% when breaks in protocol were observed (p=0.01). Haddock et al. (1985) found that when a designated nutrition nurse was responsible for catheter management, catheter-related sepsis fell from 36% to 10% when compared to a six month period during which catheter management was the responsibility of general nursing staff. Nelson et al. (1986) demonstrated that careful attention to aseptic technique during dressing changes by a specially trained nurse resulted in significantly fewer episodes of catheter-related sepsis than when dressings were performed by Surgical residents. Nehme (1980) compared 211 patients managed by a Nutritional Support team with 164 cared for by other physicians: Sepsis occurred in 1.3% versus 26.2%. Similar results were obtained by Faubion (1986) in a 6 month prospective study pre-and post inception of a parenteral and enteral nutrition team, with a sepsis rate 24% pre-team and 3.5% post team. Tomford et al. (1984) found that an IV therapy team can also reduce catheter-related complications, particularly infection.

Sherman et al. (1988) proposed that the lower infection rates seen with specialist teams was due to a lack of compliance with rigorous protocols by other staff and proposed that this could be remedied by an intensive educational programme. This has been demonstrated; Puntis et al. (1990) found a significant fall in the sepsis rate of TPN catheters (45% to 8%) following an intensive education programme.
**VI) TYPE AND USE OF THE CATHETER**

**Total parenteral nutrition**

The administration of TPN is a recognised risk factor for the development of catheter-related infection. Rose *et al.* (1988) in 596 subclavian catheters found a significant risk of infection when catheters were used for TPN: 5.7% versus 1.14% non-TPN. Gil *et al.* (1989) demonstrated a rate of colonisation of 25% of catheters used for TPN versus 13% without (p<0.05).

The reasons for this increased risk of infection are likely to be multifactorial: In ICU patients, Hilton *et al.* (1988) found the administration of TPN to be significantly associated with CRS, p=0.001, but this fell to p=0.072 when adjusted for time at risk, and p=0.492 when adjusted for other factors such as underlying disease. Patients receiving TPN have been noted typically to be severely ill or injured, catabolic, protein-calorie deficient, immunosuppressed and receiving long-term, broad-spectrum antibiotics (Haller and Rush 1992).

In examining the relationship between malnutrition and nosocomial infection in general medical and surgical patients, Gorse *et al.* (1989) found that a poor nutritional score (derived from serum albumin, total lymphocyte count and unintentional weight loss) and worsening nutritional score and serum albumin were associated with the development of infection. Nutritional measures were more abnormal in subgroups of patients developing nosocomial pneumonia, urinary tract infection, wound infection and bacteraemia than in controls. Abnormal serum albumin and transferrin levels, total lymphocyte count, creatinine-height index and arm circumference have been correlated with an increased risk of septicaemia (Harvey *et al.* 1981). Malnutrition has also been associated with an alteration in intestinal flora and an increase in bacterial translocation from the gastro-intestinal tract in experimental studies (Deitch *et al.* 1987). This also occurs in gut stasis, with feeding by parenteral nutrition alone causing a decrease in the integrity of the gut mucosa and local immunity (Alverdy *et al.* 1985). In an animal model, Shou *et al.* (1994) found a significantly higher number of bacteria-positive mesenteric lymph nodes, decreased splenocyte proliferation and decreased peritoneal macrophage function in TPN as opposed to a regular diet. Infection rates have been demonstrated to be higher in critically ill patients given parenteral vs enteral feeding (Moore *et al.* 1989). Gut
translocation is a possible, although unproven, source of catheter infection during TPN (Mughal and Leidhardt 1990).

A modest degree of uncontrolled hyperglycaemia induced by TPN appears to increase the risk of infection. Rapid and/or excessive lipid administration can interfere with reticuloendothelial system function which may also predispose the patient to infection (Pomposelli and Bistrian 1994). TPN has also been demonstrated to provide a good growth medium for bacteria and fungi (Gelbart et al. 1973).

**Single- versus Triple-Lumen Catheters**

A triple-lumen catheter (TLC) is composed of a bundle of 3 catheters of differing diameter that are infused through separate ports. This allows concomitant administration of TPN, fluid and medications, and monitoring of central venous pressure. The first triple-lumen catheters were introduced in 1983, since which time there has been an enormous growth in their use despite minimal data regarding risk, and the correct procedures required for their care (Farber 1988). Due to concerns over their safety, several studies have compared the infection rate of single- versus triple-lumen catheters as summarised in Tables 1.2 and 1.4 (page 39). Although most have been prospective, many have not been comparative and few randomised. Furthermore, it is very difficult to make direct comparison between the studies because of differences in the criteria for defining a catheter-related infection, use of parenteral nutrition, differences in patient population and severity of illness, and differences in dressing and catheter-use protocols.

**The influence of catheter type in TPN administration**

In studies of catheters used for TPN administration, the findings are typified by Yeung et al. (1988) who found the risk of infection to be three times greater in patients receiving TPN via a TLC, in comparable patient groups. Pemberton et al. (1986) found an incidence of systemic infection of 19% triple-lumen catheters used for TPN compared to 3% in single lumen catheters (SLC) used exclusively for TPN despite a similar incidence of site infection (5% versus 3%) suggesting that infection was related to the manipulations of the catheter. Similar findings were obtained by Rose et al. (1988).
TABLE 1.2: RATES OF CATHETER-RELATED SEPTICAEMIA IN TPN ADMINISTRATION VIA SINGLE- AND TRIPLE-LUMEN CATHETERS

<table>
<thead>
<tr>
<th>AUTHOR(s)</th>
<th>YEAR</th>
<th>TYPE OF STUDY</th>
<th>CATHETER-RELATED SEPTICAEMIA % (sample size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pomp et al.</td>
<td>1985</td>
<td>Retrospective</td>
<td>1.9(274)</td>
</tr>
<tr>
<td>Wolfe et al.</td>
<td>1986</td>
<td>Prospective</td>
<td>3.2(383)</td>
</tr>
<tr>
<td>Pemberton et al.</td>
<td>1986</td>
<td>Prospective</td>
<td>3.0(68)</td>
</tr>
<tr>
<td>McCarthy et al.</td>
<td>1987</td>
<td>Randomised</td>
<td>0.0(36)</td>
</tr>
<tr>
<td>Kovacevich et al.</td>
<td>1988</td>
<td>Prospective</td>
<td>5.3(756)</td>
</tr>
<tr>
<td>Pomp et al.</td>
<td>1988</td>
<td>Prospective</td>
<td>1.3(617)</td>
</tr>
<tr>
<td>Lee et al.</td>
<td>1988</td>
<td>Prospective</td>
<td>0.0(68)</td>
</tr>
<tr>
<td>Yeung et al.</td>
<td>1988</td>
<td>Prospective</td>
<td>1.5(65)</td>
</tr>
<tr>
<td>Clark-Christoff et al.</td>
<td>1992</td>
<td>Randomised</td>
<td>2.6(78)</td>
</tr>
</tbody>
</table>

*p*used only for TPN.

During a five year prospective surveillance of complications of TPN, Wolfe et al. (1986) observed a significant increase in the CRS rate following the introduction of triple-lumen catheters (Table 1.3). The patient population was unchanged. More recently, in a randomised study of catheters used for TPN administration, Clark-Christoff et al. (1992) found an incidence of catheter-related sepsis of 2.6% with SLC and 13.1% with TLC (p<0.01).

TABLE 1.3: EFFECT OF THE INTRODUCTION OF THE TRIPLE-LUMEN CATHETER ON THE INCIDENCE OF CATHETER-RELATED SEPSIS IN TPN

<table>
<thead>
<tr>
<th>YEAR</th>
<th>PATIENTS</th>
<th>SEPSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981</td>
<td>284</td>
<td>2.1%(6)</td>
</tr>
<tr>
<td>1982</td>
<td>296</td>
<td>4.4%(13)</td>
</tr>
<tr>
<td>1983</td>
<td>358</td>
<td>3.6%(13)</td>
</tr>
<tr>
<td>1984*</td>
<td>368</td>
<td>11.1%(41)</td>
</tr>
<tr>
<td>1985*</td>
<td>341</td>
<td>10%(34)</td>
</tr>
</tbody>
</table>

Wolfe et al. (*47% catheters triple-lumen*)

Influence of catheter type in catheters not inserted for TPN administration.

In 502 ICU catheters, Hilton et al. (1988) found a significant difference in rate of sepsis of TLC vs SLC even after adjusting for confounding variables such as severity and type of underlying disease (3.57 versus 1.16/100 days at risk). Similar results have been found in other studies as summarised in Table 1.4. Conversely, Farkas et al. (1992) found no significant difference in the rate or day of occurrence of sepsis between single-and triple-lumen catheters in the critically ill, although triple...
lumen catheters were in for a significantly shorter duration. The rate of CRS increased steeply in the TLC group only, after the first 15 days, the authors concluding that TLCs should be changed before this time.

Table 1.4: Rates of Catheter-Related Septicaemia in Multi-purpose Single- and Triple-Lumen Catheters.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Type of Study</th>
<th>Single (%)</th>
<th>Multi (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critically Ill</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miller et al.</td>
<td>1984</td>
<td>Prospective</td>
<td>10(10)</td>
<td>7(27)</td>
<td>NS</td>
</tr>
<tr>
<td>Hilton et al.</td>
<td>1988</td>
<td>Prospective</td>
<td>8(126)</td>
<td>32(93)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gil et al.</td>
<td>1989</td>
<td>Prospective</td>
<td>7.9(63)</td>
<td>3.8(157)</td>
<td>NS</td>
</tr>
<tr>
<td>Farkas et al.</td>
<td>1992</td>
<td>Randomised</td>
<td>8.9(68)</td>
<td>11.5(61)</td>
<td>NS</td>
</tr>
<tr>
<td>All Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeung et al.</td>
<td>1988</td>
<td>Prospective</td>
<td>0.35(580)</td>
<td>0.62(323)</td>
<td>NS</td>
</tr>
<tr>
<td>Rose et al.</td>
<td>1988</td>
<td>Retrospective</td>
<td>1.6(248)</td>
<td>4.9(232)</td>
<td>0.065</td>
</tr>
</tbody>
</table>

*not used for TPN

The reason for these observed differences is unclear: Triple lumen catheters are wider and stiffer than single-lumen catheters, causing more injury to vein walls, predisposing to thrombosis and infection (Wolfe et al. 1982). In a randomised study of single- versus triple-lumen catheters, McCarthy et al. (1987) observed that significantly more triple-lumen catheters were removed because of the presence of pus at the insertion site which they proposed may be due to the larger skin puncture site and the need for a small incision at insertion.

Other authors have proposed that the increased risk of infection is due to the potential for more frequent manipulations of the catheter increasing the likelihood of contamination (Pemberton et al. 1986, Rose et al. 1988). Clark-Christoff et al. (1992) found the rate of catheter-related infection no different in SLC vs TLC if catheters were isolated and not used for multiple accesses. Conversely, Lucas et al. (1992) suggest that whilst it is assumed that triple-lumen catheters are manipulated more frequently, perhaps one single-lumen catheter would require to be manipulated even more frequently in critically-ill patients. Powell et al. (1988) found no difference in the rate of infection between a double lumen catheter and bi-lateral single-lumen catheters.

In TPN administration, where it has been established practice that the catheter used for TPN should be inviolate, it has been asserted that provided a strict catheter
care protocol is maintained, multilumen catheters are no more liable to become infected than single-lumen catheters used exclusively for TPN (Faubion et al. 1986). However, Mughal (1989) states that is “well known” that procedures such as blood sampling, central venous pressure monitoring and the administration of drugs are not usually performed with the same regard to asepsis that pertains when parenteral nutrition solutions are set up. The impression of Clark-Christoff et al. (1992) is that little control is exercised in most hospitals over who may access the catheter. Ryan et al. (1974) in a study of 200 single-lumen catheters for TPN observed that catheters used with strict aseptic technique had a sepsis rate of 3% compared to 20% when breaks in protocol were observed (p=0.01).

Johnson and Rypins (1990) found no difference in infection rate between single- and double-lumen catheters, but catheters were excluded if the additional lumen was used for blood administration, blood sampling or central venous pressure monitoring. Two double-lumen catheters were excluded, both due to blood administration via the catheter and both developed positive catheter cultures as opposed to 1 of the remaining 51 double-lumen catheters. Dedication of one lumen to TPN administration only remains associated with an increased risk of infection: Lee et al. (1988) administered TPN as a constant uninterrupted flow through a dedicated lumen of 307 triple-lumen catheters and 68 single lumen catheters. Tips of 10.4% of TLCs were colonised versus 4.4% SLCs with sepsis rates of 1.3% and 0.0% respectively. Despite dedicating the middle port of the TLC to TPN only, McCarthy et al. (1987) found a higher incidence of sepsis with TLCs 12.8% versus SLCs, 0%.

In addition to higher rates of infection, the infectious complications of TLCs have been observed to appear sooner (Pemberton et al. 1986, Hilton et al. 1988).

**D) SUMMARY: THE CONTROVERSY OVER THE PREDOMINANT ROUTE OF CATHETER-RELATED INFECTION**

The relative importance of the different routes by which bacteria may enter the bloodstream of a patient with a central venous catheter has been debated for more than a decade (Farr 1994). As recently as 1991, Armstrong et al. state that
"the data supporting the hub hypothesis are too tenuous to necessitate inclusion of hub cultures in any study of intravascular catheter-related infections"

Conversely, Mughal and Leinhardt (1990) assert that there is no clear evidence to support the theory that organisms migrate from the exit site through the tunnel and onto the intravascular portion of the catheter. The evidence for each of the two major routes of infection as discussed in the literature review is summarised as follows:

**The administration system as a source of infection**

The fact that administration systems become contaminated in use is well established. Maki *et al.* (1974) found low numbers of organisms in 11% of administration sets in a hospital study. Sets in use for more than 48 hours were at greater risk suggesting that the risk of introduced contamination was cumulative. This was shown by one study which demonstrated that attachment of a bag to the set gave a 0.4% chance of contamination which rose to 0.74% with one additive and 1.51% with two (Kundsin 1983). More recently, Ullman *et al.* (1990) cultured each lumen of triple lumen catheters daily and found increasing colonisation with increasing time. Use of three-way taps and CVP manometers increase the risk of contamination (Hoshal 1972). The massive contamination required to cause septicaemia appears to be rare however, it has been shown that once within the system, if capable of growth in the infusion, organisms can remain for many days despite many changes of infusion bag and high rates of flow (Maki *et al.* 1977). Evidence for the intraluminal route of infection includes:

Significant reductions in the rate of catheter-related infection by

- Use of a catheter with an integral hub and improved junctional care (Stotter *et al.* 1987).

Conversely, the rate of infection is increased:
• In triple- versus single-lumen catheters (Pemberton et al. 1986, Wolfe et al. 1986).
• Following leaks in the administration system (Dietal 1983).
• Following protocol violations on catheter usage (Snydman et al. 1982, Ryan et al. 1974).
• With the use of three-way taps (Hoshal 1972).
• With outbreaks due to infusate contamination at manufacture (HMSO 1972).

The skin as a source of catheter-related infection

Evidence in favour of the skin as a major source of infection is that the majority of organisms isolated from catheter tips are part of the normal skin flora. In addition, Cooper and Hopkins (1985) found organisms most frequently present on the outside rather than the inside of the catheter on light microscopy suggesting migration from the insertion site to the tip via the subcutaneous tunnel or external surface of the catheter.

In an animal model, bacteria inoculated at the insertion site could be identified on the tip (4cm away) within 1 hour, by capillary action in a fluid phase, either at the time of catheter insertion or one week later (Cooper et al. 1988).

Many studies have demonstrated an association between organisms at the insertion site and the rate of tip colonisation or infection: Snydman et al. (1982) found that patients (on TPN) with no microbial growth at the insertion site did not go on to develop an infection. Bjornson et al. (1982) demonstrated an association between colonisation of the catheter tip and the presence of more than 1000 colony forming units at the insertion site and proposed that colonisation occurred once a threshold had been reached. Bentley et al. (1968) found that a threshold time was required to produce phlebitis with peripheral cannulae which was dependent on the amount of damage to the endothelium, number of organisms at the insertion site and host factors. Extension to septicaemia arose after a further 0-96 hours dependent on host factors and degree of local infection. Jakobsen et al. (1989) cultured skin at the insertion site of subclavian catheters every three days and found that the rate of colonisation of the skin increased with time. Conly et al. (1989) found that use of a transparent polyurethane dressing increased bacterial counts at the site and increased the rate of infection.

Further evidence suggesting the skin as a source of infection are studies in which interventions have decreased the rate of infection by:
• Tunnelling of catheters and thus increasing the distance between the external site and the catheter tip (Mitchell et al. 1992).

• The use of maximal sterile barrier precautions (sterile gowns, gloves, large drape, non-sterile mask and cap) as opposed to sterile gloves and small drapes at catheter insertion (Raad et al. 1994).

• The use of an attachable collagen cuff to encourage tissue growth to seal the entry site (Maki et al. 1988, Flowers et al. 1989). This agrees with the low rates of infection using surgically implanted Hickman and Broviac catheters.

• The use of antiseptic or antimicrobial ointments (Flowers et al. 1989).

And conversely,

• The increase of infection in burn patients, who have large bacterial populations on the skin (Maki et al. 1987).

• Outbreaks of catheter-related septicaemia traced to contaminated disinfectants or tape (Maki et al. 1987).

• The failure of trials of different intervals of administration set changes to influence the infection rate (Buxton et al. 1979, Band and Maki 1979).

• The increase in the infection rate when surgical residents were responsible for dressing changes as opposed to a specially trained nurse (Nelson et al. 1986).

**Identification of the predominant route of catheter-related infection**

Studies attempting to determine the predominant route of infection have produced conflicting results. The reasons for this can be seen when examining the studies in the light of the literature review: To provide valid results, coagulase-negative staphylococci, the most frequent isolates, must be speciated and typed by a combination of methods to determine that isolates are identical or different with a high level of probability. In addition, culture methods must be used that will detect colonisation on the internal surface of the catheter tip, which can be missed by the conventional method of rolling the external surface of the tip over an agar plate. Both insertion sites and catheter hubs must be cultured, and culture methods must be adequate to ensure any organisms present in significant numbers are detected.
To allow comparison of results between studies, information is required on the many factors outlined above which modify the infection risk. In particular, it is necessary that the type and use of catheter, insertion site, severity of illness of the patients, duration of catheterisation and a precise definition of the outcome chosen e.g. infection, are stated, and controlled for in comparative trials. Furthermore, because of the strong influence on the infection rate of different practices in the maintenance of the insertion site and administration systems, the dressing regimen and number and type of disconnections of the administration system, and techniques used need to be stated.

As summarized in table 1.6, previous studies examining the route of infection of central venous catheters have been small (all less than 50 positive central venous catheter tips) and have focused on single-lumen catheters, particularly in TPN, however of these only 3 studies speciated isolates of coagulase-negative staphylococci. Of the four studies examining triple-lumen catheters, only two cultured both the hub and the insertion site, and none used a quantititative culture method and thus may have missed infections arising from the catheter lumen. None speciated CNS. Where stated, care of the insertion site varied widely between studies (table 1.5) and with the exception of dedicated TPN catheters, the type and degree of use of the catheter was not measured.

TABLE 1.5: VARIATIONS IN CATHETER CARE BETWEEN STUDIES

<table>
<thead>
<tr>
<th>AUTHOR</th>
<th>INSERTION SITE CARE</th>
<th>TUBING CHANGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bjornson et al. 1982</td>
<td>3x weekly, ?products</td>
<td>daily</td>
</tr>
<tr>
<td>Snydman et al. 1982</td>
<td>3x weekly, ?products</td>
<td>3x weekly</td>
</tr>
<tr>
<td>Abbott et al. 1983</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Sitges-serra et al. 1984</td>
<td>2x weekly, PVI *ointment, ?dressing</td>
<td>daily</td>
</tr>
<tr>
<td>Linares et al. 1985</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Mcgeer and Righter 1987</td>
<td>3x weekly, ?products</td>
<td>?</td>
</tr>
<tr>
<td>Gollledge and McPherson 1988</td>
<td>2x weekly, PVI ointment, gauze/plastic film</td>
<td></td>
</tr>
<tr>
<td>Fan et al. 1988</td>
<td>2x weekly, PVI soaked gauze + Op-site</td>
<td>daily</td>
</tr>
<tr>
<td>Maki et al. 1988</td>
<td>48 hourly, PVI, gauze</td>
<td>48 hourly</td>
</tr>
<tr>
<td>De Cicco et al. 1989</td>
<td>3x weekly, acetone, PVI, Mepore</td>
<td>daily</td>
</tr>
<tr>
<td>Flowers et al. 1989</td>
<td>48 hourly, PVI, polyantibiotic ointment, plastic film</td>
<td>48 hourly</td>
</tr>
<tr>
<td>non-cuff control group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jakobsen et al. 1989</td>
<td>?</td>
<td>varied (trial)</td>
</tr>
<tr>
<td>Ullman et al. 1990</td>
<td>weekly, alcohol, iodophor ointment, plastic film</td>
<td>?</td>
</tr>
<tr>
<td>Armstrong et al. 1990</td>
<td>2x weekly, PVI, gauze</td>
<td>24 hourly</td>
</tr>
<tr>
<td>Cerenado et al. 1990</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Balakrishnan et al. 1991</td>
<td>3x weekly, Tegaderm plus, ?cleanser</td>
<td>?</td>
</tr>
<tr>
<td>Bozzetti et al. 1991</td>
<td>48 hourly, PVI ointment, occlusive dressing</td>
<td>?</td>
</tr>
<tr>
<td>Maki et al. 1991</td>
<td>48 hourly, antiseptic trial, gauze</td>
<td>?</td>
</tr>
</tbody>
</table>

* PVI = povidone iodine
<table>
<thead>
<tr>
<th>AUTHOR</th>
<th>+VE TIPS</th>
<th>CATHETER</th>
<th>PATIENTS</th>
<th>QL *</th>
<th>SQ *</th>
<th>QT *</th>
<th>SKIN</th>
<th>HUB</th>
<th>TIP</th>
<th>TYPED?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bjornson et al. 1982</td>
<td>19</td>
<td>TPN only</td>
<td>TPN</td>
<td>Y</td>
<td>I</td>
<td>Y</td>
<td>Y</td>
<td>13/19</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Snydman et al. 1982</td>
<td>14</td>
<td>single tunneled</td>
<td>TPN</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>61% positive predictive value (ppv)</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Mcgeer and Righter 1987</td>
<td>23</td>
<td>single</td>
<td>?</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>20/23</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Golledge+McPherson 1988</td>
<td>7</td>
<td>?</td>
<td>?</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>2/7</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Armstrong et al. 1990</td>
<td>19</td>
<td>single</td>
<td>TPN</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>significant association</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Balakrishnan et al. 1991</td>
<td>20</td>
<td>single/TLC</td>
<td>paediatric ICU</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>10/20</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Ullman et al. 1990</td>
<td>12</td>
<td>TLC</td>
<td>ICU</td>
<td>Y</td>
<td>Y</td>
<td>Y (lumen)</td>
<td>Y</td>
<td>7/12</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Bozzetti et al. 1991</td>
<td>43</td>
<td>nutricath</td>
<td>cancer TPN</td>
<td>F+I</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>37% ppv sepsis</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Abbott et al. 1983</td>
<td>24</td>
<td>central?type +arterial trauma</td>
<td>Y</td>
<td>Y</td>
<td>Y (stopcock)</td>
<td>15% skin 13% stopcock</td>
<td>NO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sitges-serra et al. 1984</td>
<td>19</td>
<td>single</td>
<td>TPN</td>
<td>Y</td>
<td>F</td>
<td>Y</td>
<td>Y</td>
<td>hub 21/23, skin 0.</td>
<td>speciated+atb</td>
<td></td>
</tr>
<tr>
<td>Linares et al. 1985</td>
<td>19</td>
<td>single</td>
<td>TPN</td>
<td>Y</td>
<td>F</td>
<td>Y</td>
<td>Y</td>
<td>CRS 14/20 hub, 2/20 skin</td>
<td>species, atb</td>
<td></td>
</tr>
<tr>
<td>Fan et al. 1988</td>
<td>28</td>
<td>single</td>
<td>surgical TPN</td>
<td>Y</td>
<td>F</td>
<td>Y</td>
<td>Y (extension tube)</td>
<td>14/28 skin, 2 hub, 5 both</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Maki et al. 1988</td>
<td>40</td>
<td>various, cuffed +non all in-patient</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>hub 4/40, skin 36</td>
<td>NO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>De Cicco et al. 1989</td>
<td>22</td>
<td>single</td>
<td>cancer, TPN</td>
<td>Y</td>
<td>I</td>
<td>Y</td>
<td>Y</td>
<td>hub 4/40, skin 36</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Flowers et al. 1989 non-cuff control group</td>
<td>10</td>
<td>TLC+PA</td>
<td>ICU</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>10/22 skin, 9/22 hub</td>
<td>biotype +Atb*</td>
<td></td>
</tr>
<tr>
<td>Jakobsen et al. 1989</td>
<td>14</td>
<td>single</td>
<td>TPN/CVP</td>
<td>Y</td>
<td>Y</td>
<td>Y (lumen)</td>
<td>9 hub, 5 skin,</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerenado et al. 1990</td>
<td>53</td>
<td>various central +peripheral in-patients</td>
<td>Y</td>
<td>I</td>
<td>Y</td>
<td>Y</td>
<td>Y (lumen)</td>
<td>9 hub, 5 skin,</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Maki et al. 1991</td>
<td>24</td>
<td>?</td>
<td>ICU</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>hub/infusate 4/14</td>
<td>CRB, catheter 10.</td>
<td>NO</td>
</tr>
</tbody>
</table>

*QL=qualitative (broth immersion) SQ=semiquantitative (roll-plate) QT=quantitative: I=immersion (intra- + extraluminal culture) F=flush (intraluminal). Atb= antibiogram
As can be seen from the above studies, there is no agreement on the relative importance of each route. The intensity of the debate is apparent from correspondence in letters to the Editor, between Sitges-Serra and Linares with Brun-Buisson and with Collignon and Munro in the *Journal of Clinical Microbiology* (1988), Segura and Sitges-Serra, with Maki in the *Lancet* (1991) and between Segura and Sitges-Serra with Armstrong *et al.* in the *Journal of Infection Control and Hospital Epidemiology* (1991).

The main proponents of the “hub theory” are the team of Sitges-Serra, Linares and Segura who assert that the hub is the most important route of central venous catheter infection (as shown by their studies in the above table). The main proponent of the “skin theory” is Maki and his team who assert that the majority of infections arise from the skin at the insertion site. Some reasons for this difference may be proposed:

- **Definition of infection.** The definition of catheter-related infection used/invented by Maki is isolation of $>15$ cfu by the roll-plating method. Other definitions of infection, including that by Sitges-Serra *et al.* require clinical symptoms of infection. Additional factors may be important between tip colonisation and the development of clinical infection (Maki 1991).

- **Definition of hub-related infection.** The definition of the Maki teams of hub/infusate related septicaemia requires negative semi-quantitative culture of the catheter tip:

  “isolation of the same species from the catheter hub and from separate percutaneously drawn blood cultures with semiquantitative culture of the catheter negative for the infecting organism” (Maki *et al.* 1988 & 1991).

This is not supported by the literature with studies showing a large proportion of cases of hub-related infection growing $>15$ cfu on roll-plating (Fan *et al.* 1988, Sitges-Serra *et al.* 1988, Kristinsson *et al.* 1989). Typically, Bozzetti (1991) demonstrated that contamination of the hub with high numbers of organisms was associated with a positive external surface in 50% of cases. Kristinsson *et al.* (1989) examining culture methods for central venous catheters found that virtually all heavily colonised catheters were colonised on both the inside and outside.
• **Culture methods a) skin.** The rate of positive skin cultures in the study by Sitges-Serra was extremely low (11%) suggesting very effective site care or low recovery due to the skin sampling method of a dry swab at the insertion site. In contrast, the method of the Maki team involved vigorous scrubbing with a transport medium moistened swab of a 10cm square area. This may recover organisms not present at the catheter-skin interface itself, but at a distance that may be in contact with the catheter hub.

• **Culture methods b) tip.** The tip culture method used by Maki is his own roll-plating technique which will miss organisms on the internal surface of the catheter only. There is also the possibility that the correlation between organisms at the insertion site skin site and tip on semiquantitative culture may be due to the pulling of the tip through the subcutaneous tract on removal.

• **Speciation of organisms.** There are no details of any speciation of CNS being performed by the Maki team.

• **Catheter type/duration of catheterisation.** As observed by Raad (1991), Maki has examined short-term catheters in situ a mean of 7.2-9.1 days versus 23.4-26.5 days by Sitges-Serra. By electron microscopy, Anaissie et al. (1991) found that with short-term central venous catheters (mean 15 days), both surfaces were colonised to an equal extent, whereas for long-term central venous catheters (mean 109 days), the degree of colonisation and biofilm formation on the internal surface has been found to be at least that of the external surface (Raad 1991). Raad et al. (1993) observed that external colonisation predominated in the first 10 days, with luminal colonisation increasing progressively becoming predominant after 30 days. In addition, after 2-4 weeks from insertion, the skin entry sites heals (Elliott et al. 1994) which may make infection from the skin more unlikely.

• **TPN administration.** Sitges-Serra’ patients were receiving TPN which is a good medium for bacterial growth as previously discussed.
Therefore, whilst a lot of information is available in the literature on risk factors for central venous catheter infection, due to differences in definitions and methodology, it is not possible to apply the results of previous studies to other clinical areas, or to identify the predominant route of catheter-related infection.

E) DEVELOPMENT OF THE STUDY

Following concern over the development of clinical septicaemia in several surgical patients with a central venous catheter, the author undertook the above literature review to evaluate current practice. The literature on catheter care was found to be confusing and contradictory. This research project was conducted in four stages that were designed to address areas in which information was not available in the literature, with each study developing from the previous study as new findings were made:

Maintaining an effective catheter care policy requires that the routes of infection be clarified, the risk of infection via each route be assessed and the most appropriate infection control measures implemented. The problem is summarised by Johnson and Oppenheim (1992) who state that there is

"no consensus concerning either a useful definition of catheter-related sepsis, or the optimal method of catheter management and prevention of infection."

A grant was obtained from the Regional Health authority to examine central venous catheter infection.

As a first step, a retrospective survey of the medical records of all patients with a central venous catheter over a six month period was undertaken to determine a baseline rate of infection, identify at-risk groups of patients, to provide details of the clinical setting and identify areas for further investigation. The results confirmed that there was a problem of catheter-related septicaemia, apparently confined to surgical patients receiving TPN. In addition there was a high rate of positive tip culture in patients receiving TPN or a triple-lumen catheter. Reasons for this were unclear due to the very limited amount of information documented on catheter insertion, and
subsequent care and use of the central venous catheter. As summarised above, the literature examining the route of these infections in short-term catheters was conflicting. Intuitively, one might expect that in a dedicated TPN catheter, in situ for 1-2 weeks, used only for TPN and disconnected only once a day using full aseptic technique, the route of infection would be via migration from the skin insertion site. In a triple-lumen catheter in for a shorter period of time but for example, attached to six infusions and disconnected 100 times with a no-touch technique, a route of infection via intraluminal contamination would appear to be the most probable. However as seen from the literature review, the teams of Linares and Maki would argue the opposite, with an intraluminal route of infection in TPN patients (Linares et al. 1985) and an extraluminal route in triple-lumen catheters (Maki et al. 1988, 1991). However, as discussed in the previous section, studies are difficult to compare or generalise to other settings because of the differences and deficits in the methodology, and differences in patient group and care and use of the catheter. The author therefore decided to undertake a prospective study to determine the routes of infection in our clinical practice using accurate recording of practice and good microbiological techniques, to define the relative importance of each route in different patient groups and to investigate the influence of site care and catheter usage on microbial colonisation. This part of the study aimed to resolve the problems of previous studies by examining the route of infection at a microbiological level, using culture methods designed to detect both intra- and extra-luminal organisms, characterised to a high level to determine their similarity with organisms colonising the catheter tip. One of the most important findings of the study was the determination of an intraluminal source of infection in our TPN patients, with, in addition, a high level of intraluminal colonisation of triple-lumen catheters. Subsequent to this study, in-line intravenous filters were introduced in the ICU. These were examined as the third part of the study, to evaluate their implementation, and to provide additional information on the problem of intraluminal contamination, which is lacking in the literature. The influence of in-line filters, changed 96 hourly, on tip colonisation had not previously been examined.

Finally, as a first step to further investigating the increased risk of clinical infection in patients receiving TPN, the ability of our Total Nutrient Admixture to support bacterial growth was compared to other commonly administered solutions in the
Intensive Care Unit, in an *in vitro* experiment using organisms most frequently recovered from the earlier studies. Information on growth in drug containing solutions, of skin flora rather than environmental contaminants was obtained that was not previously available. The development of the study is summarised on page 52.
LITERATURE REVIEW

deficits in microbiological methods of previous studies identified
lack of applicable, research based guidelines for patient care

RETROSPECTIVE SURVEY

high level of positive tip culture observed in triple-lumen and TPN catheters
high rate of septicaemia in patients receiving TPN
lack of documented information on catheter insertion and maintenance thus
reason for tip colonisation/septicaemia unknown

PROSPECTIVE STUDY OF ROUTES OF TIP COLONISATION

AIM: using good microbiological methods and recording a complete set of data, to
determine the route of tip colonisation in triple-lumen and TPN catheters to identify
areas for intervention.
RESULT: high rate of intraluminal colonisation of triple-lumen and TPN catheters,
and intraluminal source of septicaemia in TPN patients determined.
areas for improvement in practice identified

EXPLORATORY STUDIES OF
AREAS FOR FURTHER RESEARCH

triple-lumen catheters

IMPLEMENTATION OF IN-LINE FILTERS EVALUATED

high level of intraluminal contamination confirmed by filter culture
effectiveness in preventing passage of organisms demonstrated.

TPN catheters

GROWTH OF ORGANISMS IN IV SOLUTIONS TESTED

ability of our TPN admixture to support microbial growth confirmed.
other high and low risk solutions identified
CHAPTER TWO: A RETROSPECTIVE SURVEY OF THE INCIDENCE OF INFECTION IN PATIENTS WITH A CENTRAL VENOUS CATHETER

INTRODUCTION

The researcher became interested in the problem of central venous catheter infection whilst working in the Central Treatment Room (CTR) of a District General Hospital. The CTR was an area of the hospital, with its own staff, where procedures which require an aseptic environment were performed. This included wound dressings, insertion of invasive devices such as central lines and chest drains, and minor operations not requiring general anaesthesia. After it had been noticed that several central lines were removed because of suspected septicaemia, Surgical staff expressed concern over the apparently high incidence of clinical septicaemia in patients with central venous catheters, and it was suggested that current practices regarding the insertion and subsequent care of these lines should be investigated and any necessary changes made.

This raised several questions;

i) What was the baseline rate of infection?

ii) Was this a real rise in the rate of infection or merely a chance cluster of cases?

iii) Was the apparent rise distributed amongst all patients with a central line in situ, or, for example, contained within the Surgical area?

iv) Was there a common factor linking infected cases?

v) Was this factor amenable to change?

AIMS

To answer these questions a survey was undertaken to:

I. determine the rate of infection in the total population and subsets of the population with a central line,
II. observe the incidence of infection and identify any trends or short term deviations from the overall pattern.

III. identify "at risk" groups of patients and thus areas at which change may best be directed.

IV. detect the presence of common factors linking patients with infection.

V. highlight areas for further investigation.

As there was no surveillance of central venous catheter infection in the hospital at this time, the aims of the study, prior to the microbiological study, were to examine current clinical practice, highlight problem areas and act as a pilot for further research, rather than to provide new knowledge of the aetiology of central venous catheter infection.

**STUDY DESIGN**

**CHOICE OF METHOD**

The survey method was identified as being the most appropriate to address the aims of the study which were predominately descriptive and exploratory. Although having the limitation of being non-experimental, the survey method allows collection and analysis of a large amount of data on events in their natural setting to provide a description of current practice and identification of problems for further research. Furthermore, although having the limitation of being unable to prove causation directly by manipulation of the independent variable, an explanatory survey may provide evidence that establishes a relationship beyond reasonable doubt (Sartwell 1974).

It was recognised that this was an epidemiological study, examining the determinants, occurrence and distribution of disease within a population. Two methods are commonly employed: the retrospective survey and the prospective survey. As the author was unsure whether or not a problem existed, it was decided to undertake a retrospective or case-control epidemiological survey, which would allow determination of the risk factors associated with central line infection by contrasting patients with central line infection with a control group of patients without, and thus identifying
attributes more common in cases than controls. This was undertaken by review of the patients’ Medical Records by archival analysis.

**RELIABILITY AND VALIDITY OF RETROSPECTIVE MEDICAL RECORD REVIEW IN DETECTING NOSOCOMIAL INFECTION**

Sartwell (1974) argues that retrospective studies are both useful and valid, having been used extensively since the 1920’s, with well designed and conducted studies rarely disagreeing with results of equally well conducted prospective studies. As a pilot to the S.E.N.I.C. project (Study on the Efficacy of Nosocomial Infection Control), Haley *et al.* (1980) assessed the reliability and validity of a standardised method of retrospective record review in detecting nosocomial infection: Validation studies in 4 different types of hospital compared retrospective medical record review by non-physicians using standardised proformas, with intensive prospective surveillance by physician-epidemiologists. Each record was reviewed by two researchers. The inter-rater reliability was found to be 0.94, with an average specificity (defined as the probability that a patient truly uninfected will be declared uninfected) of 0.964, and a sensitivity (probability that a patient truly infected will be declared infected) of 0.74, compared with a sensitivity of 0.76 by prospective survey (specificity assumed to be 1). Similar results were recorded by Wenzel *et al.* (1976). Haley *et al.* (1980) conclude that the results of the pilot studies

"did not support the unqualified preference for prospective data collection methods, at least for research about nosocomial infections."

**LIMITATIONS OF MEDICAL RECORD REVIEW**

Advantages of obtaining data by medical record review include unobtrusiveness, non-reactivity, low cost and ability to collect large amounts of data recorded over a long time span. There is a fixed amount of data available which is stable over time and thus easily replicated (Webb *et al.* (1966). Unfortunately, the data has been written for a specific audience and specific purpose which may differ from that of the research study: Definitions of the variables for which data is available may not correspond with those of the study, descriptions may be vague or ambiguous, contain subjective statements, and
different practitioners may record conflicting findings in describing the same patient (Boyd et al. 1979). In addition the data may be incomplete, contain errors or be biased, by selectivity or by reflecting the unknown bias of the author. However, as the person writing the record has no knowledge of the use to which it will be put, the record is unbiased for the purpose of the research study. Unfortunately, because the records have not been compiled with the purposes of the research in mind, they may not always contain all the information required and even important details may have been omitted if not thought relevant at the time, and thus their adequacy and completeness in providing a representative sample of data from which to draw conclusions must be assessed, along with the authenticity, authorship and purpose of the record.

RELIABILITY AND VALIDITY OF MEDICAL RECORD REVIEW

Aaronson and Burman (1994) observe that issues of reliability and validity occur

- during the original collection of the data
- during documentation
- during extraction of the data, and
- during interpretation and coding of the data.

These will be discussed in turn to identify measures increasing the reliability and validity medical record data: Firstly, the validity of the data is dependent on the writer “knowing the truth and actually recording it” (Aaronson and Burman 1994). Skilled physicians examining a patient may disagree regarding the findings as reviewed by Koran (1975). Even if findings agree and thus are reliable, they may be proved wrong, and therefore invalid, by future events, reflecting the imperfect methods used in clinical assessment and physician judgements. Noting the imperfect nature of medical records, Boyd et al. (1979) assessed the suitability of the medical record for research on the clinical course of disease: Four physicians independently read the same 110 patient records and classified them according to distinct prognostic groups. Levels of agreement between the physicians was high (Kappa scores 0.89) and the demonstrated ability to accurately predict patient survival suggested recorded data reflected true biological differences, indicating it’s validity.
In assessing what data is recorded, Romm and Putman (1981) compared medical records with recorded patient interviews: Concordance was high for chief complaint, diagnosis, diagnostic tests, major problems and their treatment, and abnormal laboratory results. Medical history recording, which was noted to require accurate patient recall, interviewing competence and accurate recording, was found to be less valid and reliable. Aaranson and Burman found that whilst recording of normative and minor events was frequently missing, data such as physical assessment findings, chief complaint, medical diagnoses, major problems, specific tests and abnormal laboratory findings possessed acceptable reliability and validity. The availability and completeness of information and how it was obtained must therefore be evaluated, and if necessary obtained or corroborated from other sources.

In evaluating data extraction, Damiano et al. (1992) examined how reliably the components of the APACHE II score - acute physiology, age and chronic health evaluation score - were extracted from the medical records by hospital personnel compared to trained extractors. They observed that extraction of admission details, age and acute physiological variables was highly reproducible whereas extraction of chronic health data (requiring an assessment of significance) was less so (Kappa 0.66). They conclude that the most reliable components are those that require little or no judgement on the part of the extractor. In contrast, Horowitz and Yu (1984) found that problems in data coding occurred more frequently than problems in data extraction: Three technicians examined 102 records to assess the reliability of epidemiological data obtained from medical records. High rates of interextractor agreement were found demonstrating data from medical records may reliably be used for epidemiological studies, however some errors were noted to occur: Infrequently, data collection errors occurred due to conflicting reports in the record or transcription errors. The most frequent source of disagreement was in coding data that was correctly extracted from the record. The authors suggest that the use of pre-coded forms in which data is extracted and coded in one step increases the likelihood of error. Garvin et al. (1988) distinguish two types of reliability in the use of category coding systems: Unitising reliability, which is consistency in the identification of what is to be recorded, and interpretative reliability, which is the consistency with which labels are applied. These may be
increased by increasing the exhaustiveness of the coding system and providing rigid rules on their application.

In summary, retrospective review of the medical record is a valid and reliable epidemiological method which is sensitive and specific in identifying nosocomial infection. Measures taken to optimise the reliability and validity of the information obtained are detailed in the methodology section.

METHODOLOGY

STUDY POPULATION

Sampling

For the exploratory purposes of this study it was decided to include all patients in whom a central venous catheter was inserted during a defined period to eliminate sampling error by providing an intact group, and to allow the incidence and prevalence of catheter-related infection to be calculated.

Target Population

The target population consisted of the total population of adults aged sixteen and above receiving one or more central venous catheters anywhere within the District General Hospital during a six month period. A central venous catheter was defined as an intravenous catheter inserted into a large central vein. Patients with catheters inserted in the Intensive Care Unit were identifiable from registers within the department as were patients arriving in the Unit with lines inserted in the Operating Theatre or, in emergency situations, in the Accident and Emergency Department or on the wards. Patients on the wards requiring catheters routinely were identified from registers within the Central Treatment Room. A list of patients for whom total parenteral nutrition was ordered was obtained from the Pharmacy Department. To enable direct comparison of variables, Pulmonary artery catheters, Hickman/Broviac catheters, pacing wires and haemodialysis catheters, which examination of the registers identified were inserted very infrequently, were excluded.
Survey Period

Prior to the study, the number of central lines inserted in the hospital and the rate of catheter-related infection was unknown. As a primary aim of the study was to determine whether an increase in the rate of infection had recently occurred, a timespan of six months was chosen allowing three months before and three months after it had been proposed that a problem may exist. Examination of the registers suggested that this would include approximately 100 central venous catheters.

Definition of infection

Central venous catheter tips were routinely sent to the microbiology laboratory on catheter removal. It was determined that significant growth on the catheter tip (by the roll-plate method in use (Maki et al. 1977) - a positive tip culture - would be defined as a local catheter infection. This correlated with a report of scanty, moderate or heavy growth obtained on tip culture as recorded on the microbiology results. No significant growth or no growth was recorded as a negative tip culture. Catheter-related septicaemia was defined as a positive tip culture with the isolation of the same species of bacteria on blood culture, with clinical suspicion of catheter-related sepsis i.e. the patient was recorded as having clinical symptoms of systemic infection with no other probable source. Reliant purely on recorded information, it was recognised that this was a narrow definition which would only include those patients for whom a blood culture was taken and a tip culture sent.

ETHICAL APPROVAL

Permission was granted by the Ethical Committee to access the patients medical records. Information obtained from the medical records was stored anonymously on computer.

SURVEY METHOD

Data was extracted from the medical records of all patients who had received a central venous catheter during the six months period. To maximise content validity, a literature review was conducted to identify variables associated with central line infection as discussed in Chapter One. Briefly, these were:
1. demographic variables such as ward, and department inserting the line,
2. variables influencing the patients' susceptibility to infection such as age and underlying disease,
3. factors relating to insertion of the catheter such as type of catheter and site of insertion.
4. factors linked to microbial contamination of the catheter for example length of time in situ and line usage,
5. laboratory results.

To increase construct validity, data was extracted from all areas of the record, including medical, nursing and paramedical notes (Yin 1994). Sources included medical case notes, nursing cardex and care plans, anaesthetic and recovery sheets, temperature, drug and ICU flow charts and microbiology reports. To maximise internal validity, records were examined to ensure that they were genuine, accurate and consistent, and data was validated by cross-checking data from different sources and within a source. Inter-rater reliability was not assessed, however data was recorded on a proforma which consisted of a checklist of major variables identified by the literature review, to prevent omissions (Appendix 1). In addition, all comments referring to the central venous catheter were recorded. Facts were recorded in preference to opinions and a numerical value assigned where possible. To maximise reliability of the steps of data extraction and data categorisation, the proforma was not precoded.

STATISTICAL ANALYSIS

To maximise the internal validity of a retrospective survey by ensuring that a true difference is being observed whereby changes in the independent variable actually cause a significant change in the dependent variable, it is necessary to detect or avoid indirect association through the action of confounding variables. In epidemiological studies this may be achieved by selecting controls with the same characteristics as the cases in all but the attribute under study, or by statistical adjustment for differences that exist by multiple regression analysis. Both of these methods were employed: Data were entered onto a spreadsheet (Lotus 123, The Lotus corporation, version 3) and analysed using SPSS (SPSS inc. 1993, Chicago) with the assistance of a statistician, to examine
the effect of individual variables on tip culture result, and to assess the significance of their contribution by linear regression.

Statistical analysis was performed using the Mann-Whitney U-test for comparing two groups with ordinal variables, and the Chi-square test for comparing two proportions, or Fisher’s exact test as appropriate; a probability of $p < 0.05$ was considered statistically significant.

RESULTS

DEMOGRAPHIC VARIABLES

1.1 Number of patients

The total population of adults aged 16 years and above receiving one or more central venous catheters for any reason were included in the study over a six month period. Catheters were inserted in 95 patients of whom medical notes could be traced for 92 patients. Two patients were each admitted on two separate occasions, giving a total of 94 patient admissions included in the analysis.

1.2 Number of Catheters

One hundred and forty one catheters were inserted in the 94 patient admissions, with a mode of one per patient and a maximum of three simultaneously or eight sequentially per admission (Figure 2.4). Seventy-two percent of catheters were used in surgical patients and twenty-eight percent in medical patients.

The median number of days in hospital prior to catheter insertion was 3 days, with a range of 1-47 days.
1.3 Speciality

Fifty nine patients were treated by a Surgical consultant, of whom 49 were on General Surgical wards, 9 on Orthopaedic wards and 1 on the Urology ward. Thirty five patients were treated by a Medical consultant of whom 33 were on the general wards and 2 on the Geriatric unit (Figure 2.5). No patients were from Ear, Nose and Throat, Obstetric and Gynaecology or Psychiatry specialities.

![Figure 2.5 Pie Chart of Number of Patients and Number of Catheters Inserted by Hospital Speciality](image)
VARIABLES LINKED TO PATIENT SUSCEPTIBILITY

2.1 Age

The mean age of all patients was 63.0 years (SD 15.5 years). Fifty patients were male (53%) and 44 female (47%). Male patients were aged from 17 - 92 years with a mean of 61.7 years (SD 16.9). Female patients were aged from 30 - 83 years with a mean of 64.1 years (SD 13.7). Two thirds of both male and female patients were aged 60 years and above. The distribution of ages is displayed in Figure 2.6.
2.2 Severity of illness

Fifty six percent of patients underwent one or more surgical operations, and 67% were prescribed antibiotics. Forty one patients (44%) were deemed to have one or more chronic underlying diseases as shown in table two (below). Forty eight percent of catheters were inserted in these patients. Eleven catheters were inserted in patients receiving immunosuppressant therapy (Azathioprine and/or Prednisolone and/or Hydrocortisone).

**TABLE 2.7: CHRONIC UNDERLYING DISORDERS**

*Cancer:*

Stomach/oesophagus  10
Colon                5
Pancreas             2
Bladder              1
Bronchus             1
Ovary                1
Cervix               1

*Hodgkins disease*  1
*Diabetes*          6
*Asthma, emphysema, COAD*  5
*Chronic renal failure*  3
*Rheumatoid arthritis*  3
*Cardiomyopathy, myelofibrosis*  2
*Multiple sclerosis*  2
*Ulcerative colitis*  1
*Cirrhosis*          1

Twenty patients (21%) died with a catheter *in situ* which was therefore not sent for culture and thus the rate of positive tip culture in moribund patients cannot be calculated. A further 11 patients died later within the admission in which a catheter was inserted (Figure 2.7)
The high death rate - 35% of all patients receiving a central line highlights the severity of illness of the population. Sixty eight patients (72%) visited the ICU during their admission.
FACTORS RELATED TO CATHETER INSERTION

3.1 Department inserting catheter

Catheters were introduced in the Intensive Care Unit (ICU) (47), Operating Theatre (42) and for patients on the wards, the Central Treatment Room (CTR) (35). A small number were inserted on the ward (11) and in the Accident and Emergency department (6) in emergency situations (Figure 2.8).

3.2 Type of catheter

In 27% of insertions the type of catheter used was not recorded, and in 65% of cases the size of catheter employed was not stated. Of those documented, 83 catheters were single lumen catheters of which 12 were VygonR "Nutricaths" (size 14, length 35cm silicone) and 2 were "long-lines". Two were WallaceR double lumen catheters and 20 were ArrowR triple lumen catheters (size 7Fr, length 20cm polyurethane). Single lumen catheters were noted to be AbbottR "Abbocaths"(size 14G or 16G, length 14cm fluoroethylene polymer Teflon) or VygonR "Ledercaths" (size 14G or 17G, length 18cm polyethylene).

Where documented, all catheters inserted in the Central Treatment Room were single lumen (32), including 5 Nutricaths. In Theatre, 28 single lumen, 2 double lumen and 5 triple lumen catheters were inserted. In contrast, the most commonly used catheter in ICU was a triple lumen (14), with 7 Nutricaths, 2 long-lines and 10 other single lumen catheters (Figure 2.9).
3.3 Site of insertion

Catheters were inserted into the superior vena cava via the right or left subclavian vein, right or left internal or external jugular vein or the brachiocephalic vein via the antecubital fossa. The frequency of use of each site is shown in Figure 2.10. Seventy four percent of catheters inserted in CTR were subclavian with 26% jugular. In contrast, 97% of catheters inserted in Theatre were jugular with 3% subclavian. A more even distribution was found in ICU with 56% subclavian and 44% jugular. This reflects
differences in the member of staff inserting the catheters, with anaesthetists, working predominately in theatre and ICU favouring jugular lines, and medical and surgical house officers/registrar in CTR and ICU favouring subclavian lines. All tunnelled lines were subclavian.

Jugular catheters tended to be "Abbocaths", with subclavian "Ledercaths" due to their greater flexibility and longer length. All tunnelled lines were "Nutricaths".

![Figure 2.10: ANATOMICAL SITE OF INSERTION PROPORTION BY DEPARTMENT INSERTING](image)

**Figure 2.10: ANATOMICAL SITE OF INSERTION PROPORTION BY DEPARTMENT INSERTING**

**TOTAL**

- Jugular: 48.2%
- Subclavian: 11.4%
- Other: 12.6%
- Unknown: 14%

**CTR**

- Jugular: 48.2%
- Subclavian: 37.2%
- Other: 10.5%
- Unknown: 4.1%

**THEATRE**

- Jugular: 90.5%
- Subclavian: 4.3%
- Other: 0.3%
- Unknown: 0.9%

**ICU**

- Jugular: 88.5%
- Subclavian: 3.5%
- Other: 5.3%
- Unknown: 2.4%
3.4 Insertion procedure

Information on aseptic technique, antimicrobial preparations and dressings used was generally not available.

There was no written protocol for insertion of central venous catheters.

4 FACTORS INFLUENCING MICROBIAL CONTAMINATION OF THE CATHETER

4.1 Reason for insertion

Three main uses of the catheter were identified as shown in Figure 2.11 and 12.

- "Access"- ACC- (15.6% of catheters) was defined as a catheter inserted predominately to give access to a central vein, for example where no peripheral access was available, at cardiac arrest or to give large amounts of, or hypertonic drugs.
"Fluid balance" -FB-(27.7%) was where a catheter was inserted to measure central venous pressure as in dehydration or hypotension. A combination of the two, access and fluid balance, (39%) was commonly encountered for example at major operation, in severe illness and in the majority of admissions to ICU.

Catheters were also inserted to administer Total Parenteral Nutrition (TPN) either alone (10.6%) or in combination with other uses (7.1%) Twenty six catheters (18%) were used for TPN. The coding of each presenting condition is given in Appendix 3.

Figure 2.12: BAR CHART OF USE OF LINE AND TIME IN SITU

4.2 Time in situ

The average length of time in situ of all catheters was 4.9 days (SD 4.14) with a range of 1-23 days. The length of time varied with the speciality of the patient, site and type of catheter, and use of catheter as displayed in Figure 2.12. The average duration of catheterisation was 2.5 days for medical patients (range 1-13 median 2) and 5.6 days for surgical patients (range 1-23 days median 4).
Jugular catheters were in situ an average of 4.2 days (range 1-13 days) with subclavian 5.8 days, however if Nutricaths were examined separately, other subclavian catheters were in situ 4.6 days (range 1-23 days), comparable to the jugular catheters, with Nutricaths in situ 11.2 days (range 4-21 days, median).

With regard to the type of catheter, single lumen catheters excluding Nutricaths were in situ an average of 4.0 days (range 1-17 days) and triple lumen 5.9 days (range 1-23 days, median). Catheters used to measure central venous pressure and/or provide central access alone were used an average of 3.5 days (range 1-13 days). Catheters used only, or additionally, to administer TPN were used an average of 11.0 days (range 4-23 days)(figure 2.13). This difference was highly significant (Mann-Whitney U test p<0.0005).
4.3 Reason for removal

Reasons for removal of the catheter are illustrated in Figure 2.14. In the majority of cases catheters were no longer required (89). Six were blocked or leaking. Six catheters were routinely replaced after approximately one week in the ICU. Ten catheters were removed because of septicaemia suspected to be due to the central line. Hospital policy was that catheter tips were routinely sent to the microbiology laboratory for culture on catheter removal.

4.4 Microbiology results

Microbiology results are illustrated in Figure 2.15. In practice, results were not available for 28% of catheters: Twenty patients died and five were transferred to other hospitals with catheters in situ. Three catheters "fell out", three were wrongly sited and discarded, six single lumen catheters were rewired with a triple lumen and discarded, and a further four tips were not sent for culture.
5. INCIDENCE OF INFECTION

A total of one hundred central catheter tips were cultured. Forty three microorganisms were cultured from the tips of 38 central catheters, 38% of those cultured. A single organism was cultured from 34 catheter tips with two organisms from a further 4 tips as follows:

- Coagulase-negative staphylococcus*(CNS) 31
- Yeasts* 2
- *Escherichia coli 2
- CNS + *Enterococcus faecalis 2
- CNS + *Staphylococcus aureus 1
- CNS + diptheroids* 1
- CNS + yeasts 1
- *Serratia marcescens 1
- *Staphylococcus aureus 1
- *Pseudomonas species* 1

* not speciated by the laboratory.

The tips of seven from ten catheters removed on suspicion of septicaemia grew organisms of the same species as a concurrent blood culture and were thus the probable cause of the sepsis (Table 2.8 displayed in bold type). Assuming that any catheter suspected of causing septicaemia would have been removed and sent for culture, this is a rate of catheter related septicaemia of 5% in the study population. Positive tip cultures and cases of septicaemia were distributed throughout the six month period as shown in Figure 2.16.
Figure 2.16: CATHETERS REMOVED FOR SUSPECTED SEPTICAEMIA

Table 2.8: Microbiology Results: Catheters Removed for Suspected Septicaemia (N=10)

<table>
<thead>
<tr>
<th>Tip Culture</th>
<th>Blood Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serratia marcescens</td>
<td>Serratia marcescens</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>S. epidermidis</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>E. faecalis</td>
</tr>
<tr>
<td>Candida sp.</td>
<td>Candida sp.</td>
</tr>
<tr>
<td></td>
<td>Enterobacter sp</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>S. epidermidis</td>
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<tr>
<td>S. epidermidis</td>
<td>S. epidermidis</td>
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<tr>
<td></td>
<td>Viridans streptococci</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>S. epidermidis</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>No Growth</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>No growth</td>
</tr>
</tbody>
</table>
6. FACTORS INFLUENCING THE RATE OF POSITIVE TIP CULTURE

The proportion of central venous catheter tips exhibiting significant microbial growth on culture (positive tips) and no growth (negative tips) was calculated for the variables listed below as summarised in Fig 2.17. The sample size of 100 tips gave a 50% probability of detecting a 20% difference in proportion, and a 90% probability of detecting a 30% difference in proportion at a 5% level of significance. Chi-square tests have been used unless otherwise stated.

FIG 2.17: SUMMARY OF RESULTS - PERCENTAGE OF POSITIVE TIP CULTURES FOR EACH STUDY VARIABLE

MULTIVARIATE ANALYSIS

**MULTIVARIATE ANALYSIS**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Positive Tips</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPN Lines</td>
<td>Yes</td>
<td>25%</td>
</tr>
<tr>
<td>IPN</td>
<td>23%</td>
<td>0.0001</td>
</tr>
<tr>
<td>IYS</td>
<td>38%</td>
<td>0.0005</td>
</tr>
<tr>
<td>FPN Lines</td>
<td>21%</td>
<td></td>
</tr>
<tr>
<td>OTHER</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>TPN Lines</td>
<td>No</td>
<td>62%</td>
</tr>
<tr>
<td>NUTRICATH Single</td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td>FLUID BALANCE</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>ACCESS</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>ACCESS + TPN</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>TPN</td>
<td>21%</td>
<td></td>
</tr>
<tr>
<td>SUBCLAVIAN</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>REGULAR BLOOD</td>
<td>38%</td>
<td></td>
</tr>
<tr>
<td>TPN</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>UNDERLYING DISEASE</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>IMMUNOSUPPRESSANTS</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>ANTIBIOTICS</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>SEVERITY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE-EXISTING INFECTION</td>
<td>36%</td>
<td></td>
</tr>
</tbody>
</table>

UNIVARIATE ANALYSIS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Positive Tips</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN AGE</td>
<td>62.5</td>
<td>0.03</td>
</tr>
<tr>
<td>MEAN SEX</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>SPECIALITY</td>
<td>63%</td>
<td></td>
</tr>
<tr>
<td>MEAN INSERTION DAY</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>UNDERLYING DISEASE</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>IMMUNOSUPPRESSANTS</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>ANTI B</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>ANTIBIOTICS</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>SEVERITY</td>
<td>43%</td>
<td></td>
</tr>
<tr>
<td>PRE-EXISTING INFECTION</td>
<td>47%</td>
<td></td>
</tr>
</tbody>
</table>

6.1 Demographic variables

Hospital speciality

38.6% (32) catheters inserted in Surgical patients had a positive tip culture compared with 35.3% (6) Medical patients (not significant). All seven cases of catheter related septicaemia occurred in Surgical patients (p=0.10, Fisher's exact test, 2 tail).
6.2 Variables Linked to Patient Susceptibility (figure 2.18 page 77)

Age

The mean age of patients with a positive tip culture was 64.6 years (SD 14.0) compared to 63.1 years (SD 15.0) for patients with no growth on tip culture. This was not a significant difference.

Sex

43.8% (25) male patients had a positive tip versus 30.2% (13) female patients (not significant.)

Catheter number

The average number of the total of catheters/patient of positive tips was 1.7 (SD 1.4) and of negative tips 1.8 (SD 1.1) (Not significant).

Days in hospital prior to catheter insertion

Catheters with no growth on tip culture were on average inserted on day 6.5 (SD 7.2) compared to day 8.3 (SD 7.6) for catheters with a positive tip culture (not significant).

Antibiotic prescription

36% (27) tips in patients receiving antibiotics exhibited microbial growth compared with 44% (11) of patients not receiving antibiotics (not significant).

Underlying disease

33.3% (16) tips in patients with an underlying chronic disease or malignancy had a positive tip culture as opposed to 42.3% (22) tips in patients previously fit and well (not significant). There was no relationship between the use of immunosuppressant therapy and tip culture. Four episodes of catheter related septicaemia occurred in patients with malignancy and three in previously healthy patients.

Severity of illness

42% (30) tips were positive in patients visiting ICU. There was no significant difference between elective and emergency admissions. 47.0% (9) tips cultured from patients dying within the admission in which the line was inserted were positive
compared to 35.8% (29) in patients who were discharged (not significant). Tips were not cultured from the twenty patients who died with a central catheter in situ.

**Pre-existing infection**

Twenty one patients had a documented infection whilst 35 central catheters were in situ (Appendix 4). Seventeen (60.7%) of the 28 tips cultured from these catheters exhibited microbial growth as opposed to 29.2% (21) in patients with no documented infection at another body site. This was highly significant (Chi square p<0.005). In only four of these cases was the organism involved a possible source of the positive tip culture. Four episodes of catheter related septicaemia (57.1%) were associated with these 35 (24.8%) of catheters but in only one instance was the organism on blood culture previously isolated from another infection.
6.3 Variables Associated with the Central Venous Catheter (figure 2.19, page 79)

Department inserting catheter

32.0% of catheters inserted in CTR (8) and/or Theatre (12) were positive as opposed to 48.5% (16) catheters inserted in ICU. (Not significant p=0.12)

Insertion procedure.

Outwith the CTR, documentation surrounding the insertion of the central catheter was poor or absent. Information on the Grade of staff inserting the catheter, number of attempts required, use of a guidewire, level of asepsis and the urgency of insertion was not generally available. All, and only, Nutricaths were tunnelled.

Catheter type

Triple lumen catheter tips were significantly more likely to exhibit microbial growth than single lumen catheters (Chi square p=0.00038), as were Nutricaths (Fisher's exact test p=0.0137). 70.6% (12) triple lumen catheters and 63.6% (7) Nutricaths were positive as opposed to 23.6% (13) other single lumen catheters. It was not possible to examine catheter size or material independently of catheter type.

Site of insertion

Of subclavian catheters, 41.5% (17) exhibited a positive tip culture versus 37.5% (18) jugular. This was not significant, however subclavian catheters were in situ significantly longer (fig 2.19).
6.4 Variables Influencing Microbial Contamination of the Catheters.

\textit{Duration of catheterisation}

Tips exhibiting no growth were in situ a median of 3 days (range 1-23) with positive tips in situ a median of 6 days (range 1-17). The difference was highly significant (Mann-Whitney U test \( p=0.0071 \)). The rate of positive tip culture increased with duration of catheterisation as shown in Figure 2.19. In patients with catheter-related septicaemia, the median duration of catheterisation was 11 days (range 4-17).

\textit{Catheter Use}

30.4\% (24) catheters used for central access and/or to monitor fluid balance had a positive tip culture as opposed to 66\% (14) catheters used solely or additionally to
administer TPN. This was highly significant (Chi square p=0.00233). Furthermore, all seven cases of clinical septicaemia secondary to the central catheter occurred in catheters used to administer TPN, affecting 33% of patients receiving TPN.

_Care of the insertion site_

Outwith the CTR, the frequency of dressing change and antiseptic and dressing used was rarely documented therefore the effect of variations in site care could not be assessed.

**DISCUSSION**

The aims of the survey were to:

I. determine the rate of infection in the total population and subsets of the population with a central catheter,

II. observe the incidence of infection and identify any trends or short term deviations from the overall pattern.

III. identify "at risk" groups of patients and thus areas at which change may best be directed.

IV. detect the presence of common factors linking patients with infection.

V. highlight areas for further investigation.

These will be discussed in turn:

I. **RATE OF INFECTION**

Published rates of positive catheter tips range from 6.6-23%, with an incidence of bacteraemia of 6.4-21% as reviewed by Richet et al. (1990), and 11-33% (Horowitz et al. 1990). Sepsis rates for TPN administration range from 1.7-14.8% (Gill et al. 1989).

The overall rate of positive tip culture of 38% in this study is thus higher than that expected. This is of concern as a positive tip culture is a precursor to catheter-related bloodstream infection. A linear relationship has been demonstrated between the cumulative number of colonised catheters and the cumulative number of bacteraemic episodes, with bacteraemia occurring in approximately 10% of all colonised catheters.
In addition, Pinalla et al. (1983) assert that the finding of significant numbers of \( S. \) epidermidis colonies on semiquantitative culture should be regarded as a marker organism indicating the need for better catheter care.

The overall rate of septicaemia in this study of 5% was within acceptable limits, however, it should be noted that the definition of catheter-related septicaemia used in this survey i.e. clinical symptoms in the presence of a central venous catheter with the same organism isolated from cultures of peripheral blood and the catheter tip, in the absence of another likely source of infection will miss many cases of CRS and it has been observed that surveys using this definition will underestimate the problem (Henderson 1990). A further two, non-microbiologically documented, cases of septicaemia were recorded in the medical notes as clinically thought to be secondary to the central catheter. The rate of septicaemia amongst patients receiving TPN was 33% (25% of catheters), which is double the published results observed in other centres.

II  THE TEMPORAL INCIDENCE OF INFECTION

The removal of central venous catheters on suspicion of catheter-related septicaemia which prompted this study was found not to have been an increase in the incidence of infection, with cases distributed throughout the six month period, from 3 months before to two months after the cluster of infections was observed. Seven out of ten suspected infections were confirmed. This perhaps indicates a low index of suspicion. Removal of the catheter when catheter-related infection is suspected leads to unnecessary removal in upto 75% of cases (Ryan et al. 1974). Pettigrew et al (1985) noted that 30% of TPN catheters were removed for suspected sepsis of which 90% were not proven.

III  THE IDENTIFICATION OF HIGH RISK GROUPS OF PATIENTS

Four variables were significantly associated with the presence of a positive tip culture: Presence of a focus of infection, duration of catheterisation, catheter type, and administration of TPN. These variables, which will be discussed in turn, identified three groups of patients at high risk of infection- those with a pre-existing infection, those with triple-lumen catheters, and all patients receiving TPN.
Pre-Existing Infection

The finding of this study that the presence of a positive tip culture is correlated with presence of infection at another body site is in accordance with other authors although the mechanism is unclear: Ena et al. (1992) studying 353 intravascular catheters found that the presence of infection elsewhere was the strongest independent risk factor for catheter-related infection (odds ratio 8.7). In a study of triple-lumen catheters in the critically ill, Norwood and Jenkins (1990) found that there were no infections in non-septic patients, but 26.3% catheter-related infection and 9.6% catheter-related sepsis in septic patients, however the duration of catheterisation and number of days hospitalisation were greater in the septic patients.

In terms of the means of colonisation of the catheter, Chuang and Chuang (1991) found that colonisation of CVCs used for TPN was significantly higher in patients with a distant site of infection but it was not determined whether the organisms involved were the same. In addition, patients with a distant infection also had a significantly higher mortality and thus were more severely ill. By electron microscopy, catheters removed from patients with non-catheter-related bacteraemia showed no seeding of the catheter (Raad et al. 1991, and the contribution of haematogenous seeding to catheter colonisation and seeding is considered to be minimal, and rarely demonstrated (Raad 1994). Micro-organisms from distant sites of infection such as surgical wounds have been recognised to colonise the catheter tip in the absence of bacteraemia, suggesting transfer to the insertion site or administration system by staff (Maki et al. 1973, McKee et al. 1985).

In this study however, in only four of 38 tips exhibiting bacterial growth was the organism of the same species as that cultured from, or (from the author’s experience) microbiologically probable, to be from infection at another body site, suggesting that the presence of pre-existing disease may act as a marker for an increased susceptibility to infection rather than a source of tip colonisation.

Duration of Catheterisation

In this study, tips exhibiting no growth were in situ a median of 3 days compared with a median of 6 days for catheters with a positive tip culture, with the rate of positive tip culture increasing with increasing duration of catheterisation. In patients with
catheter-related septicaemia, the median duration of catheterisation was 11 days. Duration of catheterisation has been identified as a major factor in the pathogenesis of catheter-related infection as discussed in the literature review (page 30).

**Total Parenteral Nutrition**

All seven cases of microbiologically documented catheter-related septicaemia occurred in catheters used for TPN, affecting 33% of TPN patients. The administration of TPN is a recognised risk factor for the development of catheter-related infection as previously discussed (page 37).

**ASSOCIATION BETWEEN VARIABLES**

The relationship between the above variables significantly associated with a positive tip culture, i.e. catheter type, catheter use and duration of catheterisation, was further examined to determine which factors were most highly associated with a positive tip culture:

**Catheter type**

Six triple lumen and 7 single lumen catheters were used to administer TPN. When these were removed from the analysis 21.6% (11) single lumen catheter tips versus 63.6% (7) triple lumen tips were positive for growth. This remained a highly significant difference (Fisher's exact test (2 tail) p=0.0097). All "Nutricaths" were used to administer TPN thus could not be examined further.

**Administration of total parenteral nutrition**

Administration of TPN appeared to be a risk factor independent of catheter type although the number of catheters involved was too small to allow statistical comparison. 50% (2) single lumen catheters used to deliver TPN exhibited growth on tip culture versus 21.6% (11) tips of catheters inserted for other purposes. 83.3% (5) triple lumen catheters used for TPN were positive compared with 63.6% (7) triple lumen catheters not used for TPN.

A positive tip culture was associated with 61.5% (8) instances where TPN was administered alone and 63.6% (7) of administrations via a Nutricath, compared to 75%
(6) of cases where a catheter was used for multiple purposes and 83% (5) of cases where a triple lumen catheter was used.

Duration of catheterisation

The catheters used for TPN were in situ significantly longer than the non-TPN catheters (Mann-Whitney U test p<0.0005). However, there was no significant difference in time in situ for positive tips (mean 10.8 days, range 4-17) and negative tips (mean 11.4 days, range 6-23).

Likewise, in catheters not used for TPN, positive single lumen tips were in situ an average of 4.6 days (range 2-10) versus 3.9 days (range 1-9) for negative tips, with positive triple lumen tips in situ 4.0 days (range 1-12) versus 2.5 days (range 2-23) for negative tips.

There was however a highly significant difference in the proportion of positive tips when the duration of catheterisation equalled or exceeded six days (p=0.0091).

To assign the relative importance of each independent variable in predicting a positive tip culture, a stepwise logistic regression was performed using SPSS, with tip result as the dependent variable, and TPN administration, triple lumen catheter (non-TPN use) and duration of catheterisation greater than six days as the independent variables. Regression coefficients for each of the variables were calculated and compared with each other and the dependent variable in a correlation matrix. Variables were entered by a stepwise method of entry. TPN administration was found to be most strongly correlated with a positive tip result was entered into the equation at step one. The subsequent model correctly predicted 69% of tip results but only 37% of positive tips. The addition of "triple lumen catheter" improved the model, with 72% of tips correctly predicted and 55% of positive tips identified. The subsequent addition of "duration" did not produce a significant improvement in fit.

<table>
<thead>
<tr>
<th>Step One - TPN administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIP CULTURE (percent)</td>
</tr>
<tr>
<td>observed negative</td>
</tr>
<tr>
<td>observed positive</td>
</tr>
</tbody>
</table>
Step Two- Triple-lumen catheter (non-TPN)

<table>
<thead>
<tr>
<th>TIP CULTURE (percent)</th>
<th>predicted negative</th>
<th>predicted positive</th>
<th>CORRECTLY PREDICTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>observed negative</td>
<td>51</td>
<td>11</td>
<td>82.26</td>
</tr>
<tr>
<td>observed positive</td>
<td>17</td>
<td>21</td>
<td>55.26</td>
</tr>
</tbody>
</table>

**IV) COMMON FACTORS LINKING PATIENTS WITH INFECTION**

High risk groups of patients were identified as discussed above. Within these groups, it was not possible to determine practices which may have been linked to catheter-related infection.

**V) AREAS FOR FURTHER INVESTIGATION**

The results highlighted two main areas of concern: Triple lumen catheters and Nutricaths or any other catheter used to administer TPN were twice as likely to be associated with the presence of microbial growth on catheter tip culture. In addition there were seven cases of septicaemia amongst 6 of 18 (33%) patients receiving TPN. The overall rate of septicaemia was 5%. A further two cases of septicaemia were recorded in the medical notes as clinically thought to be secondary to the central catheter.

The reason for these findings was unclear. With regard to catheters used for TPN administration, the high rate of infection may be linked to the length of time the catheter is in situ (11.2 days for Nutricaths versus 4.2 for jugular, and 4.6 for other subclavian catheters, 10.6 days for TPN administration versus 3.3 days for other uses). The problem may thus be the duration of catheterisation itself, the administration of TPN admixtures, or one, or both, may be an indicator of another factor such as degree of catheter usage or severity of illness.

Additional factors may be operating with triple lumen catheters. Triple lumen catheters were as likely to exhibit a positive tip culture as Nutricaths but were in situ only 5.9 days on average. The average duration of catheterisation of positive tips was 6.6 days. Twenty two triple catheters were inserted. Of the twenty sent for culture, 12 had a
positive tip culture (60%) only 5 of which were used for TPN administration. It was not possible to evaluate the type and extent of use of the triple-lumen catheters.

Limitations of the study

Whilst the study clearly identified problem groups of patients, it was not possible to identify any common factor linking cases of septicaemia or positive tip culture due to the small number involved and limited information available. As this was a retrospective survey, items not generally recorded in the medical or nursing notes couldn't be examined. This particularly related to practice in the insertion and care of the central catheter and administration system.

These included:

- insertion technique and standard of asepsis,
- type and size of catheter used,
- antimicrobial preparations and dressing used,
- subsequent care of the insertion site,
- degree and type of use of the catheter,
- disconnections of the administration system,
- severity of illness of the patient.

A further problem was that catheters were not cultured from patients who had died, thus biasing the study by eliminating patients at high risk of infection.

In the case of dressing change for example, it was not possible to determine whether there was a lack of documentation, or whether care had not been performed. Donabedian (1969) observes that whilst skilful recording may give a false impression of quality care, omissions from the record may convey a false impression of poor care.

Factors such as the number of disconnections of the administration system and the usage of individual lumens of a triple-lumen catheter could not be assessed retrospectively. As previously discussed, the relative importance of these factors in colonisation of the central catheter remains controversial and may differ between different patient groups.

Further investigation

It was decided to address these areas by undertaking a prospective, microbiological study to determine the route of infection in patients with a central
venous catheter, and to compare the route of infection in the two groups most highly at-risk - patients with triple-lumen catheters, and those receiving TPN- to provide further information on the controversy over the predominate route of infection in these patients.

**SUMMARY**

The results demonstrated a high incidence of positive tip culture and septicaemia amongst patients with a central venous catheter. There was no evidence of a recent increase in the rate of infection, with cases occurring throughout the six month period. Patients with triple-lumen catheters and those receiving total parenteral nutrition were particularly at risk, but the reasons for this were unclear due to the limited amount of information on the care and use of the central venous catheter available retrospectively. Four factors were significantly associated with a positive tip culture, consistent with previous author’s findings:

- a pre-existing focus of infection
- a duration of catheterisation exceeding 6 days
- use of a triple-lumen catheter, and
- administration of total parenteral nutrition.
CHAPTER THREE: ROUTES OF INFECTION IN PATIENTS WITH A CENTRAL VENOUS CATHETER

INTRODUCTION

A retrospective survey by the author revealed a high incidence of catheter colonisation and septicaemia amongst patients with a central venous catheter, and in particular surgical patients receiving total parenteral nutrition and patients with a triple-lumen catheter. It was not possible to determine the source of these problems from information recorded in the patients’ medical records. Most importantly, information directly relating to microbial contamination of the catheter, notably clinical practice concerning care of the catheter insertion site and use of the central line was not available, and culture of the insertion site and catheter lumens is not routinely performed. Review of the literature, as presented above, identified several unanswered questions relating to the route of infection of the central venous catheter in short term TPN and triple-lumen catheters, due to differing definitions of infection, the use of only extraluminal culture methods, non-speciation of organisms, lack of standardisation or description of dressing change procedure and lack of assessment of the type and frequency of use of the central venous catheter. Furthermore, it may not be applicable to generalise findings on the predominant route of infection between different types of catheter and between patient groups with differing severity of illness and underlying risk factors, particularly as the majority of studies look for differences in the rates of infection rather than examining the underlying aetiology. In addition, the relationship (if any) between skin colonisation and hub colonisation has not been determined. Furthermore, the reason for the increase in infection with increasing duration of catheterisation seen in the retrospective survey and reported in the literature has not been determined at a microbiological level.

As summarised in table 1.5, page 45, previous studies examining the route of infection of central venous catheters have been small (all less than 50 positive central line tips) and have focused on single-lumen catheters, particularly in TPN, however of these only 3 studies speciated isolates of coagulase-negative staphylococci. Of the four studies examining triple-lumen catheters, only two cultured both the hub and the insertion site, and none used a quantitative culture method and thus may have missed
infections arising from the catheter lumen. None speciated CNS. Where stated, care of the insertion site varied widely between studies, (table 1.6 page 46) and with the exception of dedicated TPN catheters, the type and degree of use of the catheter was not measured.

It was therefore decided to repeat the survey, prospectively with, in addition, observation of clinical practice and microbiological sampling, to address the limitations of the previous survey and deficiencies in previous reported studies.

AIMS

The aims of the study were as follows:

I) To examine the use of a typing system in determining the epidemiology of catheter-related infection

II) To investigate the effect of site care and catheter usage on growth at the insertion site or catheter lumen, and the relationship to duration of catheterisation

III) To determine the routes of tip colonisation in our clinical practice.

IV) To define the relative importance of each route in patients with a triple-lumen catheter or receiving total parenteral nutrition.

It was also proposed that the study would supply a description of current practice and a preliminary identification of potential high risk types of equipment, practice or patient.

STUDY DESIGN

A framework was developed to identify the most important variables and aid study design. The main problems and major concepts were identified from the literature, as follows:

- Infection of the catheter tip may theoretically occur via more than one route - intraluminally or extraluminally.
- There is a need to establish the occurrence of each in routine clinical practice and the relative frequency with which each occurs.
Nursing practice related to care of the site or administration system, and the type of equipment used may influence the above.

The first stage was to attach definitions to the concepts used (such as "infection") that would accurately reflect the underlying meaning of the concepts, be understandable to other researchers, and allow empirical testing. The concept of nursing practice was defined as management of the catheter insertion site and management of the fluid administration system. Measurable variables were then identified, namely the frequency of dressing change, the type of antiseptic used, the type of dressing employed, the type of central venous catheter, the degree and type of usage and number of disconnections of the system as shown below (table 3.9).

The presence of microbial growth on the skin at the point of insertion of the catheter was taken to indicate potential tip infection via the extraluminal route, with microbial growth in intravenous fluids, administration sets, three-way taps and filters indicating tip infection via the intraluminal route (Maki et al. 1988). Tip "infection" was defined as the presence of significant microbial growth (as defined in method section) on the distal catheter tip at the time of catheter removal (Collignon et al. 1988, Kristinsson et al. 1989). These definitions also allowed the identification of the major variables in the study which were then employed in the formation of hypotheses.

<table>
<thead>
<tr>
<th>TABLE 3.9: DEFINITION OF CONCEPTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CONCEPT</strong></td>
</tr>
<tr>
<td><strong>OPERATIONAL DEFINITIONS</strong></td>
</tr>
<tr>
<td>management of catheter insertion site</td>
</tr>
<tr>
<td><strong>EMPIRICAL INDICATORS</strong></td>
</tr>
<tr>
<td>frequency of dressing change</td>
</tr>
<tr>
<td>type of antiseptic used</td>
</tr>
<tr>
<td>type of dressing employed</td>
</tr>
</tbody>
</table>
The dependent variable in this study was the presence of microbial growth at the catheter tip. The independent variables presumed to be related to this outcome were firstly, the presence of microbial growth at the insertion site, and secondly, the presence of microbial growth within the administration system. It was hypothesised that a positive relationship existed between the variables such that as one increased the other would increase as follows:

**HYPOTHESES**

i) colonization of the catheter tip occurs via the intraluminal and extraluminal routes.

ii) microbial growth at the catheter insertion site and within the administration system precedes microbial growth at the catheter tip.

iii) the presence of growth at the insertion site and within the administration system is related to clinical practice in care of the site and manipulation of the administration system.

Whilst determination of the existence and direction of the relationship was the primary aim of the study, it was recognised that many other variables could effect the magnitude of the result. To maximise content and construct validity, variables were chosen from a literature review of factors demonstrated to influence the rate of infection
in patients with a central venous catheter, and from the author’s experience as a Staff nurse now working in the Intensive Care Unit (table 3.10, page 93).

These variables were recorded to allow description of the specific sample, conditions and characteristics of the research setting in which the hypotheses were tested and the population to which any generalisations may be made. It also ensured that the conditions in which the hypotheses were tested remained constant throughout the study.

Covariables affecting both the dependent and independent variables included factors such as age and underlying disease which affect the patients susceptibility to infection. Antecedent covariables variables predominately related to the nursing care of the insertion site and administration system. Confounding variables such as presence of growth at a distant body site and antibiotic administration were acknowledged as potentially increasing or decreasing the values of the dependent variable.

**PLAN OF INVESTIGATION**

**CHOICE OF METHOD**

As in the retrospective survey, it was recognised that this was an epidemiological study, examining the determinants, occurrence and distribution of disease within a population. Having identified problem areas, a prospective survey was appropriate, whereby a cohort of patients not initially having central venous catheter colonisation was followed for sufficient time for cases to develop amongst them (Sartwell 1974). Non-obtrusive measures were chosen to do this, again to examine the mechanisms operating within the Hospital’s current clinical practice. The survey method allowed collection and analysis of a large amount of data of events in their natural setting.

In addition to data available from Medical records as previously recorded, prospective study allowed collection of data by recording of events and interview of Staff.
<table>
<thead>
<tr>
<th>COVARIABLES</th>
<th>ANTECEDENT VARIABLES</th>
<th>INDEPENDENT VARIABLES</th>
<th>CONFOUNDING VARIABLES</th>
<th>DEPENDENT VARIABLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Management of</td>
<td>Presence of</td>
<td>Presence of</td>
<td>Presence of</td>
</tr>
<tr>
<td>Sex</td>
<td>management of</td>
<td>microbial growth</td>
<td>microbial growth</td>
<td>microbial growth</td>
</tr>
<tr>
<td>Underlying disease</td>
<td>insertion site</td>
<td>at insertion site</td>
<td>at distant body site</td>
<td>at catheter tip</td>
</tr>
<tr>
<td>Severity of illness</td>
<td>Management of</td>
<td>Presence of</td>
<td>Antibiotic</td>
<td></td>
</tr>
<tr>
<td>Surgical intervention</td>
<td>administration</td>
<td>growth in system</td>
<td>administration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>system</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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LIMITATIONS, RELIABILITY AND VALIDITY OF THE PROSPECTIVE SURVEY

METHOD

Being non-experimental, the survey had the limitation of being unable to prove causation directly by manipulation of the independent variable, although as previously noted, an explanatory survey may provide evidence that establishes a relationship beyond reasonable doubt (Sartwell 1974). As in the retrospective survey, to maximise internal validity it was necessary to ensure that a true difference is being observed whereby changes in the independent variable actually cause a significant change in the dependent variable, and to detect or avoid indirect association through the action of confounding variables. These variables were identified as outlined above, and were recorded, to allow comparison between cases and controls, and to allow for statistical adjustment for differences that did exist.

Limitations of retrospective medical record review were discussed in Chapter Two. In a prospective study, there is a “running record” which gives the opportunity to overcome many of these limitations by obtaining missing data and clarifying ambiguity (Webb et al. 1966). The major problem encountered in Study Two was due to data not being recorded, due to either to poor documentation or due to care not being performed. With regard to missing data, Donabedian (1966) observes that when assessing the quality of care from a medical record one is assessing both the record and the care provided, and the two can only be separated when an alternative source of information, such as direct observation is available. Other data required for the research study was not generally recorded as not relevant to clinical practice. It was decided to address these limitations, and to increase the reliability and validity of all the data collected by using a triangulation approach.

Data triangulation - the collection of data from multiple sources- allowed validation of the data obtained (Kimchi et al. 1991). Methodological triangulation - the use of two or more research methods, allowed collection by questioning or by unobtrusive observation of physical events, and microbiological sampling, of data not available from written records (Mitchell 1986). It also had the advantage of recording events in real time and in context (Yin 1994). Use of a collection of methods which may have different methodological weaknesses increases the reliability and validity of the results obtained (Webb et al. 1966).
ETHICAL APPROVAL

All data collection techniques employed were unobtrusive, being obtained by recording of practice and from physical evidence requiring no alteration to the patients usual treatment. Guidelines from the Royal College of Physicians (1990) state that “use of personal medical records without approaching or involving the patients concerned is ethically acceptable provided confidentiality and anonymity are preserved”. This also applies to the use of biological specimens. Nevertheless approval was obtained from the ethical committee and the purpose and nature of the study was explained to those patients well enough to be aware of my visits, and their verbal consent was obtained. No patients declined to be included.

STUDY POPULATION

Medical patients were found in the retrospective survey to have a low rate of catheter-related infection due to the short time the catheters were in situ (median 2 days), with no TPN administration. All seven cases of catheter-related septicaemia were in Surgical patients. It was therefore decided to restrict the prospective survey to Surgical patients. All Surgical patients receiving one or more central venous catheters of any type and for any purpose were recruited over a six month period. The inclusion of all surgical patients reduced problems of sampling validity by providing an intact group. A previous survey (Lee 1990) and quarterly hospital statistics suggested that a sample of approximately 50 patients would be obtained in a six month period, of which approximately 40% may be expected to yield microbial growth on tip culture. All patients lost to the study were assessed to detect any methodological problems and to detect problems of attrition or selection bias.

Patients were identified from treatment registers in the Intensive Care Unit (ICU) and Central Treatment Rooms (CTR), and by daily visit to the ICU and surgical wards. A label was attached to the treatment cardex to notify staff of the patients' inclusion in the study. A protocol and instruction sheet were circulated to staff in ICU, CTR, Accident and Emergency, Theatre and the Surgical wards, and posters were displayed in areas where lines were inserted or subsequently cared for.
METHODOLOGY (i) DATA COLLECTION

The majority of factual data was obtained from written records of individual patients. The reliability and validity of archival analysis was discussed in Chapter Two. Records were examined to ensure that they were genuine, accurate and consistent, by comparing as many sources as possible, and comparing internally within each source. Sources included medical records, nursing cardex and care plans, anaesthetic and Theatre notes, temperature, drug and ICU flow charts and pathology reports which were reviewed daily. Primary sources were used wherever available. Facts were recorded in preference to opinions and a numerical value assigned where possible. Photocopies were taken for example of the temperature chart, where it was useful in an individual case.

Data relying on clinical judgment, that is to say the reason for admission and reason for line insertion which are both based on symptoms of underlying processes were recorded verbatim and categorised at the stage of analysis by which time the precise reason e.g. disease classification had been determined. The use of information recorded very recently and the concurrent collection of data from other sources including observation allowed verification of written data and the opportunity of correcting omissions and clarifying ambiguity. This method was used to record for example, age, sex, surgical intervention, use of antibiotics and laboratory parameters. Recognised instruments were used where available, for example the Apache II severity of illness scoring system.

Each patient was visited by the researcher at least daily, and recording of practice relating to the care of the insertion site or use of the administration system was used to collect supplementary data and to qualify data obtained from written records. It was not used to study behaviour, but was used throughout the course of the study to assess the progress of the study and identify any changes to procedure or intervening factors which may have influenced the outcome of the study. This method was used for example to monitor the usage of the catheter and appearance of catheter dressing on a daily basis as detailed below. Data was recorded on a proforma to ensure consistency. Any comments relating to the central line volunteered by the patient or Staff were recorded.
Documented data was validated by direct observation where practical, for example in determining the type of dressing used at a dressing change, where information in the nursing and Central Treatment Room cardex was confirmed by direct observation of the patient. This method was used, for example, to determine type of central venous catheter, frequency of dressing change and type of dressing. Disconnections of the sterile administration system were assessed by a combination of methods. Documented changes of solutions on the drug chart were compared with the observed use of the line and the presence of physical data such as administration set expiry dates, researchers markings and drug additive labels. This method was also used, for example, to determine whether central venous pressure was being recorded.

**PILOT STUDY**

A pilot study was performed for a 1 month period. No alterations were made to the data collection and the results were thus incorporated into the main study. Several changes were made to the laboratory procedures as discussed in the microbiological methodology section.

**DATA ENTRY/STATISTICAL ANALYSIS**

Data was recorded on “Epi info” version 5.01 (USD inc., Georgia, 1990), a word-processing, database and statistics package for handing epidemiological data. The accuracy and reliability of data entry was maximized by using the CHECK and VALIDATE programs: CHECK allowed ranges and legal values (those that will be accepted) to be specified, and “must enter” fields to prevent missing values. The assignment of numerical codes to variables was performed automatically from predetermined definitions. Intrarater reliability was assessed for 10 patients by entering the data on two separate occasions and comparing using the VALIDATE program. No errors were noted.

**VARIABLES RECORDED**

**Patient characteristics**

Patient characteristics such as age and sex, past medical history, details of current admission and treatment interventions along with the place, type, site of and
reason for insertion of the central venous catheter were recorded as in Chapter Two. In addition, severity of illness was recorded, using the APACHE II score (acute physiology, age and chronic health evaluation score) where routinely performed by an Anaesthetist on ICU admission (Knaus et al. 1985). This is a point score combining routine physiological measurements with previous health status and gives a predictive probability of survival. In addition a subjective severity of illness rating based on clinical judgment of likelihood of survival was assessed by the researcher for all patients. This has been shown to accurately predict mortality and risk of nosocomial infection survival (Charlston et al 1986).

Administration system

All catheters were observed once daily to determine the extent and type of usage of the catheter. The number of lumens in use (multi-lumen catheters) and type of fluid being administered via each individual lumen was recorded. Presence or absence of extension sets, three-way taps and filters was noted and these and the administration sets were inconspicuously marked and any expiry dates and times present were recorded to determine the length of time in use. Fluid administration charts and drug charts were examined for number and type of drug/fluid given, and frequency and route of administration. This allowed an estimate to be made of the number of administration set changes, bag changes and bolus injections performed and thus the number of breaks in the sterile administration system, and also which of the three catheter lumens was involved. In addition, the administration of antibiotics and immunosuppressants was noted. The prescribed and actual length of time of administration of total parenteral nutrition (TPN) was recorded. On removal of the catheter, the catheter plus any attached three-way taps, filters or extensions were retained in a sterile container for culture.

Insertion site

The integrity of the dressing and appearance of the site (where visible through the transparent dressing) were examined daily and the dressing was inconspicuously marked to identify when a change had taken place. Dressings were changed only when required due to leakage or to exposure of the insertion site. Staff were asked to record the date, antiseptic and dressing used and the presence of any inflammation on a
treatment Cardex at each dressing change. A swab of an area approximately 1cm immediately around the insertion site was taken prior to each dressing change, and immediately prior to line removal.

Microbiology

All specimens were refrigerated until collected daily from the clinical areas. Catheter tips and any associated culture and sensitivity plates, plus any other cultures from the same patient, (in particular blood culture plates and clinically requested site swabs) were collected from the clinical laboratory when no longer required (immediately after roll-plating for catheter tips).

**METHODOLOGY (ii) MICROBIOLOGY**

**CHOICE OF CULTURE METHOD**

**Blood culture**

As the end point of the study was catheter colonisation, it was not required that blood cultures be taken for study purposes. When taken because of clinical indications, blood cultures were sent to the clinical laboratory from which positive culture plates were retrieved and typed for comparison with isolates obtained from the catheter tip. As the focus of the study was the route of tip colonisation, no attempt was made to correlate the results of tip/blood culture with clinical symptoms. A positive blood culture in conjunction with an identical organism on the catheter tip was defined as a microbiologically documented catheter-related bacteraemia.

**PILOT STUDY**

To date, no agreement exists in the literature regarding the best method for assessing skin and hub colonisation or on the cut-off points used to classify positive/negative skin/hub cultures (Moro et al. 1990). Methods used by previous researchers are summarised in tables 3.11, 3.12, 3.13, pages 105-108.
Skin culture methods

Skin culture methods are summarised in table 3.11 page 105. Skin culture methods have ranged from a dry swab at the skin-catheter interface rubbed over an agar plate to vigorous scrubbing of a 10cm² area with a swab moistened in transport medium which was subsequently agitated in saline and serially diluted before plating: Overall rates of skin colonisation detected were 11% and 46% respectively, however it is not possible to assess whether this difference is due to the effectiveness of the sampling method or to differences in the management of the insertion site. In the majority of studies, any growth present at the insertion site was considered significant. A quantitative skin sampling method has been developed - the scrub cylinder method (Williamson and Kligman 1965) but this is difficult to use and has not been validated in central venous catheter infection. The efficiency of the method depends on the ability to remove organisms from the surface, and the extent to which the organisms are recovered from the sampler (Hambraeus et al. 1990). The low efficiency of the cotton swab has been shown in experimental studies (Hambraeus et al.), however it was used for all but one of the previous studies. For the purpose of this study it was recognised that the likelihood of determining a source of organisms colonising the catheter tip was related to the proportion of the different clones of organisms present at the insertion site or hub that were recovered and cultured. Nevertheless, because the researcher was physically unable to personally be present at each dressing change or catheter removal, it was necessary to utilise a method that was simple and reliable. It was therefore decided to utilise the method in current clinical use i.e. a dry swab at the skin-catheter interface, for the duration of the pilot study. From the work of Bjornson et al. (1982) demonstrating that a threshold of 1000cfu was required at the insertion site before the development of significant catheter colonisation, it was anticipated that this method would be sufficiently sensitive. Whilst previous authors have sampled a wider area around the insertion site, the researcher was unsure of the validity in assuming that organisms found e.g. 5cm away were representative of organisms in contact with the catheter, thus the swab was kept in contact with the catheter and rotated in a circle. Swabs were obtained at catheter removal, and additionally at the time of dressing changes, to determine the relationship of colonisation with duration of catheterisation.
Hub culture

Hub culture methods are summarised in table 3.12 page 106. Ullman et al. (1990) cultured fluid from each lumen of triple-lumen catheters daily. Whilst this would have provided very useful information it was recognised that this might bias the study by increasing the number of disconnections of the closed system, with the additional possibility of introducing contaminants. Jackobsen et al. (1989) sampled the catheter lumen at the first change of the administration set, however these were single-lumen catheters with set changes at 1, 3 and 5 day intervals. It was not considered feasible in this study to time hub sampling to coincide with routine set changes as these were performed 24/48 hourly at any time of the day or night, with sets on different lumens being changed at different times, as infusions were completed. Sampling of the hub was therefore restricted to following catheter removal. For the purpose of this study, any growth present within the hub - of what should be a closed sterile system- was considered significant and a possible source of tip colonisation. In the studies by Maki and Ringer (1987), Weightman et al. (1988) and Flowers et al. (1989), similar positive growth rates were obtained by a dry swab agitated in saline and serially diluted, flushing of the internal surfaces of the hub and serially diluting, and direct plating of a dry swab. For the pilot study, flushing of the hub and three way taps was compared with rotation of a dry swab.

Tip culture

Tip culture methods are summarised in table 2.13 page 107. The aim of the majority of these studies was to identify catheters likely to be associated with clinical infection, and levels of significance were chosen to detect the maximum number of true infections with the least number of false positives. Nahass and Weinstein (1990) determined that qualitative catheter cultures have minimal value in predicting catheter-related bacteraemia, and recommended that they no longer be performed in clinical microbiology laboratories. For epidemiological studies however, it is necessary to detect all isolates. Haslett et al. (1988) demonstrated that broth culture isolated more organisms, and some organisms more consistently than semi-quantitative culture, including organisms causing clinical infection: 27% of infecting organisms were recovered only by the broth immersion method - methicillin-resistant
S. aureus, Streptococcus mitis, Micrococcus sp., Serratia marcescens, Propionibacterium acnes, Eubacterium lentum, and Aspergillus fumigatus. In addition, isolates of S. haemolyticus, S. warneri, viridans streptococci, Enterococcus faecalis, Acinetobacter anitratus, Enterobacter cloacea and aerogenes, Klebsiella pneumoniae and Pseudomonas aeruginosa were recovered more consistently and on more occasions by broth immersion. For this study, a method which would detect both intra- and extraluminal colonisation was required which would differentiate contamination of the external surface of the catheter on catheter removal. The roll-plate technique of Maki and co-workers (1977) to culture the external surface was combined with flushing of the catheter lumen (Linares et al. 1985) to culture the intraluminal surface, followed by immersion and vortexing in broth, and overnight incubation to detect low numbers, adherent or fastidious organisms not detected by the other methods.

Gram-staining

Gram-staining of the internal and external catheter surface by the method of Cooper and Hopkins (1985) was attempted to distinguish the intra- and extraluminal routes of colonisation.

RESULTS OF THE PILOT STUDY

Several changes were made to the laboratory procedures following the pilot study:

Skin culture

Skin swabs were obtained from 7/12 catheters inserted in the ten patients in the pilot study. Three patients died or were transferred with the catheter still in situ. Bacterial growth from the catheter insertion site was obtained in 4 of the 7 (57%), and in 2 of the 3 colonised catheter tips, an identical organism (by preliminary biotype and antibiogram) was detected at both sites. In view of these results, the site culture method was unchanged.

Hub culture

Growth was obtained from three catheter lumens by both the flushing and dry
swab methods. Swabbing was superior at removing semi-congealed or encrusted material from the hub or three-way tap. Flushing was technically difficult in applying sufficient force without splashing and thus causing contamination from the rim or external surface of the hub/tap, or aerosol formation. Furthermore, growth obtained by flushing was lower (by a factor of 2 to 5) than that obtained by swabbing. Catheter hubs and three-way taps were thus sampled by swabbing only for the remainder of the study.

Tip culture

The aim of distinguishing intra- and extraluminal colonisation by selective culture was not realised. In practice, catheter tips were seen on receipt to be sitting in fluid and/or blood which had leaked from within the catheter tip and contaminated the external surface. In addition, because of the positioning and shape of the exit holes on the catheter tip, it was not possible to seal and flush each of the three lumens of the triple lumen catheters without contact of fluid with the external surface. The flush method of Linares et al. (1985) was therefore substituted for a quantitative tip culture method, modified from Brun-Buisson et al. (1987) whereby following roll-plating the tip was vortexed in trypticase soy broth and a quantified amount plated. From the results of Kristinsson et al. (1989) in which ultrasonication of tips was not found to improve the count of organisms obtained, vortexing was considered sufficient to break up clumps to allow enumeration of single colonies. After experimentation 5 x 10^3 drops from a 5ml volume were found to allow better differentiation of individual colonies than the original plating of 0.1ml by glass spreader.

Gram-staining

Multiple problems were encountered in examining the catheter tips under the microscope. Whilst some of these problems were anticipated, they were compounded by the triple-lumen catheters: Cooper and Hopkins (1985) noted that because of the cylindrical shape of the catheters, frequent adjustments of fine focus were required. Whilst with translucent catheters, the internal and external surfaces could be distinguished from one another by adjusting the depth of focus, more opaque catheters had to be cut longitudinally and the four sides examined separately. Coutlee, Lemieux
and Paradis (1988) found direct gram-staining laborious, with a significant number of primarily opaque catheters uninterpretable.

Three single and six triple lumen catheters were received in the pilot study. The three single lumen catheters were white, extremely thin and shiny, and were very difficult to cut, reluctant to stain and required to be selotaped to the slide. No organisms were visualised or obtained on culture. All the triple-lumen catheters were yellow and opaque. Due to lack of light penetration, it was not possible to examine the external surface intact. To examine the internal surfaces, it was necessary to dissect the catheter into three longitudinal sections to view the internal surface of each lumen. This was extremely difficult and resulted in irregular sections which were difficult to focus. The sections possessed a high degree of curvature and were too rigid to squash. Each triple-lumen catheter took approximately three hours to process and gave potentially unreliable results. It was generally not possible to enumerate organisms in individual fields, and where colonised on culture (3 catheters) organisms were visible externally and in all internal lumens. This method was therefore not continued, however some useful information was obtained: In general, Gram-positive cocci and epithelial cells were visible on the external surface, particularly where tape or the dressing had been adherent. Gram-positive cocci and erythrocytes were visible internally. The purple discoloration was visible macroscopically. With experience, this method may be of value in examining the transparent section of catheter between the hub and the catheter tip but was not found to be of value in examining individual lumens within the catheter tip.

The finalised laboratory methods are summarised on page 115.
<table>
<thead>
<tr>
<th>AUTHOR</th>
<th>CATHETERS</th>
<th>METHOD</th>
<th>REPORTED</th>
<th>SIGNIFICANCE</th>
<th>COLONISATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bjornson et al. 1982</td>
<td>central, 74 single TPN</td>
<td>moist swab, 2inch square, 20 rotating strokes, vortexed-serial dilution</td>
<td>any/sterile</td>
<td>&gt;1000 catheter colonisation</td>
<td>19/74</td>
</tr>
<tr>
<td>Snyderman et al. 1982</td>
<td>59 central single TPN</td>
<td>moistened swab rolled once forward &amp; backward at skin-catheter interface</td>
<td>+ve/-ve</td>
<td>any growth at skin ppv catheter colonisation 67%</td>
<td>overall 39%</td>
</tr>
<tr>
<td>Sitges-Serra et al. 1984</td>
<td>43 single TPN</td>
<td>dry swab 2cm diameter-plated</td>
<td>“a few colonies”</td>
<td></td>
<td>removal 11%</td>
</tr>
<tr>
<td>Linares et al. 1985</td>
<td>135 single TPN</td>
<td>“swab from skin surrounding puncture site”-plated</td>
<td>0-confluent</td>
<td>with sepsis &gt;100</td>
<td>?</td>
</tr>
<tr>
<td>Maki and Ringer 1987</td>
<td>2088 peripheral</td>
<td>10cm² vigorously scrubbed with transport medium moistened swab, agitated in saline, serially diluted.</td>
<td>colonised/not</td>
<td>not stated</td>
<td>46%</td>
</tr>
<tr>
<td>Fan et al. 1988</td>
<td>central 156 single TPN</td>
<td>skin broth moistened swab “skin-catheter junction” plated</td>
<td>?+ve/-ve</td>
<td>?any</td>
<td>43/156</td>
</tr>
<tr>
<td>Cheesbrough et al. 1988</td>
<td>64 haemodialysis</td>
<td>saline moistened swab-plated</td>
<td>&gt;5 cfu significant</td>
<td>not given</td>
<td>?</td>
</tr>
<tr>
<td>Jakobsen et al. 1989</td>
<td>40 TPN/CVP</td>
<td>dry swab rolled at insertion site-plated</td>
<td>+ve/-ve</td>
<td>not stated</td>
<td>11/40</td>
</tr>
<tr>
<td>De Cicco et al. 1989</td>
<td>central 129 single, TPN</td>
<td>“skin swab” 3cm area plated</td>
<td>? +ve/-ve</td>
<td>not stated</td>
<td>overall 29%, 14% at removal</td>
</tr>
<tr>
<td>Flowers et al. 1989</td>
<td>29 triple/PA</td>
<td>10cm² agar plate applied to site</td>
<td>&gt;10cfu/plate colonisation</td>
<td>ppv 60% for positive catheter</td>
<td>37.9%</td>
</tr>
<tr>
<td>Cercenado et al. 1990</td>
<td>139 central +peripheral</td>
<td>dry swab 10cm² area-plated</td>
<td>0-&gt;1000</td>
<td>15/plate highest sensitivity for colonised tip</td>
<td>?</td>
</tr>
<tr>
<td>Armstrong et al. 1990</td>
<td>169 single TPN</td>
<td>dry swab 2.5cm diameter-plated</td>
<td>NG/&lt;50/&gt;50</td>
<td>&gt;0 non-CNS, &gt;50 CNS sig. associated with catheter colonisation</td>
<td>38/166</td>
</tr>
<tr>
<td>AUTHOR</td>
<td>CATHETER</td>
<td>METHOD</td>
<td>REPORTED</td>
<td>SIGNIFICANCE</td>
<td>COLONISATION</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------------</td>
<td>------------------------------------------------------------------------</td>
<td>-------------------</td>
<td>-------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Sitges-Serra et al. 1984</td>
<td>43 single TPN</td>
<td>catheter hub flushed</td>
<td>sterile/infected</td>
<td>not stated</td>
<td>21/43</td>
</tr>
<tr>
<td>Linares et al. 1985</td>
<td>135 single TPN</td>
<td>catheter hub flushed</td>
<td></td>
<td>with sepsis &gt;1000</td>
<td>?</td>
</tr>
<tr>
<td>Maki and Ringer 1987</td>
<td>2088 peripheral</td>
<td>dry swab, agitated in saline, serial dilution</td>
<td>no growth/ contaminated</td>
<td>all&lt;10</td>
<td>“contamination “ 19-28%</td>
</tr>
<tr>
<td>Weightman et al. 1988</td>
<td>42 Hickman, children</td>
<td>internal surfaces flushed</td>
<td></td>
<td>non-bacteraemia mean 18 bacteraemia mean 3472</td>
<td>23.6%</td>
</tr>
<tr>
<td>De Cicco et al. 1989</td>
<td>central 129 single, TPN</td>
<td>hub-internal surface rinsed</td>
<td>?+ve/-ve</td>
<td>not stated</td>
<td>?</td>
</tr>
<tr>
<td>Jakobsen et al. 1989</td>
<td>40 TPN/CVP</td>
<td>20cm plastic obdurator inserted into lumen and rotated. Rolled on agar plate</td>
<td>&lt;10-&gt;1000, majority &lt;100, 50% &lt;10</td>
<td>not stated- identical or not</td>
<td>11/40</td>
</tr>
<tr>
<td>Flowers et al. 1989</td>
<td>29 triple/PA</td>
<td>hub-dry swab-plated</td>
<td>any growth</td>
<td>ppv 45.5% for positive catheter</td>
<td>20.7%</td>
</tr>
<tr>
<td>Cercenado et al. 1990</td>
<td>139 central + peripheral</td>
<td>hub-dry alginate swab-plated undissolved?</td>
<td>0-&gt;1000</td>
<td>5/plate highest sensitivity for colonised tip</td>
<td>?</td>
</tr>
<tr>
<td>Bozzetti et al. 1991</td>
<td>144 single TPN</td>
<td>hub immersed in broth-vortexed-plated-incubated-plated</td>
<td>600-10⁵</td>
<td>positive first culture associated with colonised catheter, median 22,500</td>
<td>74/144</td>
</tr>
<tr>
<td>AUTHOR</td>
<td>CATHETER</td>
<td>METHOD</td>
<td>COLONISATION</td>
<td>BACTERAEMIA</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------</td>
<td>---------------------------------------------</td>
<td>-----------------------</td>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td>Irwin <em>et al.</em> 1973</td>
<td>211 peripheral</td>
<td>1) tip streaked across BA plate</td>
<td>growth in 1 or 2</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) thioglycollate broth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maki <em>et al.</em> 1977</td>
<td>250 (of which 33 central)</td>
<td>1) 5cm rolled/smeared across BA plate</td>
<td>1) &gt;/= 15 local inflammation</td>
<td>1) &gt;1000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) triptolate soy broth (TSB)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cleri <em>et al.</em> 1980</td>
<td>189 (of which 35 central)</td>
<td>intravascular segment immersed and flushed in broth.</td>
<td>1) &lt;1000 indeterminate</td>
<td>1) &gt;1000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1) serially diluted and plated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) incubated and plated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moyer <em>et al.</em> 1983</td>
<td>101 (of which 90 central)</td>
<td>1) roll plate</td>
<td>1) 0-14 = negative</td>
<td>1) &gt;25 = positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) incubated and plated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bjornson <em>et al.</em> 1982</td>
<td>74 central</td>
<td>1) immersed in both, centrifuged, serially diluted</td>
<td></td>
<td>1) &gt;1000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) incubated and plated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linares <em>et al.</em> 1985</td>
<td>135 central</td>
<td>1) roll plate</td>
<td></td>
<td>1) &gt;7 cfu</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) catheter lumen flushed with 2ml TSB, serially diluted</td>
<td></td>
<td>2) &gt;1000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) incubated and plated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pettigrew <em>et al.</em> 1985</td>
<td>195 central</td>
<td>1) rinsed, flushed with broth, diluted, pour plates</td>
<td></td>
<td>1) &gt;1000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) incubated and plated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooper and Hopkins 1985</td>
<td>330 (of which 272 central)</td>
<td>1) roll plate</td>
<td>2) 1 organism/20 oil immersion fields</td>
<td>1) &gt;15</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) gram stain catheter tip</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheesbrough <em>et al.</em> 1986</td>
<td>64 haemodialysis</td>
<td>1) roll plate</td>
<td>1/2) &lt;30</td>
<td>1/2) &gt;30 generally &gt;100</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) flushed, plated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) incubated and plated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collignon <em>et al.</em> 1986</td>
<td>780 ICU</td>
<td>roll plate</td>
<td></td>
<td>&gt;5 higher sensitivity, same specificity as 15.</td>
<td></td>
</tr>
<tr>
<td>Collignon <em>et al.</em> 1987</td>
<td>322 (of which 132 central)</td>
<td>1) roll plate</td>
<td>1-10 organisms per slide</td>
<td>1 colony per 5 immersion fields</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) Gram-stained impression smears, external surface only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUTHOR</td>
<td>CATHETER</td>
<td>METHOD</td>
<td>COLONISATION</td>
<td>BACTERAEMIA</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
<td>---------------------------------------</td>
<td>--------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>Brun-Buisson et al. 1987</td>
<td>331</td>
<td>vortexed in sterile water, calibrated amount plated</td>
<td>30-500</td>
<td>&gt;1000</td>
<td></td>
</tr>
</tbody>
</table>
| Kristinsson et al. 1989 | 193              | 1) roll plate  
2) flushing, pour plate  
3) ultrasonification, pour plate | 1) >15ppv 46% npv 99%  
>100 ppv 56% npv 96%  
2) >15 ppv 66% npv 94%  
>100 ppv 71% npv 93% | 1, 2 and 3) >100 |
| Gil et al. 1989   | 220 (of which 157 TLC) | roll-plate                            |              | >15         |
| Rello et al. 1991 | 91 central and arterial | 1) roll plate  
2) flushed, serially diluted, plated |              | 1) >25  
2) >1000 |
As the aim of this study was to investigate the route of tip colonisation in patients with a central venous catheter, this required an assessment as to whether organisms cultured from the catheter tip were likely to be the same organism as cultured from the skin at the point of catheter insertion, or from the administration system. This is due to the fact that we are only able to test a fraction of the biochemical and genetic properties of an organism and thus are better able to determine unrelatedness than relatedness. The ability to determine that two organisms are of the same phenotype is a function of both the number of phenotypes recognised by the characterisation system and the proportion of the total microbial population each phenotype represents.

From the results of the retrospective survey and a review of the literature, it was anticipated that the majority of isolates would be coagulase-negative staphylococci. Christensen *et al.* (1983) proposed that to identify individual strains of coagulase-negative staphylococci, a characterisation system must identify between a sufficiently large number of phenotypes such that the probability of isolating any two phenotypes by chance alone is $p<0.05$. Assuming each phenotype is equally represented, this would require at least twenty different phenotypes. In practice, type distribution is unequal, greatly increasing the number of types required. They compared a number of characterising systems using a collection of 143 isolates of coagulase-negative staphylococci, to determine the number of phenotypes identified by each method. In addition, they calculated the probability of assigning two randomly selected strains to a single phenotype or species by chance alone: Combinations of systems increased the sensitivity of the results: Use of API-Staph with antibiotic sensitivity testing (10 agents) differentiated between fifty-six phenotypes. Most effective was the combination of biotyping, antibiograms and phage typing (95 phenotypes) but it require a "prodigious effort". Production of extracellular slime was found to be independent of biochemical reactions and phage type, and was strain stable enabling further differentiation. Archer *et al.* (1984) examined the effectiveness of plasmid profiles in differentiating *S.epidermidis* and calculated an assignment probability of 0.028. These results are tabulated below (Table 3.14).
In addition to sensitivity, there are several requirements of an ideal typing system: It must be applicable to the type of organism in question, and of proven value in epidemiological investigation i.e. it must be valid. Secondly, it must be reliable. Methods must be standardised to be readily reproducible, appropriate quality control measures must be built in and the characters used must be stable over time. Lastly, it must be practical i.e. available and at reasonable cost. As discussed in Chapter One, bacteriophage typing and plasmid analysis are technically complex, difficult to standardise, labour intensive and generally limited to research and reference laboratories.

For the purpose of this study, it was therefore decided to characterise isolates predominately by antibiotic sensitivity and biochemical (API) profile to combine reliability and feasibility with an assignment probability of less than 0.05 by chance alone. This probability is commonly accepted as being sufficiently specific, as it implies that the typing system must generate at least 20 categories, with sufficient distribution of organisms within the categories (Birnbaum et al. 1991). Should this prove inadequate it was proposed to use plasmid profiling in individual cases, which was the subject of the researcher’s M.Sc. dissertation.

**RELIABILITY AND VALIDITY OF TYPING METHODS**

**BIOLOGICAL PROFILES**

Biological profiles are formed from a series of biochemical tests as discussed in Chapter One. Results of a test of an individual organism are either positive (1) or negative (0). As the ratio of positive to negative of the species approaches 0.5, the validity of the test becomes nil. Reliability can be estimated by analysis of the variance
of replicates (test error). As many tests as possible should be employed, with a test error of <10% for each test (Amato et al. 1991). With regard to sensitivity, separation figures which represent the differentiating power of a test are available ranked on computer. Tests are selected for commercial systems which maximise these requirements. As most identification systems are based on biochemical reactions, a biotype is automatically produced which with commercial systems is reduced to a numerical code.

Sensitivity of biotyping is however limited at the level of strain characterisation. For example, using the Baird-Parker based systems, the vast majority of clinical isolates are classified as *S. epidermidis* biotype SII. Even with the more extensive kit systems, the number of biochemical profiles identifying *S. epidermidis* are limited and the majority of isolates are contained within one or a few profile numbers (Aber and Mackel 1981). In addition, the commercially prepared biotyping systems are not necessarily 100% reproducible at the individual biochemical test level, although they may quite reliably determine genus and species level identification (Bentley et al. 1968). This is due to variations in the composition of media or reagents used, lack of standardisation of methods such as inoculum size and duration of incubation, and to variations between individual micro-organisms themselves. Biotyping schemes have been devised especially for strain identification (Bentley et al. 1970) but have not proved any more successful than systems such as API-Staph which are primarily taxonomic in nature, again with the majority of strains in clinical trials falling into a single large phenotype or species (Bentley et al. 1968). Furthermore, the biotype is not a stable genetic property of the organism, being influenced by environmental conditions and the presence or absence of plasmids for example.

In summary, biotyping is feasible, standardised, valid and reliable using commercial systems but at strain level may not be stable and can lack sensitivity for epidemiological studies.

**BIOTYPING METHODOLOGY**

**API 20E for Enterobacteriaceae and other Gram-negative rods**

Five mls of water were water added to the incubation tray to create a humid atmosphere. From a purity plate, one single well isolated colony was removed with a sterile pipette and emulsified in 5mls sterile water to produce a homogenous bacterial
suspension. The individual test wells containing dehydrated substrates were inoculated with the bacterial suspension and incubated at 37°C for 18 hours, after which reagents were added to the test wells and the strip read in accordance with the interpretation table and comparison with positive and negative controls. Results were recorded on report sheets. The pattern of reactions was coded into a seven digit numerical profile and manually compared with the analytical profile index.

**API Staph for Staphylococci and Micrococcis**

A similar method was used for Staphylococci with the exception that organisms were subcultured onto blood agar and incubated overnight, and a standard inoculum (by turbidity) was added to 5mls Staph Medium (API) and applied to the test wells. The reactions used in API STAPH and API 20E are given in Appendix 5.

**ANTIBIOTIC SUSCEPTIBILITY PATTERNS**

Antibiotic sensitivity testing is carried out routinely in the clinical laboratory and thus has the advantage of being readily available, standardised, reproducible and relatively inexpensive. An antibiogram is based on determination of the pattern of susceptibility or resistance of an organism to a panel of antibiotics. The level of sensitivity for epidemiological purposes is dependent upon the organism, number and type of antibiotics tested and the manner in which the results are reported (Pfaller and Herdwaldt 1988). Most testing panels are designed for clinical rather than epidemiological purposes and there is no consensus as to how much difference between antibiograms is necessary to distinguish unrelated strains (Pfaller and Herdwaldt 1988). As antibiograms are influenced by the antibiotics in use within the hospital, this method of strain identification is generally useful only within a localised environment and at a particular time. At strain level there is instability due to antibiotic pressure, environmental factors, and gain and loss of plasmids. Loss of resistance plasmids by *S. epidermidis* isolated from patients within a period of hospitalisation (Parisi and Hecht 1980) and in the laboratory following subculture has been demonstrated (Ludlam *et al.* 1989). The recording of actual zone size using a disc diffusion technique in addition to recording resistance or sensitivity increases the utility of the antibiogram for epidemiological studies (Bentley *et al.* 1968). ATB Staph (API-Biomerieux) has been
demonstrated to possess an agreement of 96.3% with agar dilution methods and has the advantage of using 16 antibiotics of clinical and epidemiological interest (as shown in table 3.15 page 114) with the ability to record positive, negative and intermediate sensitivities (Gayral et al. 1986).

**ANTIBIOGRAM METHODOLOGY**

Antimicrobial sensitivity testing was performed by a disc diffusion technique, whereby a source of antimicrobial agent is applied to the surface of a solid medium through which diffusion occurs, inhibiting the growth of an organism growing upon it. The size of the zone produced is dependent upon the sensitivity of the organism, but also on other variables which were controlled to produce valid and reliable results:

- The ingredients and mineral content of the culture medium.
- pH of the culture medium.
- Viscosity and depth of the culture medium.
- Size of the inoculum (Cremer 1984).

Commercially produced impregnated single discs, sufficiently widely spaced to prevent overlapping of zones, were employed on “Iso-sensitest” agar, applied firmly to the medium to ensure even diffusion. Plates were poured flat with an even depth of medium. From a purity plate, a loopful of several different colonies (to allow for variation in sensitivity) was spread evenly over the plate with a dry, sterile cotton swab, to produce not quite confluent growth (Felmingham and Stokes 1972 in Stokes and Ridgeway 1983). A comparative method was used: This is one of 3 disc diffusion methods which have been accepted as sufficiently reliable to give comparable results when performed in different laboratories (Stokes and Ridgeway 1983). To assess resistance, zone sizes were compared with those of an appropriate known sensitive control, on the same plate for specimens from the clinical laboratory and on a separate plate for study specimens. For gram-positive organisms, this was the “Oxford Staphylococcus” NCTC 6571.
ATB Staph and ATB Strep

The ATB test is a breakpoint antimicrobial sensitivity test in a semi-solid medium. Factors recognized to affect the validity of the results are:

- A mixed or contaminated inoculum
- Use of media other than those recommended
- Incorrect standardization of the inoculum density
- Too great an interval between inoculum preparation and use
- Outdated or incorrectly stored tests, or
- Incubation at incorrect temperatures or for too short a period (API).

These were avoided by following the manufactures instructions: Isolated colonies from purity plates were suspended in sterile distilled water until the desired turbidity using the McFarland scale (0.5 for Staphylococci and 2.0 for Streptococci). 50μl of the suspension were added to an ampoule of the appropriate supplied ATB medium and thoroughly mixed. 135μl of the medium was inoculated into each of the test wells and incubated at 37°C. After 24 hours, each well was examined for growth and scored as positive or negative and interpreted according to the guidelines.

Because the additional tests provided in ATB Strep i.e. ampicillin, streptomycin and a first generation Cephalosporin gave no further discriminatory ability, being generally always sensitive (ampicillin and streptomycin) or resistant (cephalothin), ATB Staph strips were used for Enterococci in the first instance and repeated with ATB Strep where required.

<table>
<thead>
<tr>
<th>ANTIMICROBIAL AGENT</th>
<th>ATB STAPH</th>
<th>ATB STREP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PENICILLIN</td>
<td>0.25 mg/l</td>
<td>0.25 and 16 mg/l</td>
</tr>
<tr>
<td>OXACILLIN</td>
<td>2 mg/l</td>
<td>2 mg/l</td>
</tr>
<tr>
<td>TOBRAMYCIN</td>
<td>4 and 8 mg/l</td>
<td>2 and 8 mg/l</td>
</tr>
<tr>
<td>LINCOMYCIN</td>
<td>2 and 8 mg/l</td>
<td>2 and 8 mg/l</td>
</tr>
<tr>
<td>TETRACYCLINE</td>
<td>4 and 8 mg/l</td>
<td>4 and 8 mg/l</td>
</tr>
<tr>
<td>FUCIDIC ACID</td>
<td>2 and 16 mg/l</td>
<td>2 and 8 mg/l</td>
</tr>
<tr>
<td>CO-TRIMOXAZOLE</td>
<td>2 and 8 mg/l</td>
<td>2 and 8 mg/l</td>
</tr>
<tr>
<td>VANCOMYCIN</td>
<td>8 mg/l</td>
<td>4 mg/l</td>
</tr>
<tr>
<td>KANAMYCIN</td>
<td>8 and 16 mg/l</td>
<td>500 mg/l</td>
</tr>
<tr>
<td>GENTAMICIN</td>
<td>4 and 8 mg/l</td>
<td>500 mg/l</td>
</tr>
<tr>
<td>ANTIMICROBIAL AGENT</td>
<td>ATB STAPH</td>
<td>ATB STREP</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>ERYTHROMYCIN</td>
<td>1 and 4 mg/l</td>
<td>1 and 4 mg/l</td>
</tr>
<tr>
<td>PRASTINAMYCIN</td>
<td>2 and 4 mg/l</td>
<td>2 mg/l</td>
</tr>
<tr>
<td>PEFLOXACIN</td>
<td>1 and 4 mg/l</td>
<td>4 and 16 mg/ml</td>
</tr>
<tr>
<td>RIFAMPICIN</td>
<td>4 and 16 mg/l</td>
<td>4 and 16 mg/ml</td>
</tr>
<tr>
<td>FOSFOMYCIN</td>
<td>32 and 64 mg/l</td>
<td>32 and 64 mg/ml</td>
</tr>
<tr>
<td>AMPICILLIN</td>
<td>4 and 16 mg/l</td>
<td></td>
</tr>
<tr>
<td>CEPHALOTHIN</td>
<td>8 and 32 mg/l</td>
<td></td>
</tr>
<tr>
<td>STREPTOMYCIN</td>
<td>1000 mg/l</td>
<td></td>
</tr>
</tbody>
</table>

**SUMMARY OF LABORATORY METHODS**

Swabs were commercially supplied cotton swabs in an Amies (charcoal) modified Stuart transport medium, (Oxoid Ltd) which is a non-nutritional medium for the preservation of fastidious organisms. This has been demonstrated to be effective for specimens in transit for 3-5 days, (Stuart 1959) and refrigerated for up to 6 days (Wilkinson 1958). Swabs were plated directly using a conventional reducing inoculum technique. On completion of the infusion, TPN fluid was aspirated aseptically from the 3rd (previously unused) port of the three-litre bag. A 1 µl volume was plated using a disposable plastic loop. The internal surface of the luer connections of each catheter lumen, filter, extension set and the distal ports of three-way taps were swabbed with a sterile cotton tipped applicator then plated. Swabs were not pre-moistened to avoid inadvertent contamination. After roll culture, a 4 cm section was cut from the catheter tip, added to 5 mls tryptone soya broth, agitated and with a microtitre pipette 5x10⁻µl drops plated before and after overnight incubation to ensure detection of intraluminal tip colonisation. All specimens were plated onto 7% sheep blood agar to encourage growth of clinical isolates, and cysteine lactose electrolyte deficient agar to maximize differentiation of colonies from different species/strains. These were incubated aerobically at 37°C. The presence or absence of haemolysis and lactose fermentation was used as a first stage of characterization of Gram-positive organisms (Ludlam 1989). Colonies of each morphological type were subcultured to obtain pure cultures and single colonies and identified to genus level using the standard microbiological techniques of Cowan and Steel (1965). Variables such as incubation time and temperature were kept constant. All results were checked for transcription errors or post analytic variability.

A positive tip culture (catheter colonisation as opposed to contamination) was defined as the presence of >5 colonies on roll-plate culture, or any growth on broth
culture prior to incubation (1 colony equivalent to 100 colonies in 5ml sample). Growth in broth culture after incubation in the absence of any organisms on roll-plate culture was considered unlikely to be due to external contamination of the catheter on removal (which would be expected to yield organisms on roll-plating), and considered significant.

Gram-positive organisms were distinguished by morphology, appearance on staining and production of catalase in the presence of hydrogen peroxide. Enterococci were distinguished by aesculin hydrolysis. All Gram-negative organisms were speciated using API 20E. Cultures of similar morphological characteristics were tested for antimicrobial sensitivity by a disc diffusion method using eight agents currently in use in the clinical laboratory (appropriate to that species) to enable comparison with laboratory specimens. Zone size was recorded to increase sensitivity. Those organisms of patients with positive tip or blood cultures were identified to species level and further characterized using a biotyping system (API 20E or API Staph): Organisms were subcultured from purity plates onto blood agar and incubated overnight, then inoculated, incubated and interpreted according to the manufacturers instructions. All cultures from an individual patient were tested together in one batch, to enable standardisation of conditions such as incubation time and temperature, and visual comparison of test strips. A further antibiogram was performed on isolates from an individual patient of the same species and biochemical profile using a broth diffusion method - ATB Staph or ATB Staph (API-Biomerieux).

All cultures were stored in glycerol broth at -40 C to enable further differentiation if required.
RESULTS

The results will be discussed in the following sections:

- Study population
- Factors influencing extraluminal colonisation
- Factors influencing intraluminal colonisation
- Factors influencing tip colonisation
- Microbiology results.

STUDY POPULATION

Seventy eight catheters were inserted in fifty patients over the six month period. The characteristics of the patient population are summarised in Table 3.12.

| TABLE 3.16 : SUMMARY OF PATIENT CHARACTERISTICS ASSOCIATED WITH THE INSERTION OF CENTRAL VENOUS CATHETERS |
|-------------|-------------|-------------|-------------|
| AGE         | Range = 21-87 | mean = 63.68 | SD = 15.5 |
| SEX         | male = 66   | female = 12 |
| ANTIBIOTICS | no = 9      | yes = 69   |
| IMMUNO-SUPPRESSANT | yes = 10 | no = 68 |
| UNDERLYING DISEASE | none = 37 | chronic = 17 | cancer = 24 |
| SEVERITY OF ILLNESS | discharged = 56 | died within admission = 8 | died<1 week = 5 | died catheter in situ = 9 |
| APACHE SCORE | min = 7 | max = 33 | mean 16.5 | SD 6.4 |

Variables associated with the central venous catheter are summarised in Table 3.13.

| TABLE 3.17: VARIABLES ASSOCIATED WITH THE CENTRAL VENOUS CATHETER |
|---------------------|---------------------|---------------------|---------------------|
| INSERTING DEPT      | CTR = 8             | Theatre = 39        | ICU = 20           | A&E = 5, wards = 6 |
| SITE                | jugular = 63        | subclavian =14      | don't know=1       |
| (81.8%)             | (18.2%)             |
| CATHETER TYPE       | single = 38         | triple = 30         | nutricath = 10     |
| (48.7%)             | (38.5%)             | (12.8%)             |
| REASON              | fluid balance =10   | access = 0          | access+fluid       |
|                     |                     |                     | balance = 50       |
| DAYS IN-SITU        | mean = 5.03         | SD = 3.56           | median = 4         | range = 1 - 17    |
| REASON FOR REMOVAL  | not req’d = 38      | fell out = 3        | ?septicaemia = 14  |
|                     | not working=2       | rewired = 10        | died = 9           |
| CULTURE             | no growth = 36      | positive = 23       | bacteraemia = 7    |
|                     |                     |                     | none = 19          |
Six single-lumen catheters were re-wired to a triple-lumen catheter within hours of insertion and were not evaluated further, leaving 72 catheters that were prospectively examined from insertion to removal. The length of time the catheters were in situ is illustrated in Figure 3.20.

**Figure 3.20: Bar Chart of Length of Time In-Situ**

![Bar Chart of Length of Time In-Situ](image)
RESULTS: FACTORS INFLUENCING EXTRALUMINAL COLONISATION

CATHETER INSERTION

Thirty six checklists were completed at the time of catheter insertion by the assisting Doctor or Nurse, 16 in the operating theatre, 15 in ICU, 3 in A/E and 2 in CTR. These catheters were inserted by 16 different Doctors comprising 5 Senior House Officers, 5 Registrars, 1 Senior Registrar and 5 Consultants. The time taken for catheter insertion ranged from 2-40 minutes for single-lumen catheters (mean 12 minutes), 6-30 minutes for triple-lumen catheters (mean 18 minutes) and 30 and 35 minutes for the two Nutricaths. The time taken decreased with increasing experience as shown in Table 3.18. The number of attempts taken is shown in Table 3.19. As only 10 of these 36 catheter tips were positive on culture it is not possible to assess the effect of these variables on tip colonisation.

TABLE 3.18: TIME TAKEN TO INSERT CATHETER

<table>
<thead>
<tr>
<th>GRADE OF STAFF (number timed)</th>
<th>MEAN TIME (MINUTES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHO (n=3)</td>
<td>27 mins</td>
</tr>
<tr>
<td>Registrars (n=11)</td>
<td>19 mins</td>
</tr>
<tr>
<td>Senior Registrars (n=3)</td>
<td>18 mins</td>
</tr>
<tr>
<td>Consultants (n=9)</td>
<td>8 mins</td>
</tr>
</tbody>
</table>

TABLE 3.19: NUMBER OF ATTEMPTS TAKEN TO INSERT CATHETER

<table>
<thead>
<tr>
<th>NUMBER OF ATTEMPTS</th>
<th>TIP POSITIVE</th>
<th>TIP NEGATIVE</th>
<th>NOT CULTURED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=28)</td>
<td>8 (42%)</td>
<td>11 (58%)</td>
<td>8</td>
</tr>
<tr>
<td>2 (n=5)</td>
<td>0</td>
<td>4 (100%)</td>
<td>1</td>
</tr>
<tr>
<td>3 (n=2)</td>
<td>1 (100%)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6 (n=1)</td>
<td>1 (100%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
The degree and type of aseptic technique taken at catheter insertion is given in Table 3.20. As this was highly variable with small numbers in each category it was not possible to link clothing or equipment practice to subsequent tip culture.

**TABLE 3.20: ASEPTIC PRECAUTIONS AT CATHETER INSERTION**

<table>
<thead>
<tr>
<th>CLOTHING</th>
<th>THEATRE</th>
<th>ICU</th>
<th>A &amp; E</th>
<th>CTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mask only</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gloves Only</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Gloves &amp; gown</td>
<td></td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gloves, gown &amp; mask</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gloves, apron &amp; mask</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gloves &amp; mask</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| EQUIPMENT               |         |     |       |     |
| None                    | 2       |     |       |     |
| Back of glove packet    | 1       |     |       |     |
| Linen towels only       | 2       |     |       |     |
| Dressing pack only      | 3       | 3   |       |     |
| dressing pack & towels  | 4       | 12  |       |     |
| Minor operation pack    | 3       | 1   | 2     |     |

| HANDWASH                |         |     |       |     |
| None(wore Gloves)       | 2       | 3   | 3     |     |
| Soap, no gloves         | 1       |     |       |     |
| Antiseptic              | 13      | 12  | 2     |     |

| SKIN PREPARATION        |         |     |       |     |
| None                    | 3       |     |       |     |
| Iodine                  | 13      | 2   |       |     |
| Betadine                | 2       | 2   |       |     |
| Alc. Chlorhexidine      | 14      |     |       |     |

Despite the small sample, a large difference was observed in the result of tip culture with the different skin cleansers employed as shown in Table 3.21:
Iodine was associated with a greater proportion of positive tip cultures than alcoholic chlorhexidine (p=0.08 fishers exact test 2 tail). As seen above, alcoholic chlorhexidine was only used in theatre whilst iodine solution was used predominately in ICU. There were thirteen catheters inserted in ICU and 12 in Theatre for which a tip culture and a checklist were available. Ten of the 12 central lines inserted in theatre went to ICU from theatre. There was no significant difference in the duration of catheterisation (Theatre mean 4.7 days vs ICU 3.7 days) or severity of illness (mean Apache score theatre 15.6 vs ICU 15.3) between the two groups, and it would not appear from the above checklists that catheters were inserted in Theatre with a higher degree of asepsis, suggesting a true difference in the effectiveness of the skin cleansers. All dressings used were Opraflex/Op-site.

RESULTS OF SITE CULTURE

Eleven insertion site swabs were obtained at dressing change with 37 swabs being obtained immediately prior to removal of the central line. Bacterial growth was obtained from 8 swabs at change of dressing (73%) and 23 swabs at line removal (62%). Forty six isolates of predominately Gram positive cocci were obtained from these 31 positive sites as shown in table 3.22. A single isolate was recovered from 19 (61%) insertion sites, two isolates from 9 sites (29%) and three isolates from 3 sites (10%).
TABLE 3.22: ORGANISMS ISOLATED FROM INSERTION SITE SWABS.

<table>
<thead>
<tr>
<th>ORGANISMS AT INSERTION SITE</th>
<th>NO. OF ISOLATES</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. faecalis</em></td>
<td>13</td>
</tr>
<tr>
<td>Unspeciated CNS</td>
<td>9</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>8</td>
</tr>
<tr>
<td><em>S. haemolyticus</em></td>
<td>5</td>
</tr>
<tr>
<td>Yeasts</td>
<td>2</td>
</tr>
<tr>
<td><em>Acinetobacter sp</em></td>
<td>2</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>2</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>1</td>
</tr>
<tr>
<td><em>S. warneri</em></td>
<td>1</td>
</tr>
<tr>
<td>&quot;diphtheroids&quot;</td>
<td>2</td>
</tr>
<tr>
<td>&quot;coliforms&quot;</td>
<td>1</td>
</tr>
</tbody>
</table>

**INFLUENCE OF NURSING PRACTICE**

**Care of the insertion site**

Site dressings were only changed if leaking or failing to adhere. The insertion site was cleaned with "Betadine" solution and redressed with "Op-site". The dressing was left intact throughout the life of the catheter in 79% of cases (maximum 15 days). It was therefore difficult to assess the effect of dressing change on the rate of positive tip culture, however insertion sites not exhibiting microbial growth were redressed 7 times in 77 catheter days with an average of 4 days without a change of dressing as opposed to 9 dressings in 170 days with an average of 6 days without a dressing change for sites possessing microbial growth. Where not redressed ≥ 7 days (10 catheters), 3 were on the general ward, 1 in ICU, and six had care split between the two.

Twenty one percent of sites were redressed, at an interval of 1-9 days, mean 2 days, with, most frequently (mode) 1 redress per catheter (range 1-5). The reasons (where stated: n=25) for redressing the insertion site were: site exposed 77%, site leaking/bleeding 16% and site inflamed 8%. There was no evidence of regular, routine redressing of insertion sites. Two sites were noted by the author to be inflamed, 1 with visible pus (not redressed for 13 days). Of 17 insertion sites noted by the author to be
exposed, 10 were redressed that day, 2 on the second day and 1 on the third day. Four were not redressed before catheter removal (>3 days). Of these 17 catheters, 75% were positive on site culture. The microbiology results of insertion site culture for sites redressed and not redressed are compared in Tables 3.23 and 3.24.

**TABLE 3.23: RESULT OF SITE CULTURE, SITES NOT REDRESSED**

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>FREQUENCY (NUMBER OF SITES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>9</td>
</tr>
<tr>
<td>CNS + <em>S. aureus</em></td>
<td>1</td>
</tr>
<tr>
<td>CNS + <em>E. faecalis</em></td>
<td>1</td>
</tr>
<tr>
<td>Coliforms</td>
<td>4</td>
</tr>
<tr>
<td>Coliforms + <em>E. faecalis</em></td>
<td>1</td>
</tr>
</tbody>
</table>

**TABLE 3.24: RESULTS OF SITE CULTURE, SITES REDRESSED*†**

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>BEFORE REDRESS (SITES)</th>
<th>REISOLATED BEFORE NEXT REDRESS/CATHETER REMOVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>CNS + <em>E. faecalis</em></td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Yeast</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*All redressed with Betadine/iodine solution or N/saline with Betadine spray

Site of insertion

It was not possible to assess the effect of site of insertion on tip colonisation as 79% of catheters with a tip culture were jugular, and 9/12 subclavian catheters were Nutricaths. From removal site swabs, 6/10 (60%) subclavian insertion sites were positive with a mean duration 8.3 days (vs negative 5.3 days), and 16/28 (57%) jugular sites were positive, with a mean duration 6.7 days (vs negative 4.8 days).

Duration of catheterisation

There was a significant increase in the rate of positive skin culture when the duration of catheterisation exceeded six days (p=0.01, Chi-square, Yates correction) as shown in Figure 3.21.
FACTORS INFLUENCING INTRALUMINAL COLONISATION

INTRALUMINAL GROWTH

Forty eight central venous catheters were saved for culture on removal. Microbial growth was obtained from 18 of 73 3-way taps (25%), on 13 catheters, and from 16 catheter hubs on 14 catheters (30%). Eighteen samples of TPN were received from which growth was obtained in 3 samples (17%). Nutricaths and catheters used for the administration of TPN were associated with very high rates of intraluminal growth of 87% and 92% as shown below (Table 3.25).
TABLE 3.25: RATES OF INTRALUMINAL GROWTH IN DIFFERENT CATHETER TYPES.

<table>
<thead>
<tr>
<th>TYPE OF CATHETER</th>
<th>CATHETERS SAVED</th>
<th>GROWTH IN HUB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Lumen (n=32)</td>
<td>20</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Triple Lumen (n=30)</td>
<td>20</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Nutricath (n=10)</td>
<td>8</td>
<td>7 (87)</td>
</tr>
<tr>
<td>TPN (All Lines) (n=18)</td>
<td>13</td>
<td>12 (92)</td>
</tr>
</tbody>
</table>

Fifty organisms were isolated from the administration system as follows (table 3.26)

Single isolates were obtained from 16 taps/hubs, two isolates from 11 taps/hubs and three isolates from 5 taps/hubs. More isolates were obtained from the tap than the catheter hub, and heavy growth in the tap was frequently moderate or scanty in the hub. Growth in the catheter hub was heavy in 4 instances, moderate in 3 instances, and scanty in 7.

TABLE 3.26: ORGANISMS ISOLATED FROM THE ADMINISTRATION SYSTEM.

<table>
<thead>
<tr>
<th>ADMINISTRATION SYSTEM</th>
<th>NO. OF ISOLATES:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAP</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>7</td>
</tr>
<tr>
<td><em>S. hominis</em></td>
<td>4</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>4</td>
</tr>
<tr>
<td><em>S. warneri</em></td>
<td>2</td>
</tr>
<tr>
<td><em>S. haemolyticus</em></td>
<td>2</td>
</tr>
<tr>
<td><em>S. capitis</em></td>
<td>0</td>
</tr>
<tr>
<td>Unspeciated CNS*</td>
<td>3</td>
</tr>
<tr>
<td><em>Acinetobacter sp.</em></td>
<td>0</td>
</tr>
<tr>
<td>micrococci</td>
<td>2</td>
</tr>
<tr>
<td>yeasts</td>
<td>1</td>
</tr>
</tbody>
</table>
TPN ADMINISTRATION

Type of catheter

TPN was administered via 18 catheters, 8 triple-lumen and 10 Nutricath. Of the 13 out of 18 lines used for TPN that were saved for culture (5 triple-, 8 Nutricath) 12 exhibited bacterial growth (92%). Of the 8 triple-lumen catheters, all had a lumen dedicated to TPN administration only at the time of catheter insertion. However, only four of these remained dedicated, one of which was cultured with no growth. One lumen blocked and TPN was switched to another lumen. Three were used for multiple purposes. Of these three were cultured, with all lumens exhibiting microbial growth.

Delivery time

The prescribed and actual time taken to administer (hang time) a 3 litre bag of TPN is given in Table 3.27. The prescribed administration time was exceeded in 85% bags by an average of 5.75 hours.

<table>
<thead>
<tr>
<th>PRESCRIBED TIME (HOURS)</th>
<th>ACTUAL DELIVERY TIME (HOURS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MINIMUM</td>
</tr>
<tr>
<td>24 (N=64)</td>
<td>20.50</td>
</tr>
<tr>
<td>30 (N=3)</td>
<td>34.50</td>
</tr>
<tr>
<td>36 (N=19)</td>
<td>24.50</td>
</tr>
</tbody>
</table>

TPN samples were taken by the researcher from the bag, whilst still hanging, at the end of the infusion. This was achieved for 18 of 86 bags (21%) from 7/13 patients, of which 3 were positive for CNS (17%).

DISCONNECTIONS OF THE CATHETER

The effect of disconnections of the sterile administration system was examined by adding the number of administration set changes, infusion bag/syringe changes, top-ups of in-line burettes and bolus injections from the drug chart, and observation of the line, and calculating the number of disconnections/day. The largest proportion of
disconnections were commonly for bolus injections, most frequently of antibiotics. The
total number of disconnections was greatest in the acute phase (generally at the time of line
insertion) and progressively decreased. To illustrate the potential extent of use of the
catheters, the highest number of disconnections was 203 over 10 days, with the highest
rate 103 over 4 days (26/day), 55 of which were bolus injections. Both catheters were
triples-lumen. The average number of disconnections was less for Nutricaths (1.25/day)
than for single lumen catheters (5.6) or triple lumen catheters (12.2). Catheter hubs
exhibiting microbial growth were disconnected more frequently than negative catheter
hubs as illustrated in Table 3.28:

TABLE 3.28 : RELATIONSHIP BETWEEN NUMBER OF DISCONNECTIONS AND GROWTH IN
HUB

<table>
<thead>
<tr>
<th>TYPE OF CATHETER</th>
<th>NUMBER OF DISCONNECTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GROWTH IN HUB</td>
</tr>
<tr>
<td>SINGLE (n=20)</td>
<td>14</td>
</tr>
<tr>
<td>TRIPLE (n=20)</td>
<td>59</td>
</tr>
<tr>
<td>NUTRICATH (n=8)</td>
<td>6</td>
</tr>
</tbody>
</table>

Administration sets were generally changed according to policy (24 hours for
lines containing drugs, 48 hours for clear fluids), with a maximum for a single catheter
of 48 set changes over 4 days. Nine administration sets were observed to have expired
in 7 patients and used for 1 extra day, of which 5 were CVP manometers. Surprisingly,
three-way taps were not changed with the set unless faulty and remained unchanged for
the duration of catheterisation. The influence of the number of disconnections of the
catheter hub for administration set changes and bolus injections, the number of
disconnections/breaks to the administration system for bag/syringe changes and top-up
of in-line burettes, the total number of disconnections, and the mean total number of
disconnections per day, on the result of hub culture are compared. The total number of
disconnections per duration of catheterisation was most highly associated with a
positive hub culture (p=0.027, t-test). This is illustrated in Figure 3.22 (page 128) .

The effect of the number of disconnections of triple-lumen catheters on the
result of hub culture is illustrated in Figure 3.23 (page 129).
FIGURE 3.22: BOX-WHISKER PLOT OF VARIABLES ASSOCIATED WITH A POSITIVE TRIPLE-LUMEN HUB CULTURE

Hub Disconnections (y=total number)

Bag changes/top ups (y=total number)

Total disconnections/bag changes

Rate of disconnections per day
Duration of catheterisation

A positive hub culture did not appear related to time in situ, as illustrated in figure 3.24. This was confirmed by comparison of the number of disconnections of individual positive hubs of single triple-lumen catheters, with hubs negative for growth acting as controls for time in situ, as shown in figure 3.25.
FIG 3.24: BAR CHART OF DURATION OF CATHETERISATION AND HUB CULTURE

FIGURE 3.25: TOTAL NUMBER OF DISCONNECTIONS: COMPARISON OF POSITIVE AND NEGATIVE HUBS OF INDIVIDUAL TRIPLE-LUMEN CATHETERS
RESULTS: FACTORS INFLUENCING TIP COLONISATION

RESULTS OF CULTURE

Thirty six isolates of predominately coagulase-negative Staphylococci were isolated from the 23 positive catheter tips (table 3.29). Single isolates were obtained from 12 tips, two isolates from 9 tips and three isolates from 2 tips.

TABLE 3.29: ORGANISMS ISOLATED FROM CATHETER TIPS

<table>
<thead>
<tr>
<th>ORGANISM ON TIP</th>
<th>NO. OF ISOLATES</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis</td>
<td>17</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>6</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>6</td>
</tr>
<tr>
<td>S. warneri</td>
<td>3</td>
</tr>
<tr>
<td>S. hominis</td>
<td>1</td>
</tr>
<tr>
<td>yeasts</td>
<td>1</td>
</tr>
<tr>
<td>diphtheroids</td>
<td>1</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>1</td>
</tr>
</tbody>
</table>

Type of catheter

The rate of tip culture varied with the type of catheter, with Nutricaths and triple-lumen catheters associated with significantly higher rates of tip colonization than single lumen catheters as in the previous study despite similar numbers of catheters (table 3.30).

TABLE 3.30: RESULTS OF TIP CULTURE

<table>
<thead>
<tr>
<th>TYPE OF CATHETER</th>
<th>NUMBER CULTURED</th>
<th>NUMBER OF POSITIVE TIPS (%)</th>
<th>CATHETER-RELATED BACTERAEMIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Definite#</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Probable*</td>
</tr>
<tr>
<td>SINGLE LUMEN</td>
<td>27</td>
<td>3 (11)</td>
<td>0</td>
</tr>
<tr>
<td>TRIPLE LUMEN</td>
<td>22</td>
<td>13 (59)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>NUTRICATH</td>
<td>10</td>
<td>7 (70)</td>
<td>3 (30)</td>
</tr>
</tbody>
</table>

# microbiologically documented  * primary nosocomial bloodstream infection
Time in situ

The average length of time a catheter was in situ was 5.2 days (range <1-17). Triple lumen catheters, and Nutricaths (tunneled single-lumen catheters) tended to remain in situ longer than single lumen catheters as illustrated in Figure 3.20 page 118. Catheters yielding a positive tip culture were on average in place longer than those with a negative tip culture and in situ for more than one week (Table 3.31). It was observed that in the majority of patients returning to the wards from ICU, the central venous catheter was not connected to a manometer and was used solely in place of a peripheral cannula for an average of a further 3 days. Of 21 patients discharged from ICU with central venous catheters in place; 5 were removed next day, 11 were not connected to CVP manometer, 14 were used only for drugs/fluids which could go peripherally for a mean extra 2.8 days (range 2 - 7) and 2 were unused.

<table>
<thead>
<tr>
<th>TYPE OF CATHETER</th>
<th>POSITIVE TIPS</th>
<th>MEAN DAYS IN SITU</th>
<th>NEGATIVE TIPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SINGLE LUMEN</td>
<td>7.3</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>TRIPLE LUMEN</td>
<td>7.1</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>NUTRICATH</td>
<td>10.0</td>
<td>6.0</td>
<td></td>
</tr>
</tbody>
</table>

These results are displayed in figures 3.26-3.29:
Figure 3.26: Distribution of tip culture results - Triple lumen

Figure 3.27: Distribution of tip culture results - single lumen
Figure 3.28: Distribution of tip culture results - Nutricath

Figure 3.29: Distribution of tip culture results - TPN
Severity of illness

Forty two catheters in patients in the Intensive Care Unit were compared for severity of illness by Apache II scoring. Positive triple-lumen catheters tips were associated with a higher Apache score, and a longer time in situ than negative catheters, as shown in table 3.32.

**TABLE 3.32: FACTORS RELATING TO TIP CULTURE IN ICU PATIENTS**

<table>
<thead>
<tr>
<th>CATHETER TYPE</th>
<th>MEAN APACHE SCORE +VE TIPS -VE</th>
<th>MEAN DAYS IN SITU +VE TIPS -VE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SINGLE (17)</td>
<td>9 13</td>
<td>5 4</td>
</tr>
<tr>
<td>TRIPLE (17)</td>
<td>18 13</td>
<td>7 4</td>
</tr>
<tr>
<td>NUTRICATH(8)</td>
<td>18 19</td>
<td>9 7</td>
</tr>
<tr>
<td>TPN - ALL LINES (10)</td>
<td>16 17</td>
<td>9 7</td>
</tr>
</tbody>
</table>

**GROWTH AT THE INSERTION SITE AND TIP CULTURE**

Of sites redressed (or catheter removed) within 7 days, 50% of tips exhibited microbial growth, compared to 100% positive tips where the insertion site was not redressed for 7 or more days as illustrated in Figure 3.30. This was a significant difference (Fishers exact test p=0.024). Of sites redressed, 62% of tips were positive. The number of days since insertion/redress was a mean of 4.8 days (SD 1.3) for negative tips, 6.9 days (SD 3.6) for positive tips, and 8.8 days (SD 3.8) where an identical organism was isolated from the insertion site and tip. Where identical isolates were found, growth at the insertion site was heavy in 5 instances, moderate in 1 and scanty in 1. E. faecalis was isolated from the tip in 75% of cases where found at the insertion site.
GROWTH WITHIN THE ADMINISTRATION SYSTEM AND TIP CULTURE

As with hub colonisation, the number of disconnections of the catheter hub, and the total number of breaks in the system were most highly correlated with a positive tip culture as shown for triple-lumen catheters in figure 3.31. For all catheters, there was a highly significant difference in the number of disconnections of catheters with positive tips and negative tips (Mann-Whitney U test p=0.003). All catheters disconnected more than 60 times were associated with a positive tip culture as shown in figure 3.32 (page 139). Where identical organisms were found at the tip and on the catheter hub, growth was heavy on 2 occasions, moderate on 2 and scanty on three.

Where TPN was administered via a triple-lumen catheter, the dedicated lumen was used for multiple purposes in 3 cases, and switched to a lumen previously used for multiple purposes in a further case: 3 of these catheters were cultured and all were positive with the same organism at lumen and tip.
FIGURE 3.31: BOX WHISKER PLOT OF RELATIONSHIP BETWEEN BREAKS IN THE ADMINISTRATION SYSTEM AND TIP CULTURE OF TRIPLE-LUMEN CATHETERS

Triple Lumen Tips

Hub Disconnections (y=total number)  Bag Changes/Top-ups (y=total number)

Total Disconnections/bag changes

Rate/Day
FIGURE 3.32: BAR CHART OF TOTAL NUMBER OF BREAKS IN THE ADMINISTRATION SYSTEM AND RESULT OF TIP CULTURE

TOTAL NUMBER OF BREAKS IN ADMINISTRATION SYSTEM
ROUTE OF TIP COLONISATION

The following organisms were isolated from the catheter tip, site and/or hub/three-way tap. (table 3.33).

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>TIP</th>
<th>SITE</th>
<th>HUB/TAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis</td>
<td>16</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>7</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>S. warneri</td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>S. hominis</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>S. capitis</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Micrococcus</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Kl. pneumoniae</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Acinetobacter sp.</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>unspeciated coliforms</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>diphtheroids</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Yeasts</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

The relationship of isolates from the insertion site and within the administration system to isolates obtained from the catheter tip is shown in the following table (Table 3.34). Of six cases of microbiologically documented catheter-related bacteraemia and one case of primary nosocomial bacteraemia (probable catheter-related bacteraemia), a complete set of samples was available for six, and a source was identifiable in 5. All five cases of bacteraemia due to the catheter in which a source could be identified were due to intraluminal contamination in patients receiving total parenteral nutrition. Identical organisms were isolated from the TPN administration bag in one instance.
### TABLE 3.34: ALL ISOLATES ASSOCIATED WITH A POSITIVE TIP/BLOOD CULTURE

<table>
<thead>
<tr>
<th>PT</th>
<th>SITE</th>
<th>TAP</th>
<th>HUB</th>
<th>TIP</th>
<th>BLOOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>S.epid*(1)</td>
<td>S.epid (2)(p)</td>
<td>NG</td>
<td>S.epid*(1)</td>
<td>Not done</td>
</tr>
<tr>
<td>6</td>
<td>K.pneu*</td>
<td>S.hominis(d)</td>
<td>E.faecalis(d)</td>
<td>NG(d)</td>
<td>E.faecalis*(m)*</td>
</tr>
<tr>
<td>10</td>
<td>E.faecalis</td>
<td>E.faecalis(d)</td>
<td>S.haem*</td>
<td>NG(d)</td>
<td>S.epid(1)*</td>
</tr>
<tr>
<td>11</td>
<td>Not done</td>
<td>S.epid(2)(d)</td>
<td>NG(p)</td>
<td>S.epid(1)*</td>
<td>S.epid(1)*</td>
</tr>
<tr>
<td>15</td>
<td>NG</td>
<td>S. hominis(d)</td>
<td>E. faecalis(d)*</td>
<td>S.epid</td>
<td>S.epid</td>
</tr>
<tr>
<td>16</td>
<td>S. warneri*</td>
<td>NG</td>
<td>S. warneri*</td>
<td>Not done*</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Acinetobacter</td>
<td>NG</td>
<td>Acinetobacter</td>
<td>S.epid</td>
<td>Not done</td>
</tr>
<tr>
<td>25</td>
<td>S.epid(2)</td>
<td>NG</td>
<td>S.epid(1)</td>
<td>S.epid</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Not done</td>
<td>S. hominis</td>
<td>S.epid(1)</td>
<td>Not done</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>S. haem</td>
<td>None(m)</td>
<td>S. epid*(1)(m)</td>
<td>S.epid*(1)*</td>
<td>NG</td>
</tr>
<tr>
<td>44</td>
<td>S. haem(2)</td>
<td>None</td>
<td>S.epid(1)</td>
<td>S.epid(1)*</td>
<td>Not done</td>
</tr>
<tr>
<td>45</td>
<td>S. hominis</td>
<td>S. hominis</td>
<td>S. hominis</td>
<td>S. hominis</td>
<td>S. hominis</td>
</tr>
<tr>
<td>46</td>
<td>E. faecalis*</td>
<td>NG</td>
<td>S. warneri*</td>
<td>S. warneri*</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>Not done</td>
<td>S. epid(2)</td>
<td>S. epid(1)*</td>
<td>Not done</td>
<td></td>
</tr>
</tbody>
</table>

**KEY:** isolates identical to tip and blood asterisked and bold type. *(p)= strain number within catheter. **(p)= proximal, **(m)= medial, **(d)= distal
Of 24 positive tip/blood cultures, a complete set of samples was available for 19. Of these, a source of tip colonisation could be identified in 12 catheters (13 organisms). In a further two catheters, an identical organism to the tip was present at the insertion site, but the catheter was not saved. One specimen of TPN exhibiting microbial growth was associated with a positive blood culture. Twenty organisms were isolated only from the catheter tip. In six catheters (7 organisms) an identical organism was present at insertion site and tip, and in six catheters (7 organisms) identical organisms were present in the catheter hub/three-way tap and tip. With 1 catheter (2 organisms) an identical organism to the tip was present at both the insertion site and catheter hub/three-way tap. A further 16 organisms were isolated from the insertion site only, and 15 from the catheter hub/3-way tap. Two organisms were isolated from both the catheter hub and insertion site. Both the intra- and extraluminal routes of tip colonisation were therefore of equal importance in our clinical practice, but there were variations in frequency with type of catheter as follows (tables 3.35-3.37):

**TABLE 3.35: SOURCE OF ORGANISM ON TIP/BLOOD CULTURE FOR DIFFERENT TYPES OF CATHETER WHERE A COMPLETE SET OF SPECIMENS WAS AVAILABLE**

<table>
<thead>
<tr>
<th>CATHETER TYPE</th>
<th>COMPLETE SPECIMENS</th>
<th>INSETION SITE</th>
<th>HUB/TAP</th>
<th>BOTH</th>
<th>UNKNOWN</th>
</tr>
</thead>
<tbody>
<tr>
<td>SINGLE n=3, 3</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>TRIPLE n=13</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>NUTRICATH n=8</td>
<td>7</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>TPN - ALL LINES n=12</td>
<td>11</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

**TABLE 3.36: SOURCE OF POSITIVE TIP/BLOOD CULTURES OF NUTRICATHS (all subclavian)**

<table>
<thead>
<tr>
<th>DAYS IN SITU</th>
<th>REDRESSES</th>
<th>BREAKS IN SYSTEM</th>
<th>SKIN SITE</th>
<th>HUB/TAP</th>
<th>TIP</th>
<th>BLOOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td>0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 3.37: SOURCE OF POSITIVE TIP/BLOOD CULTURES OF TRIPLE-LUMEN CATHETERS (all jugular)

<table>
<thead>
<tr>
<th>DAYS IN SITU</th>
<th>REDRESSES</th>
<th>BREAKS IN SYSTEM</th>
<th>SKIN SITE</th>
<th>HUB/TAP</th>
<th>TIP</th>
<th>BLOOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2(bleeding)</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3(exposed)</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>113</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4(exposed)</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>148</td>
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<td></td>
</tr>
</tbody>
</table>

The relationship between isolates is illustrated in figure 3.33:

FIGURE 3.33: RELATIONSHIP BETWEEN ORGANISMS ISOLATED FROM DIFFERENT SITES

![Diagram](image)

The results of two patients are summarised below which illustrate the complex epidemiology of tip colonisation: The first demonstrates that more than one route of tip colonisation may occur concurrently in a single patient (figure 3.34). The second demonstrates that the route may be different with the same organism in successive catheter (both triple lumen) in the same patient (figure 3.35).
### FIGURE 3.34: PATIENT NO. 6 - ORGANISMS CULTURED ON REMOVAL OF TRIPLE LUMEN CATHETER

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>SITE</th>
<th>TAP</th>
<th>HUB</th>
<th>TIP</th>
<th>BLOOD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. pneumoniae</em></td>
<td>proximal</td>
<td>proximal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>medial</td>
<td>distal</td>
<td>medial</td>
<td>proximal</td>
<td></td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>proximal</td>
<td>proximal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. haemolyticus</em></td>
<td>medial</td>
<td>distal</td>
<td>medial</td>
<td>proximal</td>
<td></td>
</tr>
<tr>
<td><em>S. hominis</em></td>
<td>medial</td>
<td>distal</td>
<td>medial</td>
<td>proximal</td>
<td></td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>proximal</td>
<td>proximal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micrococcus</td>
<td>medial</td>
<td>distal</td>
<td>medial</td>
<td>proximal</td>
<td></td>
</tr>
</tbody>
</table>

### FIGURE 3.35: PATIENT NO. 41 - ORGANISMS CULTURED ON REMOVAL OF SUCCESSIVE TRIPLE-LUMEN CATHETERS

#### CATHETER ONE

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>SITE</th>
<th>TAP</th>
<th>HUB</th>
<th>TIP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. haemolyticus</em></td>
<td>proximal</td>
<td>proximal</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>proximal</td>
<td>proximal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>diphtheroids</td>
<td>proximal</td>
<td>proximal</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. warneri</em></td>
<td>proximal</td>
<td>distal</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. epidermidis (1)</em></td>
<td>proximal</td>
<td>medial</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. epidermidis (2)</em></td>
<td>proximal</td>
<td>proximal</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### CATHETER TWO

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>SITE</th>
<th>TAP</th>
<th>HUB</th>
<th>TIP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. haemolyticus</em></td>
<td>proximal</td>
<td>proximal</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>proximal</td>
<td>proximal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>diphtheroids</td>
<td>proximal</td>
<td>proximal</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. warneri</em></td>
<td>proximal</td>
<td>distal</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. epidermidis (1)</em></td>
<td>proximal</td>
<td>medial</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. epidermidis (2)</em></td>
<td>proximal</td>
<td>proximal</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
MICROBIOLOGY RESULTS

The microbiology results are given in more detail below: The preliminary (pre-identification) results obtained are shown in Table 3.38 (page 145).

TIP CULTURE

Seventy two catheters were inserted in 50 patients over a six month period. Of these 72 catheters a total of 59 catheter tips were sent to the clinical laboratory for culture. Microbial growth of 5 or more colony forming units (a positive tip culture) on roll-plating was obtained from 20 tips. Growth was heavy (>100 colonies) on 16, moderate (>15 colonies) on 3 and scanty (>5<15 colonies) on 1. In all cases of catheter-related bacteraemia tips positive by roll-plating exhibited heavy growth. On 3 occasions, tips exhibiting no growth by roll-plating exhibited scanty growth on broth culture, 1 S. epidermidis and 2 S. warneri. On a further occasion, moderate growth of an additional organism to that found on roll plating was found on broth culture (S. hominis). In three of these four cases, the organism was also found in the three-way tap and/or catheter hub, and in one instance on blood culture suggesting these represented genuine colonisation of the internal surface only. This gave significant growth on a total of 23 tips (39%). Thirty six isolates of predominately Gram-positive cocci were isolated from the 23 positive catheter tips. Single isolates were obtained from 12 tips, two isolates from 9 tips and three isolates from 2 tips.

BACTERAEMIA

Gram-negative bacteraemias where no similar organisms were isolated from the catheter tip, hub/tap or insertion site are not reported. There were 5 instances (5 patients) of microbiologically documented bacteraemia secondary to the central venous catheter in which a concurrent blood culture grew the same organism as the catheter tip (8.5%). In a further 2 instances (1 additional patient) organisms isolated from blood culture (S. hominis and S. haemolyticus) were identical to isolates from the catheter lumen and three-way tap but no organisms were recovered from the catheter tip. These meet the definition of Maki et al. (1986 ) of hub related bacteraemia. In a further 1 instance (1 patient) S. haemolyticus was isolated from the blood but identical isolates were not
recovered from any other source. This meets the definition of Raad (1994) of probable-catheter related bacteraemia, or primary nosocomial bloodstream infection.

**HUB/THREE-WAY TAP CULTURE**

Of the 59 catheters for which the tip was sent for culture, 47 catheters (80%) were saved for the study, from which 24 isolates were obtained. Nineteen triple-lumen, 20 single-lumen catheters and 8 Nutricaths were received giving a total of 85 hubs sampled, of which 18 exhibited bacterial growth (21%). Seventy three-way taps were attached of which 18 exhibited bacterial growth (26%). A further catheter and attached three-way taps was saved but the tip was contaminated on removal.

**INSERTION SITE CULTURE**

Eleven insertion site swabs were taken prior to dressing change from which 9 isolates were obtained. Insertion site swabs were received from 37 of the 59 catheters (63%) on removal, from which 37 isolates were obtained. Those missed were generally short-term catheters.

**COMPLETE CULTURES**

Of the 23 tips exhibiting bacterial growth, the complete set of microbiological data was available for 18 (78%). In 2 instances the catheter was not saved, on two occasions a swab wasn’t taken, and in 1 instance neither was obtained. As the study as entirely reliant on goodwill, this represented excellent co-operation by Ward staff.

**IDENTIFICATION OF ISOLATES**

All organisms present on the catheter tip were speciated, together with organisms of similar morphology or presumptive species present either at the insertion site or within the catheter hub/three-way tap. Six isolates were Gram-negative (GNB). A total of 102 isolates associated with a positive catheter tip were Gram-positive cocci (GPC) of which twenty were Enterococci.
<table>
<thead>
<tr>
<th>PATIENT</th>
<th>TAP (S)</th>
<th>LUMEN(S)</th>
<th>INSERTION SITE</th>
<th>TIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>2</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>3</td>
<td>GPC</td>
<td>NG</td>
<td>GPC x 2</td>
<td>GPC x 2</td>
</tr>
<tr>
<td>4</td>
<td>NG</td>
<td>NG</td>
<td>GPC x 2</td>
<td>NG</td>
</tr>
<tr>
<td>5</td>
<td>NG</td>
<td>NG</td>
<td>NONE</td>
<td>NG</td>
</tr>
<tr>
<td>6</td>
<td>GPC x 6</td>
<td>GPC x 4</td>
<td>GNB x 2, GPC</td>
<td>GNB, GPC</td>
</tr>
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<td>8</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
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<td>9</td>
<td>Diphtheroids</td>
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<td>GPC x 3</td>
<td>GPC x 3</td>
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<td>12B</td>
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<td>12C</td>
<td>NG</td>
<td>NG</td>
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<td>yeast</td>
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</tr>
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<td>42</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>44A</td>
<td>NOT USED</td>
<td>GPC x 2</td>
<td>GPC x 2</td>
<td>GPC</td>
</tr>
<tr>
<td>44B</td>
<td>NONE</td>
<td>NONE</td>
<td>NONE</td>
<td>NG</td>
</tr>
<tr>
<td>44C</td>
<td>NONE</td>
<td>NONE</td>
<td>NONE</td>
<td>NG</td>
</tr>
<tr>
<td>44D</td>
<td>GPC x 2</td>
<td>GPC x 2</td>
<td>GPC x 2</td>
<td>GPC x 2</td>
</tr>
<tr>
<td>45</td>
<td>GPC</td>
<td>GPC</td>
<td>NG</td>
<td>GPC</td>
</tr>
<tr>
<td>46A</td>
<td>NONE</td>
<td>NONE</td>
<td>NONE</td>
<td>NG</td>
</tr>
<tr>
<td>46B</td>
<td>NOT USED</td>
<td>NG</td>
<td>GPC</td>
<td>GPC x 2</td>
</tr>
<tr>
<td>46C</td>
<td>NG</td>
<td>NG</td>
<td>GPC</td>
<td>GPC</td>
</tr>
<tr>
<td>47B</td>
<td>GPC x 2</td>
<td>GPC x 2</td>
<td>GNB</td>
<td>GPC</td>
</tr>
<tr>
<td>48</td>
<td>NONE</td>
<td>NONE</td>
<td>NONE</td>
<td>NG</td>
</tr>
<tr>
<td>49</td>
<td>GPC x 2</td>
<td>NG</td>
<td>GPC</td>
<td>GPC</td>
</tr>
<tr>
<td>50</td>
<td>GPC x 2</td>
<td>GPC x 2</td>
<td>NG</td>
<td>NG</td>
</tr>
</tbody>
</table>
Eighty Gram-positive, catalase-positive, coagulase-negative isolates were speciated using API-Staph. Of these a significant proportion did not have a profile number corresponding to a species in the API analytical profile index (1987 edition), or were of low discrimination as detailed below. In view of this, and the resulting uncertainty as to the reliability and validity of the method for typing isolates, the Staphylococcal Reference Laboratory at Colindale was visited for advice, and kindly offered to 'phage type the isolates. As a first step, this involved re-biotyping the isolates at Colindale using a different method (Schleiffer and Kloos classification with Baird-Parker sugars - Dr. Richardson, personal communication). This enabled the two sets of results to be compared, to assess the reliability and validity of the original micromethod:

API Staph micromethod

This was performed as detailed on page 112.

Staphylococcal reference laboratory (SRL) methodology (Marples et al. 1978).

Two successive subcultures from a mixture of several colonies of the original sample were made on nutrient agar and incubated at 30°C for 2.5 days and 1 day. A tube of nutrient broth was inoculated from the second subculture, incubated at 30°C for 1 day and used as the inoculum for the biochemical tests: liquid media were inoculated with 2-3 drops of culture, onto solid media by multipoint inoculator to give a volume of 0.1ml.

All strains were tested for their ability to produce acid within 7 days at 30°C from arabinose, fructose, lactose, maltose, mannitol, ribose, sucrose, trehalose, xylitol and xylose (0.05% w/v in Baird-Parker’s basal medium) and from glucose, aerobically and anaerobically. Solid media were used for the detection of phosphatase and DNAse, and liquid media for reduction of nitrate and production of acetoin and coagulase.

Bacteriophage typing

This was performed by Colindale using the methods and 'phages as described by Dean et al. (1973).
COMPARISON OF RESULTS

To assess the significance of differences in test results between the two methods, the essential and usual features of the classification schemes of Schleifer and Kloos, which forms the basis for the API test, and the scheme of Baird-Parker, as reported by the reference laboratory, were determined (Baird-Parker 1963, Kloos 1990) as follows:

Baird-Parker

This scheme was modified by Marples (1981), identifying variable characters used for defining biotypes within the species:

SI  coagulase-positive

SII  essential:  phosphatase and acetoin positive, mannitol negative
other:  glycerol and maltose positive
usual:  galactose and lactose positive
(Marples: xylose and trehalose negative, phosphatase and lactose variable)

SIII  essential:  phosphatase positive, acetoin, maltose and mannitol negative
other:  glycerol positive
usual:  galactose and lactose positive
(Marples: xylose negative, maltose and mannitol variable)

SIV  essential:  phosphatase, lactose and mannitol negative, acetoin positive
other:  glycerol positive
(Marples: maltose and trehalose positive, xylose negative, fructose, nitrate, arginine and urea variable)

SV  essential:  phosphatase and mannitol negative, acetoin, lactose and maltose positive
other:  glycerol positive
(Marples: trehalose positive, xylose negative, fructose, urea and arginine variable)

SVI  essential:  phosphatase negative, acetoin and mannitol positive
other:  Group One: lactose and maltose positive
Group Two: lactose negative, maltose positive
Group Three: lactose and maltose negative.
(Marples: SVI(e)- maltose positive, trehalose and xylose negative, lactose variable.  SVI(h)- trehalose positive, xylose negative, lactose, maltose, fructose, sucrose, nitrate and urea variable).
Schleiffer and Kloos

*S. epidermidis:*
- **essential:** phosphatase positive, trehalose negative
- **usual:** urease, mannose, maltose and sucrose positive, arginine negative

*S. haemolyticus:*
- **essential:** mannose negative
- **usual:** trehalose, maltose and sucrose positive, phosphatase and urease negative

*S. hominis:*
- **essential:** mannose negative
- **usual:** urease, maltose and sucrose positive, phosphatase and mannitol negative

*S. warneri:*
- **essential:** mannose negative
- **usual:** urease, maltose, sucrose and trehalose positive, phosphatase negative

*S. capitis:*
- **essential:** mannitol positive, trehalose and maltose negative
- **usual:** mannose and sucrose positive, phosphatase, urease and lactose negative.

*S. simulans:*
- **essential:** mannitol and maltose positive
- **usual:** urease and sucrose positive

*S. saprophyticus:*
- **essential:** phosphatase and mannose negative
- **usual:** urease and sucrose positive.

Comparison of results differing between the API and reference laboratory results are tabulated for specific examples in the following sections, where the essential and usual characters of the proposed species are highlighted. In addition, the percentage of strains expected to exhibit that characteristic are indicated, as calculated by Schleiffer and Kloos (1991) and Bergey’s Manual of Determinative Bacteriology (1994). Some tests (centre sections) were common between API and the reference laboratory, others were specific to each method. Only tests which possessed some differentiating ability are recorded.
**S. epidermidis**

This was the most frequent isolate, of which 42 (95%) were correctly identified as *S. epidermidis* by API, and 2 were incorrect. Both of these had a high percentage probability by API classification. As shown in Figure 3.36, the difference was in phosphatase production in two instances and acid production from maltose in one instance. In both cases the strain was only present at one site, therefore the difference was not of significance.

**FIGURE 3.36: DIFFERENCES IN CLASSIFICATION: S. EPIDERMIDIS AND S. CAPITIS**

<table>
<thead>
<tr>
<th>Patient 14 (1 strain)</th>
<th>Classification API</th>
<th>S. epidermidis 98.9%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference lab.</td>
<td>S. capitis</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient 15 (1 strain)</th>
<th>Classification API</th>
<th>S. capitis 94.1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference lab.</td>
<td>S. epidermidis</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substrate/Product (left to right) = ribose, deoxyribonuclease, acetylmethylcarbinol, phosphatase, nitrate, mannitol, lactose, maltose, fructose, trehalose, mannose, N-acetyl glucosamine, arginine dihydrolase, urease</th>
</tr>
</thead>
<tbody>
<tr>
<td>epidermidis*</td>
</tr>
<tr>
<td>+/-</td>
</tr>
<tr>
<td>-w</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+w</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>+/-</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>(+)</td>
</tr>
<tr>
<td>+w</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>API capitis</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>-</td>
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<td>+</td>
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<tr>
<td>+</td>
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<td>+</td>
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<tr>
<td>-</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>Reference lab.</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
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<td>-</td>
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<td>-</td>
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<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>capitis*</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>w</td>
</tr>
<tr>
<td>+/-</td>
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<tr>
<td>+</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+/-</td>
</tr>
<tr>
<td>-</td>
</tr>
</tbody>
</table>

*Bergey’s manual of determinative bacteriology (1994)*

Schleifer and Kloos (1991)

usual character

Baird-Parker (1963), Kloos (1990)

essential character

+ = > 90%  +/-  11-89%  - < 10%  +, - = + or - (strains differ)

w or ± = weak  -w = negative to weak  +w = weak to positive

result differs from species definition

result differs from reference laboratory

**S. hominis and S. warneri** (figure 3.37)

*S. hominis* or *S. warneri* were isolated from 9 catheters (20 isolates). Five isolates from two catheters were correctly identified (25%) In six cases (three catheters) the two species were confused with each other: Of six instances of *S. hominis* reclassified
as *S. warneri*, 5 differed in the phosphatase reaction, and one was phosphatase positive by both methods, phosphatase negativity being an essential characteristic of both *S. hominis* and *S. warneri*. A further 4 isolates (1 catheter) were classified as *S. haemolyticus*: In the case of all four, all tests were in agreement by both methods, and the strain was atypical (fructose negative). Three isolates of *S. hominis* (one catheter) were identified as *S. epidermidis*, differing in phosphatase and trehalose reactions. Two isolates (two catheters) were not in the API index: in both cases all tests were in agreement by both methods and the strains were atypical. In all cases of multiple strains from a single catheter or patient, the isolates were consistently mis-classified and had identical API profiles, and therefore the accuracy in discriminating strains from different sources was not affected by the actual species name given.

**FIGURE 3.37: DIFFERENCES IN CLASSIFICATION: S. HOMINIS and S. WARNERI**

**Patient 10 (4 strains): Classification API**  *S. haemolyticus* (2) 92.3%

**Reference lab. SV - S. hominis**

<table>
<thead>
<tr>
<th>RIB</th>
<th>DNA</th>
<th>VP</th>
<th>PASE</th>
<th>NIT</th>
<th>MAN</th>
<th>LAC</th>
<th>MAL</th>
<th>FRU</th>
<th>TRE</th>
<th>MSE</th>
<th>NAG</th>
<th>ADH</th>
<th>URE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>haemolyticus</strong></td>
<td>+/-</td>
<td>+w</td>
<td>+/-</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/+</td>
<td>+/+</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>API strip</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>reference lab</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>hominis</strong></td>
<td>-</td>
<td>-w</td>
<td>+/-</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/+</td>
<td>+/+</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Patient 45 (4 strains): Classification API**  *S. hominis* (2) 65% and

**Patient 11 (1 strain)**:  *S. hominis* (2) 71.2%

**Reference lab. S. warneri SVI 1/2**

<table>
<thead>
<tr>
<th>RIB</th>
<th>DNA</th>
<th>VP</th>
<th>PASE</th>
<th>NIT</th>
<th>MAN</th>
<th>LAC</th>
<th>MAL</th>
<th>FRU</th>
<th>TRE</th>
<th>MSE</th>
<th>NAG</th>
<th>ADH</th>
<th>URE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>hominis</strong></td>
<td>-</td>
<td>-w</td>
<td>+/-</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/+</td>
<td>+/+</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>API 65%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>reference lab</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

| **warneri** | +/- | +w | - | +/- | +/- | +/- | +/+ | +/+ | +/- | + | + | + |

Key see figure 3.36 page 150
Patient 16 (1 strain): Classification API - none, not in index

Patient 15 (1 strain): S. warneri 80.5%
Reference lab. S. warneri SVI 2

<table>
<thead>
<tr>
<th>RIB</th>
<th>DNA</th>
<th>VP</th>
<th>PASE</th>
<th>NIT</th>
<th>MAN</th>
<th>LAC</th>
<th>MAL</th>
<th>FRU</th>
<th>TRE</th>
<th>MSE</th>
<th>NAG</th>
<th>ADH</th>
<th>URE</th>
</tr>
</thead>
<tbody>
<tr>
<td>warneri</td>
<td>+/-</td>
<td>+/</td>
<td>-</td>
<td>-w</td>
<td>+/-</td>
<td>+/</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>+/</td>
<td>+</td>
</tr>
<tr>
<td>not in index</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

| API 80.5% | + | - | - | + | - | + | - | - | ± | + |
| Reference lab | + | - | + | ± | + | - | + | + | - | + |

Patient 6 (1 strain): Classification API S. epidermidis 98.6%
Reference lab. SV- S. hominis

<table>
<thead>
<tr>
<th>RIB</th>
<th>DNA</th>
<th>VP</th>
<th>PASE</th>
<th>NIT</th>
<th>MAN</th>
<th>LAC</th>
<th>MAL</th>
<th>FRU</th>
<th>TRE</th>
<th>MSE</th>
<th>NAG</th>
<th>ADH</th>
<th>URE</th>
</tr>
</thead>
<tbody>
<tr>
<td>epidermidis</td>
<td>+/-</td>
<td>-w</td>
<td>+</td>
<td>+</td>
<td>+w</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>(+)</td>
<td>+w</td>
<td>+</td>
</tr>
<tr>
<td>API strip</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Reference lab</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

S. haemolyticus (figure 3.38)

There were 16 isolates of S. haemolyticus, (10 catheters) of which 7 isolates (5 catheters) were correctly identified (44%). In 3 instances (3 catheters), profiles not in the index were found by the reference laboratory to be mixed cultures of S. haemolyticus and E. faecalis, both of which produced translucent colonies with a zone of haemolysis on blood agar plates. Three isolates (one catheter) were misclassified by the API system as S. simulans, albeit with a very low probability. All tests were in agreement with the reference laboratory. Two isolates were misclassified as S. saprophyticus, again with a low probability. The only difference from the reference laboratory was nitrate production which was not an essential or usual character for either species. Again, all misclassifications were consistent within an individual catheter/patient and did not effect the results.
Further observations

The problem of *E. faecalis* present in mixed culture has been noted. In addition, two cultures of *S. epidermidis* were found to consist of 2 strains. Weidenfeller and Fegeler (1990) comparing API-Staph with their own micromethod noted that frequently non-coded numbers indicated unnoticed mixed cultures, and introduced a cellobiose test to detect hidden contamination with Enterococci which were often part of mixed culture with staphylococci from their clinical material.

One organism identified as *S. auricularis* was found to be a yeast, and one isolate with a profile not in the index was a Micrococcus sp.

Typing of other isolates

Only one Gram-negative organism was isolated from the catheter tip, with the same organism by API profile and antibiogram present at the insertion site only. Enterococci detected in mixed culture by the staphylococcal reference laboratory were passed on to the streptococcal reference laboratory where they were serotyped and phage typed. Further isolates were sent directly by the author to enable comparison to be made.
The problems of nomenclature are illustrated in table 3.39:

**TABLE 3.39: BIOTYPES BY API STAPH (where necessary, original profile numbers have been altered to reflect phosphatase and sugar reactions found different by the reference laboratory(SRL))**

<table>
<thead>
<tr>
<th>API NUMBER</th>
<th>ISOLATES</th>
<th>CATHETERS</th>
<th>SPECIES(SRL)</th>
<th>API</th>
</tr>
</thead>
<tbody>
<tr>
<td>2234113</td>
<td>1</td>
<td>1</td>
<td>warneri</td>
<td>not in index</td>
</tr>
<tr>
<td>6220113</td>
<td>3</td>
<td>1</td>
<td>warneri</td>
<td>85%</td>
</tr>
<tr>
<td>6232111</td>
<td>5</td>
<td>2</td>
<td>warneri</td>
<td>haemolyticus 46.3%</td>
</tr>
<tr>
<td>6232153</td>
<td>1</td>
<td>1</td>
<td>warneri</td>
<td>saprophyticus 32.7%</td>
</tr>
<tr>
<td>6236013</td>
<td>1</td>
<td>1</td>
<td>warneri</td>
<td>62%</td>
</tr>
<tr>
<td>2612152</td>
<td>4</td>
<td>1</td>
<td>hominis</td>
<td>haemolyticus 92.3%</td>
</tr>
<tr>
<td>6612152</td>
<td>2</td>
<td>1</td>
<td>hominis</td>
<td>60.7%</td>
</tr>
<tr>
<td>6712150</td>
<td>3</td>
<td>1</td>
<td>hominis</td>
<td>79.8%</td>
</tr>
<tr>
<td>6122113</td>
<td>1</td>
<td>1</td>
<td>capitis</td>
<td>98.3%</td>
</tr>
<tr>
<td>6206110</td>
<td>1</td>
<td>1</td>
<td>epidermidis</td>
<td>91.1%</td>
</tr>
<tr>
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<td>4</td>
<td>2</td>
<td>epidermidis</td>
<td>93.1%</td>
</tr>
<tr>
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<td>1</td>
<td>epidermidis</td>
<td>85.3%</td>
</tr>
<tr>
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<td>2</td>
<td>epidermidis</td>
<td>99.6%</td>
</tr>
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<td>6</td>
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<td>epidermidis</td>
<td>97.6%</td>
</tr>
<tr>
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<td>5</td>
<td>4</td>
<td>epidermidis</td>
<td>97.6%</td>
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KEY: e=epidermidis, he=haemolyticus, ho=hominis, c=capitis, w=warneri
Identical isolates on tip and skin/hub/tap
Identical isolates skin and hub/tap only
Antibiotic sensitivity

Due to uncertainty over the reliability of the speciation at that stage, all isolates associated with a positive catheter tip were antibiogrammed by ATB Staph as a confirmatory measure. Antibiograms were also performed by the reference laboratory using the following: penicillin G, methicillin, streptomycin, neomycin, gentamicin, tetracycline, chloramphenicol, erythromycin, lincomycin, novobiocin, fucidin, trimethoprim, tobramycin, kanamycin, and ciprofloxacin.

Differentiation of isolates from different sites: Differences between methods

API Staph recognised 5 biotypes of *S. warneri*, 3 of *S. hominis*, 14 of *S. epidermidis* and 6 of *S. haemolyticus* as shown in Table 3.41. In contrast, the reference laboratory recognised only 5 biotypes of *S. warneri*, 3 of *S. hominis*, 9 of *S. epidermidis*, and 3 of *S. haemolyticus*, however their antibiograms (selected for discrimination in epidemiological studies) were more sensitive, with 21 different patterns recognised for *S. epidermidis* compared to 10 by ATB Staph. In general, once the two methods were combined at each site the results were confirmatory in the majority of instances and complementary on occasion. From Table 3.40 (page 155);

Catheter 6: 2 isolates of *S. epidermidis* from skin and catheter lumen (6706152 and 6606152) differed only by the mannose reaction by API which was not tested by the reference laboratory. The strains were identical by antibiogram at both sites and not phage typable. As neither was present on the catheter tip they were not tested further.

Catheter 11: isolates present in the three-way tap, blood and on the catheter tip possessed the same biotype at the reference laboratory but slightly different API numbers (6606112 vs 6606113). This difference was confirmed to be valid by differences in antibiograms at both sites.

Catheter 17B: two isolates of *S. haemolyticus* in skin and blood samples, and isolates from the catheter tip and three-way tap of catheter 49, with the same API numbers differed in ribose production at the reference laboratory (not tested by the API strip). The isolates were different by antibiogram at both sites.

Catheter 25: Isolates on the skin and catheter tip, and catheter 44D, skin and catheter lumen possessed the same API number, and identical biotypes at the reference laboratory, however they were different by antibiogram at both sites.
TABLE 3.41: COMPARISON OF TYPING METHODS

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c = contaminated  *=not done  NT= not typable. Agents (left to right) acetylmethylcarbinol, phosphatase, deoxyribonuclease, nitrate, mannitol, lactose, maltose, fructose, sucrose, trehalose, ribose, penicillin G, methicillin, trimethoprim, gentamicin, kanamycin/tobramycin, erythromycin, lincomycin, tetracycline, neomycin, streptomycin, chloramphenicol, ciprofloxacin.
### S. haemolyticus BPVI(1)

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ANTIBIOTIC RESISTANCE

The antibiotic resistance of CNS isolates performed by Colindale are shown in table 3.42. Strains identical within the same patient or between patients have only been included once.

TABLE 3.42: ANTIBIOTIC RESISTANCE OF COAGULASE-NEGATIVE STAPHYLOCOCCI

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<th>haemolyticus</th>
<th>hominis</th>
<th>warneri</th>
<th>capitis</th>
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<td>SII+ (n=5)</td>
<td>SVepi (n=4)</td>
<td>Overall (n=26)</td>
<td>(n=9)</td>
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<td>Methicillin</td>
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<td>50%</td>
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<td>40%</td>
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<td>Kanamycin + Tobramycin</td>
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Coagulase-negative staphylococci isolated from tip culture were resistant to an average of seven antibiotics after a stay in ICU as opposed to three antibiotics in patients on the general ward (although the number of organisms is too small to be statistically significant).

The variation in antibiotic sensitivity between isolates within the same patient is shown in table 3.43.
TABLE 3.43: COMPARISON OF ANTIBIOTIC RESISTANCE OF ISOLATES WITHIN PATIENTS

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* Key as table 3.41.
DISCUSSION

AIM I: THE USE OF A TYPING SYSTEM IN DETERMINING THE EPIDEMIOLOGY OF CATHETER-RELATED INFECTION

i) RELIABILITY OF CLASSIFICATION

Comparison of the results of API-Staph with those of the Staphylococcal Reference Laboratory, demonstrated differences in the results of individual tests. This has been previously observed: Comparing API-Staph with conventional methods, Brun et al. (1978) examined ten type strains ten times: reproducibility ranged from 91-100%, the lowest being for mannitol. Marples and Richardson (1982) compared API Staph with both the methods of Baird-Parker and Kloos et al: The greatest number of variable results with the micromethod were seen in the warneri-hominis species group and the haemolyticus-capitis group as seen in this study. Reproducibility of individual tests were less than 95% for maltose, mannose, mannitol, N-acetyl-glucosaminine and arginine, and less than 90% for urea. 19.5% of results with the phosphatase test were discordant along with 10.8% tests for acetoin production. Compared to the conventional methods, false-positives were found for lactose (2.4%), mannose (8.6%), maltose (6%), phosphatase (12%) and acetoin (14.9%). False negatives were 6.6% for phosphatase and 11.4% for urea. The nitrate reduction test gave a number of false negative results due to reduction past nitrate having occurred. Late-positives (>24 hours) were seen for lactose (4.6%), mannose (5.4%), maltose (5.2%), mannitol (7.2%), arginine (7.6%) and urea (17.1%). The phophatase, urea and acetoin tests were considered to be unsatisfactory. Overall, an identification from the index supplied could be made in less than 30% of isolates.

In this study, the most significant problem of reliability observed was with the phosphatase test which gave a total of 17 false-positive results (20%) and 2 false-negative results (2.4%). This is in agreement with the findings of Marples and Richardson (1982). The higher rate of false positive results may be due to the fact that each discordant strain occurred and was tested at several sites. In several instances this resulted in mis-classification of organisms as illustrated above. There was one false negative nitrate reduction and one false positive acetoin reaction. Three results were
falsely positive for lactose production and one falsely negative for utilisation of mannitol. On retrospective review, these tests gave orange (as opposed to yellow or red) results after incubation and were inconsistently coded. All were however recorded on the record sheet as orange. Saccharose and N-acetyl-glucosamine tests frequently gave orange results, with some coded positive and some negative. As these tests were not performed by the reference laboratory, it is not possible to determine the accuracy of the coding. Lactose reactions were orange in 4 instances of which two were negative and two positive at the reference laboratory. In all 4 cases lactose production was equally difficult to determine on the C.L.E.D. plate. Three mannitol reactions gave orange results of which 2 were coded positive and one negative: all were in fact positive at the reference laboratory, although two only became positive on day six of incubation. All these findings are in concordance with those of Marples and Richardson. Their suggestion of reading the tests at both 24 and 48 hours may eliminate these problems of interpretation. Differences in test results did not however reduce the usefulness of the system as a typing method for determining the route of tip colonisation of an individual catheter, as isolates from all sites were processed together and where one gave an orange result due to insufficient incubation time for example, in all cases identical isolates gave the same results. Indeed, the visual appearance of the strip with its variations within the red and yellow results as well as the presence of orange, gave an immediate indication of the similarity or otherwise of isolates without the need to calculate an analytical profile. The typing method was therefore effective as an epidemiological tool for examining isolates within patients.

ii) VALIDITY OF CLASSIFICATION

Differences in speciation were observed between API-Staph and the reference method: Using the simplified scheme of Kloos and Schleifer (1975), Marsik and Brake (1982) found that exact identification of species was not always possible, and it was necessary to fit the results to the closest choice. Using blood isolates, Aldridge et al. (1983) found an 79% agreement with Staph-Ident (a 10 test strip). Baldellon and Megraud (1985) noted 86.4% of clinical strains were correctly identified. In a recently published study, of 217 isolates sent to the Centers for Disease Control reference laboratory, only 60% had been correctly identified by Staph-Ident (Rhoden and Miller
1995). In this study, in the majority of cases where species were reclassified by the reference laboratory, where parallel tests were performed they were found to be identical, differences resulting from additional tests performed, differences in the classification system used, and the superior ability of the reference laboratory over the API index in classifying atypical strains, and in assessing the importance of particular results. In terms of species identification, approximately one-third of isolates were mis-classified by the API Staph system when compared to the reference laboratory using a Baird-Parker based classification system. Even when the original profile numbers were altered in light of differing reactions at the reference laboratory, strains of *S. warneri*, *S. hominis* and *S. haemolyticus* remained incorrectly named, of low discrimination, or not in the index as shown in table 3.39, page 154. However, in terms of the validity of the system as an epidemiological typing method, no errors occurred in the differentiation of different strains as the mis-classifications were consistent within strains, with each isolate possessing the same profile number, albeit wrongly named on occasion.

In summary, in one catheter the API system was more discriminatory, and in two the reference system. In all these cases ATB Staph was sufficiently sensitive to differentiate the strains. Whilst useful as a confirmatory tool, the antibiogram was only required to distinguish isolates on three occasions. No isolates required phage typing, or were shown to be different on phage typing when identical by biotype and antibiogram. This was due to the fact that the method needed only to distinguish between isolates on a single catheter rather than between isolates on different catheters/patients. Fifteen of the 27 biotypes identified were only present on one catheter. Nevertheless, the necessity for a sensitive typing system is highlighted by the results: Of 28 isolates of coagulase-negative staphylococci on the catheter tips, only nine (32%) were demonstrated by biotype and antibiogram to be identical to isolates from another source: 4 from the skin, and 5 from the catheter lumen. Classified only as CNS as in other studies, only 22 separate isolates would have been noted on the catheter tip, of which CNS were present at another site in 15 (68%). By species only, there would thus have been six false-positives. This necessity for a discriminatory typing scheme is illustrated in figure 3.39 by the similarity on initial testing of isolates
from catheter 44D and catheter 49 where three different isolates of *S. epidermidis* are present at the three sites, as distinguished by biotyping and extended antibiograms.

**FIGURE 3.39: MULTIPLICITY OF ISOLATES OF *S. EPIDERMIDIS*: SIMILARITY ON INITIAL TESTING OF SIX DIFFERENT ISOLATES**

<table>
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<tr>
<th>NO</th>
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<th>CATHETER TIP</th>
</tr>
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<td><em>S. epidermidis</em></td>
<td><em>S. epidermidis</em></td>
</tr>
<tr>
<td></td>
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<td>® penicillin, oxacillin, gentamicin, co-trimoxazole, kanamycin, tobramycin</td>
<td>® penicillin, oxacillin, gentamicin, erythromycin, lincomycin, kanamycin, tobramycin</td>
</tr>
<tr>
<td>49</td>
<td><em>S. epidermidis</em></td>
<td><em>S. epidermidis</em></td>
<td><em>S. epidermidis</em></td>
</tr>
<tr>
<td></td>
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<td>® penicillin, methicillin, gentamicin, kanamycin, tobramycin</td>
<td>® penicillin, gentamicin, kanamycin, tobramycin</td>
</tr>
</tbody>
</table>

Cross-infection

Whilst it was not the aim of the study to determine the source of organisms found at the insertion site or within the catheter lumen, the comprehensiveness of the typing systems in conjunction with the reference laboratories enabled comparison of strains between patients. In particular, 11 of 12 isolates of *E. faecalis* sent to the Streptococcal reference laboratory were found to belong to serotype 9 (37% of all isolates) and phage type VII (less than 1% of isolates - personal communication, Dr. Morrison). On further examination, where an ATB Strep strip had been used, these isolates were found to have been resistant to 500 mg/l gentamicin. The original isolates typed by the reference laboratory and isolates from other patients not typed but exhibiting the same ATB resistance pattern were retrieved from storage and sent to the Antibiotic Reference Laboratory for testing where they were confirmed to be high-level gentamicin resistant (>2000 mg/l). Combining the rare phenotype with the low prevalence of gentamicin resistant enterococci within UK district general hospitals in 1991 (Grey and Pedlar 1992), it would appear that this was an epidemic strain transmitted from patient to patient.

From table 3.41 page 157, atypical isolates of *S. epidermidis* and *S. haemolyticus* isolated from different patients appear to suggest a common source. In order to assess the significance of these similarities, the antibiotic sensitivities have
been tabulated from left to right in decreasing order of their prevalence, as compiled from the findings of Marsik and Brakes (1982), Archer et al. (1984), Haslett et al. (1988), Dryden et al. (1992) and Martin De-Nicholas et al. (1995), where penicillin resistance was found to be the most frequent, and fucidin resistance the least. These similar strains are highlighted in table 3.41. These may be an underestimate as only strains identical by biotype, antibiogram and phage type, or considered indistinguishable by Colindale, have been considered similar. In practice, changes of biochemical reactions and antibiotic susceptibility have been noted to occur within the same strain over time due to loss or acquisition of plasmids or due to changing environmental conditions (Etiene et al. 1990), or to loss on subculture in the laboratory (Ludlam et al. 1989). Mickelsen et al. (1993) found 7 antibiotic profiles from 20 isolates of a single epidemic strain due to changes in erythromycin, clindamycin, gentamicin and chloramphenicol resistance, corresponding to loss of plasmids on plasmid profiling, and to varying expression of methicillin resistance. Dryden et al. (1992) found the antibiogram too discriminatory on occasion, when isolates were indistinguishable by all other means including whole-cell protein typing. Caution has therefore been suggested in assuming successive clinical isolates are not of significance due to typing differences (Vandenesch et al. 1993). There is unfortunately no consensus on the degree of difference required to signify a true difference in isolates. Furthermore, it is uncertain whether isolates phenotypically different represent different strains or clonal variants from the same parent (Deighton et al. 1992). Guidelines exist for comparison of phage types whereby a strong reaction vs no reaction in two isolates is considered to represent different strains (Blair and Williams 1961). In practice, only 46% of CNS in this study were typable: 34% of S. epidermidis were non-typable (43% SII, 50% SIIL- and 9% SVepi), with 60% S. haemolyticus, 88% S. warneri and 100% S. hominis and S. capitis non phage-typable. New techniques of comparing chromosomal DNA in studies of CNS epidemiology conducted since this study may resolve this issue (Heubner et al. 1994, Degener et al. 1994).

The isolation of indistinguishable Gram-positive isolates from separate sources is illustrated in figure 3.40.
### FIGURE 3.40: ILLUSTRATION OF POSSIBLE CROSS-INFECTION OF GRAM-POSITIVE ISOLATES: ISOLATION OF INDISTINGUISHABLE ISOLATES FROM DIFFERENT PATIENTS

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### iii) EPIDEMIOLOGY OF INFECTION

**SOURCE OF COLONISING COAGULASE-NEGATIVE STAPHYLOCOCCI**

The results of this study add to the little information available on the epidemiology of CNS infection, and whether the source is endogenous or exogenous. The epidemiology of tip colonisation was shown to be extremely complex, with up to three species of CNS and three different strains of both *S. epidermidis* and *S. haemolyticus* present at the catheter insertion site, hub or tip within a single patient with multiple catheters. From the results of patients with more than one catheter, and
from the demonstration of cross infection of *E. faecalis* and CNS between patients, it would appear from this study that both endogenous and exogenous strains resulted in tip colonisation, and in addition, those strains acquired during ICU admission which became part of the patients bacterial flora. Overall, five species of CNS were recovered. The majority of isolates were multiply antibiotic resistant, particularly within the ICU. These results are in accordance with previous studies:

From surveillance cultures of nares, throat, skin, rectum and urine in a haematology unit, Herwaldt *et al.* (1992) determined that patients were colonised by a median of 5 unique strains of CNS. The species distribution was 82.9% *S. epidermidis*, 6.4% *S. haemolyticus*, 3.9% *S. warneri*, 3.1% *S. hominis* and 1.4% *S. capitis*. In an experimental study, Brown, Wenzel and Hendley (1989) found that whereas several different strains could be found at the skin surface, one or two strains were found repeatedly in the underlying stratum corneum, suggesting these were the resident flora, with strains on the skin surface derived from several sources. In examining the epidemiology of CNS infection in patients undergoing continuous ambulatory peritoneal dialysis, Ludlam *et al.* (1989) demonstrated that infecting strains could be demonstrated on patients upto 12 weeks before clinical infection. The source of these strains is not known, but may represent organisms in numbers too low to be previously detected, organisms from an unscreened site, or organisms acquired by cross-infection (Dryden *et al.* 1992):Menzies *et al.* (1991) found no evidence that strains present in low numbers on admission emerged following antibiotic prophylaxis, with all nosocomial strains found on more than one patient or from a patient and the environment. Archer and Armstrong (1983) found 1 of 26 patients with *S. epidermidis* resistant to both methicillin and gentamicin on admission, rising to 80% ten days after surgery, suggesting nosocomial acquisition. Furthermore, strains were identical to those carried by the nursing staff. Hedin and Hambraeus (1991) examined the distribution of ciprofloxacin and/or gentamicin resistant strains over a one week period in a bone marrow transplant unit: Twenty-three percent of staff were colonised, but 82% of staff clothes were contaminated. Two strains were widely dispersed in the air, and found in the corridor and in every isolation room. More recently, environmental cross-colonization was implicated in transmission of ciprofloxacin resistant strains by air-borne spread in a renal clinic. Staff were not
colonised. This is in agreement with the findings of Oppenheim et al. (1989) in a haematology unit. Cross-infection by *S. haemolyticus* and *S. simulans* in a surgical ICU has been documented (Perl et al. 1989).

As reviewed by Birnbaum, Keely and Chow (1991), less than 10% of endemic CNS infections may be explained by transmission between patients, however nosocomial transmission of CNS has been increasingly recognised: (Heubner et al. 1994) Van-den Broek (1985) and Boyce et al. (1990) demonstrated common source outbreaks of *S. epidermidis* wound infection and endocarditis linked to carriage by a cardiac surgeon. Using pulsed-field gel electrophoresis of digested whole chromosomal DNA from blood culture isolates, Heubner.(1994) showed that distinct clones of *S. epidermidis* were endemic in a neonatal ICU persisting for a ten year period, and could be isolated from hands of staff. An outbreak strain of *S.epidermidis* over a two year period, (Carlos et al. 1991) and persistence of an endemic strain of *S. haemolyticus* over a five year period (Low et al. 1992) have previously been demonstrated in the NICU.

**TIP COLONISATION**

The most common isolates on catheter tips and in catheter-associated septicaemia, as reviewed in 1979, were “*S. epidermidis*, *S. aureus*, *Klebsiella sp*, *Enterobacter sp*, *Serratia sp*. and enterococci, all of which were noted to be ubiquitous on the skin of hospitalised patients. From 203 clinical isolates of CNS, Oren and Merzbach (1990) found no significant difference in the overall distribution of species and their distribution by source: 65% of isolates on catheter tips were *S. epidermidis*, 23% *S. haemolyticus*, 7.5% *S. capitis* and 4% *S. hominis*. With the exception of *S. capitis*, these results are identical to those found in this study. From 502 central lines in ICU patients, Haslett et al. (1988) found 40% CNS (21% in combination with other organisms), 13% *E. faecalis*, 11% *Klebsiella*, 6.5% *Pseudomonas aeruginosa*, 5% *S. aureus*, and 5% *Acinetobacter calcoaceticus* plus small numbers of ten further species on catheter tips. Whilst most colonising organisms occurred singly, most infecting organisms were found in combination. Gram-negative organisms were only found as infecting organisms. The results of this study support these findings, the most common isolates being coagulase-negative.
staphylococci, enterococci and Klebsiellae. Two or more isolates were found on the majority of colonised tips. Significant colonisation of prosthetic devices by more than one strain of CNS simultaneously was recognised by Holt (1969), examining ventriculo-atrial shunts of children with persistent bacteraemia. In addition, in some cases, successive recolonisation of replaced shunts was caused by different strains. This study demonstrates that subsequent central venous catheters were frequently colonised by a different species/strain.

iv) ANTIBIOTIC SUSCEPTIBILITY

Marsik and Brake (1982) found clinical isolates of S. epidermidis resistant to a wider range of antibiotic agents than other CNS. In contrast, Gill et al. (1983) and more recently, Froggart et al. (1989) found nosocomial isolates of S. haemolyticus more resistant than other CNS, although they appeared to share a common resistance gene pool with S. epidermidis. This is confirmed by the results of this study demonstrating higher resistance in S. haemolyticus. In vitro, transfer of gentamicin resistance plasmids has been shown to occur from S. epidermidis, S. haemolyticus and S. hominis to S. capitis, S. epidermidis, S. haemolyticus, S. hominis, S. warneri and S. simulans with ease (Noble and Naidoo 1986). Archer (1988) observes that S. epidermidis and S. haemolyticus are the two species found predominately to be multiresistant (resistant to methicillin plus three classes of unrelated antibiotics), and account for 60-80% and 10-20% of all clinical isolates respectively. Menzies et al. (1991) found that oxacillin, gentamicin, kanamycin and tobramycin resistance signified nosocomial strains of S. epidermidis. Hospital strains of S. epidermidis highly resistant to antibiotics have been proposed to be spread by cross-infection (Hedin and Hambreus 1991). The results of this study would support that assumption. Marples et al. (1978) found antibiotic resistance low in S. epidermidis isolates among nurses in the operating theatre, and senior medical staff with a median of 1 resistance mechanism, but high in ICU nurses, with a median of 4 separate resistance mechanisms. John et al. (1989) found a significantly higher carriage of methicillin-resistant S. epidermidis in nursing staff in ICU than on the general wards, and significantly higher than support staff.
ENTEROCOCCI

Cross infection with a high-level gentamicin resistant (HLGR) strain of *E. faecalis* was demonstrated. *S. faecalis* and *S. faecium* were separated from the Streptococci and redesignated Enterococci in 1984 (Schleifer 1984). Nineteen species are currently recognised: *E. faecalis, E. faecium, E. avium, E. casseliflavus, E. cecorum, E. columbae, E. dispar, E. durans, E. flavescens, E. gallinarum, E. hirae, E. malodoratus, E. mundtii, E. pseudoavium, E. raffinosus, E. saccharolyticus, E. seriolicida, E. solitarius* and *E. sulfureus*, of which *E. faecalis* accounts for 80-90% of clinical isolates. (Kaufhold and Ferrieri 1993) The first Enterococcal isolates highly resistant to gentamicin (HLGR) were reported in 1979 in France (Horodniceanu et al.).

Risk factors for colonisation/infection with Enterococci include previous antimicrobial therapy, increasing duration of hospitalisation, severe underlying disease and invasive procedures (Korten and Murray 1993), and for HLGR *E. faecalis*, treatment with cephalosporins or aminoglycosides, and previous surgery, intravenous or urinary catheterisation and need for specialised nursing care (Zervos et al. 1986, 1987). The spread of a highly gentamicin resistant strain of *E. faecalis* between patients in adjacent beds in an Intensive Care Unit has been demonstrated by Zervos et al. (1987). The strain was also identified on the hands of personnel and on environmental surfaces. Hussain *et al.* (1988) documented an outbreak of *E. faecalis* bacteraemia occurring in a Critical Care Centre over a 1 month period.

In this study, all four patients with the HLGR strain were in the Intensive care unit following surgery over a 4 month period, two concurrently. All received cefotaxime and three out of four gentamicin. Occurring in 1991, this represents one of the first UK outbreaks of a HLGR strain documented in a District General Hospital, and causing cross-infection of central venous catheters.

**AIM II: THE EFFECT OF SITE CARE AND CATHETER USAGE ON GROWTH AT THE INSERTION SITE AND CATHETER LUMEN, AND RELATIONSHIP TO DURATION OF CATHETERISATION**

*i) SKIN COLONISATION*

This study noted a high level of skin colonisation from dressing change and catheter removal swabs. In 1994, Moro *et al.* demonstrated a “complex causal model”
of skin colonisation where four strongly interacting factors were responsible: increasing age of the patient, use of a transparent dressing, the jugular insertion site and increasing duration of catheterisation. This model was earlier demonstrated by the results of this study, which showed an increase in skin colonisation to a high level, with increasing duration of catheterisation (figure 3.21, page 124) using only transparent dressings in patients who were predominately elderly, and with jugular catheters. Several reasons for these findings are suggested in the literature:

**Duration of catheterisation**

The highest risk of skin colonisation with a transparent dressing is duration of catheterisation (Maki and Will 1990, Moro et al. 1994). In a prospective study of 345 central venous catheters in ICU, heavy colonisation of the insertion site (>100cfu), local inflammation, difficulty in insertion and duration >4 days were strongly predictive of a positive tip culture (>15cfu), with heavy colonisation and duration >4 days predictive of bacteraemia. Reasons for this have been unclear:

It has been observed that as transparent dressings enable health care personnel to visualise the catheter insertion site without having to remove the dressing, they may remain in place as long as the catheter itself (Richet et al. 1990). This practice, which was observed in the present study, has been demonstrated to be significantly associated with catheter-related infection (Andersen et al. 1986, Kelsey and Gosling 1984). Maki (1991) cites a trial of Tegaderm by his team on central venous catheters in high risk ICU patients, which observed a significant build up of skin flora associated with a 50% increase in catheter-related infection when left on for up to 7 days. These results are extended by this study to Opraflex and Op-site dressings: of sites redressed within 7 days, 50% of catheter tips exhibited growth compared to 100% where the insertion site was not redressed for 7 or more days (figure 3.30). Site colonisation and the likelihood of isolating an identical organism on tip culture were demonstrated to increase with increasing duration of catheterisation.

**Jugular insertion site**

Significantly heavier colonisation, with a greater prevalence of colonisation by *S. aureus*, Gram-negative bacilli or yeasts has been found at internal jugular compared
to subclavian sites (Maki 1982). In a retrospective study of TPN patients Kemp et al. (1994) found colonisation rates of triple lumen catheters of 5% subclavian, 17% internal jugular and 36% femoral (p<0.01).

**Insertion site care**

Bjornson et al. (1982) demonstrated that a threshold level of skin colonisation (of 1000cfu) was required before catheter colonisation occurred. Colonisation can be effectively suppressed by effective site care (Maki and Ringer 1987). For example, Jarrard et al. (1980) found that daily dressings with povidone iodine, antibiotic ointment and gauze eliminated all colonisation of subclavian insertion sites, with no positive skin cultures in 242 patient days. Effective site care was not performed in the present study. It was unclear from the retrospective survey whether there was a lack of site care or a lack of documentation. It was apparent from the prospective study that redressing of insertion sites was not regularly performed if the dressing was intact. It was unlikely that a dressing change was missed by the researcher because of daily recording of the appearance of the dressing, inconspicuous marking of the dressing to detect change, and the requirement, to obtain a dressing change, to book a patient into the CTR, transport them from the ward to CTR, and document the procedure performed on a treatment cardex accompanying the patient back to the ward.

Once skin colonisation had occurred, as demonstrated by a positive swab at dressing change, the same organism, or combination of organisms were re-isolated at the subsequent dressing change/catheter removal in 50% of cases, (table 3.24, page 123) suggesting that skin antisepsis was not effective. All sites were redressed with iodine or povidone iodine solution, or 0.9% sodium chloride followed by povidone iodine spray. The persistence of *S. epidermidis* on the skin despite careful cleaning with povidone-iodine solution has been demonstrated (Sitges-Serra 1980). From-surveillance site swabs, McGeer et al. (1987) and Snydman et al. (1982) found that-bacterial growth once established persisted until removal of the catheter (catheter care not stated). Snydman et al. observed a mean duration of skin carriage in an infected course of 11.8 days. In the present study, the isolation of *E. faecalis* from the insertion site was associated with tip colonisation in 75% cases vs 25% *S. epidermidis* or *S. haemolyticus*. 

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There have been very few reports examining the relative effectiveness of different antiseptic preparations for site care of central venous catheterisation. In a large trial by Maki and Ringer (1991) 2% aqueous chlorhexidine was significantly more effective than 10% povidone iodine or 70% alcohol in preventing local catheter-related infection and septicaemia. In a retrospective survey of disinfectants used for site care and tubing connections of Broviac catheters, Rannem et al. (1990) reported 0.58 cases per catheter year with povidone iodine, vs ≤0.28% with 0.5% alcoholic iodine or chlorhexidine. In consecutive studies of peripheral cannulae, spraying the insertion site with 0.5% chlorhexidine gluconate in 70% industrial methylated spirit reduced the incidence of skin and tip colonisation and bacteraemia (Macfarlane et al 1981). These findings support the observation in this study of a lower rate of tip colonisation in catheters inserted with alcoholic chlorhexidine skin preparation as opposed to povidone iodine (Table 3.21 page 121).

*ii) INTRALUMINAL COLONISATION*

Contamination of TPN solutions

Although only 18 bags of TPN were sampled in this study, 3 were found to exhibit bacterial growth (17%), and one contaminated bag resulted in clinical septicaemia, with a blood culture positive for an identical organism. Contamination rates of 0.2 to 38% have previously been observed: A rate of contamination of three-in-one bags of 0.2-4% was found by Bozzetti (1990) where of ten patients found to have (TPN) infusate contamination (over a four year period), six developed sepsis of which one died. Dolin et al. (1987) sampled 99 completed bags of 3-in-1 TPN solution: 4 bags (4%) were contaminated with low numbers of skin flora. Deeb and Natsios (1971) found microbial growth from in-use samples, in 38% (of 85) bottles of TPN (sterile after preparation) and 3.8% (of 236) bottles of other IV fluids. Armstrong et al. (1986) cultured fluid from administration sets and bottles of patients receiving TPN: 5 bottles and 3 administration sets (6.2%) were positive for organisms, including CNS, *Proteus mirabilis* and *Bacillus sp*.

In this study, the organisms in each contaminated TPN bag were of different species and unlikely to have originated from a common source, suggesting contamination at ward level. The high rate of contamination observed may be due to
the practice of adding vitamin, lipid and mineral supplements to the bag on the ward by the nursing staff, rather than in pharmacy under laminar flow.

**Contamination of three-way taps**

Three-way taps remained *in situ* for the duration of catheterisation unless faulty. On culture, twenty-five percent of three-way taps in this study exhibited microbial growth. Contamination rates of three-way taps/stopcocks of upto 48% have previously been reported (Walrath *et al* 1983). Reasons for this are suggested in a survey by Brosnan *et al.* (1988) of 363 nursing staff in the American Association of Critical Care Nurses. The nurses estimated that an average of 58% of the arterial line stopcocks and 46% PA stopcocks in their area were contaminated by touching, dropping or misplacing the side caps, leaving ports uncovered, touching side ports while turning the lever or whilst inserting the syringe.

**Colonisation of the catheter hub/lumen**

Use of a single-lumen TPN catheter for other purposes has long been associated with catheter-related infection (Snydman *et al* 1982, Ryan *et al*.1974). Whilst it has intuitively been assumed that the increase in the rate of infection seen with triple-lumen catheters is due potential for multiple manipulations (Clark Christoff *et al*. 1993) this has not previously been tested. Little information is documented on the rate or effect of disconnections of the triple-lumen catheter: A study by the Association of German hospital Pharmacies, showed an average surgical ICU patients had six IV administration systems in use per day, to/through which an additional 155 drugs were injected per day (= 26/set) (Illgen and Koetchel in Kirkpatrick 1988). Clark-Christoff *et al.* (1992) reported 15-20 instances of catheter access per day. This compares closely with the mean of 26 disconnections per day observed in ICU in this study suggesting that this was a typical sample.

In this study, 30% of catheters exhibited bacterial growth of the catheter hub. Similar results were obtained in the few previous studies around this area: Ullman *et al.* (1990) cultured fluid in each of the three lumens of triple-lumen catheters daily in ICU patients over a six month period. 28/93 lumens exhibited bacterial or fungal growth and catheter colonisation preceded sepsis in 3 of 4 patients who became
bacteraemic. Unfortunately site cultures were not taken and the type and degree of use of the line was not examined. Franceschi et al. (1989) swabbed hubs at time of set change (24 vs 48 hour trial) and found 30% hub cultures positive at 24 hours, 39% at 48 hours and 41% at 72 hours. Bozzetti (1991) found 52% of Nutricath hubs positive for growth. Multiple organisms were recovered from triple-lumen catheters in this study, which is in agreement with the findings of Haslett et al. (1988) of twice as many organisms being recovered from triple- as opposed to single-lumen catheters.

Previous studies have suggested that hub colonisation may be related to catheter usage but these have been by statistical review rather than by microbiological sampling: Lucas et al. (1992) examined 87 central venous catheters in ICU: By univariate analysis, total hospitalisation days and total numbers of intermittent infusions were found to be the best predictors of infection. Also significantly associated with sepsis were the number of laboratory blood samples via the catheter, number of types of solution, and heparin-locked ports. Durations of catheterisation between infected and non-infected group not given and severity of illness was not allowed for. Rose et al. (1988) retrospectively surveyed 496 subclavian catheters and found a significant risk of infection if the catheter was used for TPN, administration of antibiotics, or removal of blood samples. Glowachi et al. (1990) examined 17 cases of primary nosocomial bacteraemia associated with the use of PA catheters, matched with uninfected controls by retrospective review. An increased number of piggy-back infusions was one of ten factors associated with bacteraemia by univariate analysis.

Only one previous study has reported colonisation rates of individual lumens: Miller et al. (1984) examined 27 triple-lumen catheters, where the proximal port was used for drugs and fluids, medial for TPN, and distal for pressure monitoring and blood sampling: 33% of catheters had lumen contamination of which in 25% of catheters was the proximal lumen, and 11% medial and distal. This was not significant, no comment was given and the extent of use of each of the lumens unknown. In this study 25% of triple-lumen catheters had hub contamination of which 50% was the distal lumen, 30% proximal and 20% medial. McCarthy et al. (1988) suggest that the higher rate of contamination of the proximal port found in Miller et al.’s study may be due to propagation of organisms along the catheter to the intravascular segment. However the use of both the proximal port in their study and
the distal port in this study for the administration of drugs, suggests it may be due to the high number of disconnections caused by bolus drug injection, as seen in this study. This study is the first to demonstrate a relationship between the number of disconnections, and hub and tip colonisation. Furthermore, colonisation was found to be related to the total number of disconnections rather than rate/day, or duration of catheterisation.

AIMS III AND IV: THE ROUTE OF TIP COLONISATION IN PATIENTS WITH TRIPLE-LUMEN CATHETERS OR RECEIVING TOTAL PARENTERAL NUTRITION

Previous studies examining or observing the route of infection in patients with a central venous catheter were summarised in table 1.6 page 46 and the problems of comparing studies with different sampling and culture methods, different levels of characterisation of isolates, differing definitions of infection, types of catheter, catheter maintenance and patient groups, were discussed. Where both routes were observed, a roll plate and a broth culture method used and organisms characterised at least to the level of species and antibiogram, the frequency of the intra- and extra-luminal routes of previous studies are given in table 3.44.

<table>
<thead>
<tr>
<th>AUTHOR</th>
<th>NO. TIPS</th>
<th>CATHETER TYPE</th>
<th>CATHETER USAGE</th>
<th>ROUTE OF COLONISATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitges-serra et al. 1984</td>
<td>19</td>
<td>single</td>
<td>TPN</td>
<td>hub 21/23, skin 0.</td>
</tr>
<tr>
<td>Linares et al. 1985</td>
<td>19</td>
<td>single</td>
<td>TPN</td>
<td>sepsis 14/20 hub, 2/20 skin</td>
</tr>
<tr>
<td>De Cicco et al. 1989</td>
<td>22</td>
<td>single</td>
<td>cancer, TPN</td>
<td>10/22 skin, 9/22 hub</td>
</tr>
<tr>
<td>Cerenado et al. 1990</td>
<td>53</td>
<td>various central *peripheral</td>
<td>in-patients</td>
<td>57% skin, 27% hub, 15% both</td>
</tr>
</tbody>
</table>

Since this study, two studies have examined the problem using molecular methods: Tan et al. 1994, examined 27 episodes of bacteraemia in children with a central venous catheter (?type), by multilocus enzyme electrophoresis. 7/17 originated from the hub, 4/17 skin, and 2 both. As catheters were not removed, tips were not cultured. Mermel.
Stolz and Maki (1991) examined 297 pulmonary artery catheters in ICU; 69% of local catheter infection arose from the skin, and 17% from the hub, but only a roll-plate tip culture was used.

As can be seen from these studies, there is no consensus over the predominant route of infection. The debate between proponents advocating a predominately intra-luminal versus extraluminal route of infection was outlined in Chapter One (pages 41-49), where the main differences were found to be:

- definition of infection
- definition of hub related infection
- skin and tip culture methods
- speciation of organisms
- catheter type/duration of catheterisation
- TPN administration.

These microbiological variables were standardised and optimised in this study, and the study group was defined, and consisted of short-term catheters, with and without TPN administration. This allowed the following, new observations to be made:

- An association between skin colonisation and hub colonisation. This was demonstrated in 5/23 positive catheters in this study, and demonstrates that the two routes of infection may not be totally separate. This was confirmed since this study by Moro et al. (1994) who found that skin colonisation and hub colonisation were highly correlated with skin correlation a high risk for hub colonisation. Indeed, from the results of a trial of antiseptics for insertion site care by Maki, Ringer and Alvarado (1991) it is apparent that chlorhexidine, which was demonstrated to be the most effective at reducing colonisation of the insertion site, also resulted in less colonisation of the catheter hub. This suggests that organisms contaminating the catheter hub may originate from the patients skin. The demonstration of cross-infection between patients in this study (figure 3.40 page 166) demonstrates that organisms are also transmitted via hands of staff. A previous study identified two patients treated in ICU in the same period with hub-related bacteraemia caused by
an identical *P. aeruginosa* O11, suggesting the hub was colonised during hand manipulations (Douard et al. 1989). In addition, organisms grown from stopcocks have since been shown to be identical to those in patients sputum or wound drainage (McArthur et al. 1995). Only one study has examined the source of hub colonisation: In a study to assess the effectiveness of tunnelling (in single, TPN catheters only), De Cicco et al. (1989) took pre-insertion skin, rectal and pharyngeal swabs from patients, and weekly skin and oropharyngeal swabs from nursing staff. Hub contamination was responsible for 10/22 cases of tip colonisation, of which in six cases were isolated from skin of nurses changing the bag, and 5 patients had the same, indigenous organism colonising both hub and insertion site. The results of this study would support a similar aetiology but for triple-lumen not TPN catheters.

- **Hub colonisation may be transient.** Organisms may be intermittently introduced into the administration system, and the effect may be cumulative: In this study all catheters with a positive hub culture had a positive tip culture, but not always with the same organism, and no source was found for 12/22 organisms on the catheter tip despite high recovery of organisms from skin swabs. There is some evidence in support of this: Surveillance hub cultures of Broviac/silicone catheters in neonates, three times per week found that hub colonisation was common (45%) but often transient (Salzman et al. 1993). Bozzetti et al. (1991) looked at TPN administration via Nutricaths. Positive hubs carried a higher risk of a colonised catheter tip not only with the same organism isolated from the hub, but also with other organisms, and thus acted as an indicator of "violation of the lines sterility".

- **Two-way travel of organisms via the extraluminal route.** The possibility has been proposed that organisms colonising the catheter tip may subsequently colonise the insertion site: Segura et al. (1990) inoculated the catheter hub in a rabbit model and subsequently cultured the same organism (*Pseudomonas aeruginosa*) from the insertion site. They postulated that this may be due to retrograde colonisation or capillary action. The results of this study confirm a relationship between skin and hub colonisation, but also demonstrate for the first time the simultaneous

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colonisation of the tip from different species of organism from the insertion site and catheter hub.

- **TPN administration.** The results of this study showed intraluminal colonisation of 87% of Nutricaths versus 25% triple-lumen catheters, which may explain the preponderance of the intraluminal route of infection in TPN patients.

- **Association of hub colonisation with bacteraemia.** There is evidence to suggest that a higher proportion of hub colonisations lead to septicaemia than skin colonisation: Since this study, in short-term central venous catheters in ICU and Surgical units, Moro et al. (1994) found that whilst simultaneous hub and tip colonisation was only present in 3.5% of catheters, 13% resulted in septicaemia (incidence 0.48%), compared to 26% skin and tip colonisation of which 1% were responsible for septicaemia (incidence 0.16%). In a study of paediatric Hickman catheters, of 11 cases of bacteraemia, a positive site culture was obtained in six, of which none were associated with infection, with a positive hub culture in ten, of which six were associated with bacteraemia (Weightman et al. 1988). The results of this study demonstrate the association of intraluminal colonisation and bacteraemia with 100% of microbiologically documented bacteraemias originating from hub colonisation, from an overall 30% of hubs colonised, as opposed to none from skin colonisation despite 62% of insertion sites being colonised.

This study therefore agrees with the findings of Sitges-Serra/Segura/Linares that the predominant route of infection of TPN catheters is hub colonisation, and extends it from long-term Nutricaths, to short-term single- and triple-lumen catheters.

**AREAS FOR FURTHER RESEARCH**

The aetiology and consequences of hub colonisation require further investigation. In addition, the factors influencing development of clinical infection from skin/hub colonisation are currently unknown. A limitation of this study was the inability to directly determine a source of tip colonisation in 46% triple-lumen
catheters. Given the persistent nature of skin colonisation, and the high recovery from skin cultures obtained, plus the strong statistical association demonstrated between positive hub colonisation and tip colonisation, this may be due to the transient nature of hub colonisation. The association between hub colonisation and bacteraemia, and the effect of growth medium and the species/virulence of the organism on the consequences of hub contamination require further study.

These questions will be further examined by the use of in-line filters in the next study (Chapter 4), and by examination of the ability of commonly isolated organisms to grow in commonly used intravenous solutions in Chapter 5.

**SUMMARY**

This study demonstrated the following:

**MICROBIOLOGY**

- A combination of a micromethod (API Staph) and extended antibiogram (ATB Staph/Strep) simply and reliably allows differentiation of isolates from different sources within an individual patient.
- Both endogenous and exogenous sources of tip colonisation were observed.
- Both the intraluminal and extraluminal routes of infection may occur simultaneously in the same patient at one time, or different routes in the same patient at different times.

**SKIN COLONISATION**

- Colonisation of the insertion site increased with duration of catheterisation.
- High levels of colonisation were found due to the use of transparent dressings which were left *in situ* until catheter removal.
- Colonisation persisted at the insertion site despite cleansing with povidone-iodine solution.
- Significantly less tip colonisation was observed where catheters were inserted with alcoholic chlorhexidine skin preparation.
The presence of *E. faecalis* at the insertion site was linked to subsequent tip colonisation in the majority of cases.

**INTRALUMINAL COLONISATION**

- Intraluminal colonisation increased with increasing number of disconnections. This has not previously been demonstrated. It was not dependent on duration of catheterisation.
- A high level of intraluminal colonisation of catheters used for TPN was observed despite low numbers of disconnections.
- In-use contamination of TPN solutions was occurring, and a cause of bacteraemia.
- Three-way taps were left *in situ* for the duration of catheterisation, with 25% exhibiting microbial growth.

**TIP COLONISATION**

- Both intra- and extraluminal routes of tip colonisation were observed.
- There was no significant difference in the level of skin colonisation between single- and triple-lumen catheters and Nutricaths, suggesting that the higher rates of tip colonisation seen with triple-lumen catheters and Nutricaths were due to the higher rates of intraluminal colonisation demonstrated.
- Cross infection between patients in the ICU was observed.

- All five cases of microbiologically documented bacteraemia in which a source was identified were due to intraluminal colonisation in patients receiving Total Parenteral Nutrition.

The predominant route of infection of TPN catheters is hub colonisation, extending the findings of Sitges-Serra/Segura/Linares from long-term Nutricaths, to short-term single- and triple-lumen catheters.
CHAPTER FOUR: THE EFFECT OF IN-LINE FILTERS ON INTRALUMINAL CONTAMINATION OF CENTRAL VENOUS CATHETERS

INTRODUCTION

Study II (Chapter 3) demonstrated the presence of bacterial growth in 25% of three-way taps and 25% of triple lumen catheter hubs in central venous catheters removed from surgical patients in a District General Hospital. Furthermore, there were five cases of microbiologically documented bacteraemia in which the organism isolated from the blood was identical (by biotype, antibiogram and 'phage type) to that isolated from the administration system. Subsequent to this study, in-line intravenous filters were introduced in the Intensive Care Unit.

A 0.22\(\mu\)m filter prevents passage of micro-organisms and, if positively charged, endotoxin in laboratory simulations (Baumgarten et al. 1986). Whilst original filters needed to be replaced to prevent endotoxin release every 24 hours, causing a break in the closed system, ELD96(Pall) has been shown to retain micro-organisms and endotoxin for 168 hours (Horibe et al. 1990).

Whilst the use of in-line filters is not generally recommended as an infection control measure (Spencer 1990, Gurevich 1989) it has been suggested that they may be of benefit in selected groups of high risk patients (Francombe 1988, Weinstein 1987). No previous studies have examined the effectiveness of a 0.2\(\mu\)m in-line filter, changed 96 hourly, in reducing tip colonisation of central venous catheters. To the researchers knowledge, only two previous studies have examined the effectiveness of in-line filters in reducing the incidence of catheter-related infection: Collins et al. (1973), examined a 0.45\(\mu\)m filter, changed 48 hourly on a “variety of cannulae”, (but pre-introduction of triple-lumen catheters): of 84 filters used, 45 were replaced at less than 48 hours due to blockage. The length of time the catheters were in situ is not stated. Of 80 cultured, 17 isolates were obtained from 17 filters (21%). On qualitative broth culture, 55% of the filter group had a positive tip compared to 61% without filters (not significant). There were no episodes of septicaemia in either group. In this small study of presumably mainly short-term peripheral cannulae, the high frequency of filter change may have negated any benefit in reducing contamination. In addition,
a 0.45 μm filter membrane has since been shown by Rusmin et al. (1975) to be penetrated by *E. coli* and *Pseudomonas aeruginosa* after 6 hours, and by Holmes et al. (1980) to be penetrated by *Enterobacter agglomerans, Serratia marcescens, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (all the species tested), within 72 hours, who suggested similar results may be observed with other species. No penetration was observed with a 0.2μm membrane.

Quercia et al. (1986) randomly assigned patients in a surgical ICU to receive Ivex-2 (Abbott laboratories) filtersets with or without an internal 0.22μm membrane, on “all their IV lines” replaced every 24 hours. Again the type of cannulae was not stated, but the study was prior to the introduction of triple-lumen catheters. Thirteen nosocomial bacteraemias occurred during the study period, of which 10 were in patients with blank filtersets and 3 in those with active filters. As the catheter tips or hubs were not cultured, it can only be postulated that these were catheter-related. Although 5 of these are stated to have had the same organism isolated from blood culture and the blank filterset, 4 were reported as “*S. epidermidis*” and the details of any typing method used were not given. In addition, the comparability of the two groups in terms of susceptibility to infection and duration of catheterisation is not stated. As the catheter wasn’t cultured, the effectiveness of the filters in preventing passage of bacteria can only be assumed.

The effectiveness of in-line filters in preventing colonisation of central venous catheters is therefore unknown.

**AIM**

The aim of this study was to

I. test the effectiveness of filters in preventing passage of bacteria,
II. further examine intraluminal contamination of catheters in the Intensive Care Unit,
III. determine the effect of in-line filtration on the rate of catheter tip and hub colonisation in an Intensive Care Unit.
**STUDY DESIGN**

**SAMPLING**

Filters were used on, and obtained from, all consecutive patients with a central venous catheter of any type in a general medical and surgical ICU over a ten week period. This was selected to provide a sample of approximately 50 filters for each triple-lumen catheter per continuously occupied bed-space, and approximately 25 catheters. No patients were excluded. Filters were only used for the duration of the patients stay in ICU.

**ETHICAL APPROVAL**

As filters were used on all patients, as a result of an agreed change in Unit practice, ethical approval was not required. No patient contact was involved. ICU patients from the previous prospective study were used as a control group. Only patient details necessary to enable comparison of risk factors and to link filters to a period of catheterisation were recorded.

**STATISTICAL ANALYSIS**

Data was recorded anonymously on a spreadsheet as previously. Assuming 50% of tip colonisation is due to intraluminal contamination as shown in the previous study, a reduction in the previously observed rate of tip colonisation of 59% to 29.5% would require a sample of 40 in each group at a significance level of p<0.05 and a power of 80%. In practice, as only twenty catheters were in the control group, this would give a 50% chance of assigning statistical significance to a 30% difference with a similar sized group.

**METHODOLOGY**

**MATERIALS**

Positively charged 0.22μm posidyne filters (charge-modified hydrophilic nylon) (Pall ELD96) were used for this study. This was the only filter available designed to be left in situ 4 days - replacing the filter daily would have significantly reduced the
potential for reducing contamination of the administration system. Filters were attached to each of the infusion hubs of pulmonary artery catheters and all lumens of central venous catheters at the time of catheter insertion, or on the patients admission to ICU, using aseptic technique. The time of attachment (and for later removal) was written on the label provided on the filter. The filter was removed after 96 hours or when indicated in practice due to blockage, catheter change or removal, for the administration of TPN, emulsions, blood or blood products or other substances not compatible with filtration, or for the attachment of pressure transducers. Catheter use was recorded as in the previous study. Filters were changed by nursing and anaesthetic staff using a no touch technique after spraying the hub with alcohol and allowing to dry. On removal, filters and any attached three-way taps or extension sets were capped with a new sterile cap and refrigerated until cultured. On catheter removal, the tip was sent to the clinical laboratory.

MICROBIOLOGY

Exposed ports were swabbed with a sterile cotton bud and directly plated on blood agar and cysteine lactose electrolyte deficient agar, incubated and identified as in the previous study. The ports of three-way taps, female luer lock of filter and male luer lock attachment to the catheter hub were sampled as illustrated in Figure 4.41. Culture of the filter attachment to the catheter lumen was considered to represent culture of the catheter hub (Jakobsen et al. 1989).

![Figure 4.41 Diagram of Pall ELD96 Filter](image)

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In addition, for the first fifty filters, a sterile needle was introduced into the patient side of the filter and fluid was withdrawn for culture to check the effectiveness of the filters in preventing passage of bacteria. For the second fifty and subsequent filters, organisms present on the filter membrane were cultured. Microorganisms retained in in-line filters have been demonstrated to proliferate on the membrane surface and in the fluid present in the filter set (Holmes et al. 1980). Examples of previous filter culture methods are shown in Table 4.45.

**TABLE 4.45: FILTER CULTURE METHODS**

<table>
<thead>
<tr>
<th>AUTHOR</th>
<th>FILTER CULTURE METHOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collins et al. 1973</td>
<td>filled with trypticase soya broth (TSB) and incubated</td>
</tr>
<tr>
<td>Newman et al. 1975</td>
<td>opened with hot wire, membrane placed on TSB soaked filter pad and incubated</td>
</tr>
<tr>
<td>Brismar et al. 1984</td>
<td>membrane removed and placed on blood agar plate</td>
</tr>
<tr>
<td>Quercia et al. 1986</td>
<td>2.5ml TSB injected into set with syringe, left on as cap, and incubated</td>
</tr>
<tr>
<td>Geiss 1988</td>
<td>orthograde and retrograde flush with 10mls brain-heart broth</td>
</tr>
</tbody>
</table>

As found by Collins et al., (and on discussion with the manufacturers) it was not possible to remove the filter from the holder without contamination, to enable quantitative culture. There is no evidence that retrograde flushing provides a representative sample of organisms on the filter membrane or adherent to the plastic casing. Therefore, the most sensitive method was considered to be the addition of culture medium to the filterset followed by incubation by a modified method of Quercia et al.: after swabbing, the female filter lumens were cleaned with a 70% isopropyl alcohol moistened cotton swab and allowed to dry, following which 1ml trypticase soya broth was introduced to completely fill the filter, using a sterile syringe which was left attached. The use of the syringe in this way prevented contact of the broth with the luer lock connector. After overnight incubation at 37°C, 1ml was withdrawn with the syringe and 1ul plated with a disposable loop and incubated. Organisms were identified to species level using the standard microbiological techniques of the previous study. As the aim of the filters was to prevent organisms from reaching the catheter tip, no attempt was made to determine the similarity of isolates from within the filter and on the catheter tip. Tips were however, retrieved from the clinical laboratory and broth cultured.
to detect any intraluminal colonisation missed by the roll-plate method (as previously discussed).

RESULTS

AIM I. THE EFFECTIVENESS OF THE FILTERS IN PREVENTING PASSAGE OF BACTERIA

Fifty filters (from 50 total) were collected from 2 single lumen and 12 triple-lumen central venous catheters, and 4 pulmonary artery catheters over a total of 146 filter days. The average time a filter remained in situ was 2.92 days with a range of <1 to 5 days. Three-way taps were attached to 39 filters.

As shown in Table 4.46, bacteria were isolated from the port of one three-way tap (2.6%) and the male luer lock of one filter (2%). No organisms were isolated from fluid leaving the filter.

AIM II. INTRALUMINAL CONTAMINATION

A further 52 filters were collected from 1 long line, 11 triple-lumen and 3 pulmonary artery catheters over a total of 130 filter days. The average time a filter remained in situ was 2.59 days with a range of <1 - 5 days. Forty six three-way taps were attached to the filters.

Bacteria were isolated from the port of 3 three-way taps (6%) and the proximal lumen of one filter, and one or more organisms were isolated from the membrane of twenty filters (38%) as documented in Table 4.46. No contamination was detected on the distal side of the filter.

<table>
<thead>
<tr>
<th>TABLE 4.46: SUMMARY OF RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUMBER OF FILTERS</td>
</tr>
<tr>
<td>50</td>
</tr>
<tr>
<td>52</td>
</tr>
</tbody>
</table>

* +ve = bacterial growth obtained  ND= not done

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Twenty four isolates were obtained from 20 filter membranes. Isolates were predominately skin flora - coagulase negative staphylococci and diphtheroids, but in addition, more virulent organisms such as *S. aureus* and *Klebsiella pneumoniae* were also obtained as shown in Table 4.47. A list of organisms present at each source is given in Appendix 7.

**TABLE 4.47: RESULTS OF FILTER CULTURE**

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>NUMBER OF FILTERS POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. EPIDERMIDIS</em></td>
<td>10</td>
</tr>
<tr>
<td><em>S. HAEMOLYTICUS</em></td>
<td>4</td>
</tr>
<tr>
<td>DIPHTHEROIDS</td>
<td>4</td>
</tr>
<tr>
<td><em>E. FAECALIS</em></td>
<td>2</td>
</tr>
<tr>
<td><em>S. AUREUS</em></td>
<td>1</td>
</tr>
<tr>
<td><em>K. PNEUMONIAE</em></td>
<td>1</td>
</tr>
<tr>
<td><em>S. SIMULANS</em></td>
<td>1</td>
</tr>
<tr>
<td><em>S. HOMINIS</em></td>
<td>1</td>
</tr>
</tbody>
</table>

Filter use

The relationship between type of attached infusion equipment, number of disconnections, length of time *in situ* of the filter and the result of filter culture was examined. Two filters which were found to be broken were excluded from further analysis. The type, extent and duration of use for each of the 52 filters cultured is given in Appendix 8.

Duration of use

The average length of time *in situ* was 3.0 days (SD 1.1) for filters positive for bacterial growth (median 3), and 2.2 days (SD 1.6) for negative filters (median 1), as illustrated in Figure 4.42. This was a significant difference (t-test, p<0.05).
Disconnection of the system

As administration sets could remain in place for the life of the filter once attached, the number of disconnections observed was low with the exception of filters used for the administration of bolus injections. The mean number of disconnections was 17.7 for filters used for bolus injections, and 0.2 for other filters. As the number of disconnections was small, it was not possible to examine statistically the relationship between the number of disconnections and resulting filter culture. However, all of the filters not used for bolus injections that had been disconnected were positive, and none of the negative filters had been disconnected.

Thirty filters were not disconnected once initially attached to administration sets. Growth was obtained from 10 of these (30%) suggesting contamination occurred upstream from change of infusion bags or syringes, or in topping up burettes.
Extent of use

To compare the extent of use of the filter, in the absence of disconnections at the level of the filter in many instances, a score of one was arbitrarily assigned to each administration system attached to the filter, and one for use for bolus injections. There was no relationship between the extent of use as assessed by the amount of attached equipment and filter culture, but a tendency for total use (extent by duration - Appendix 9) to be related to filter culture (Chi-square p<0.1) as shown in Figure 4.43.

FIGURE 4.43: SCATTER PLOT OF RESULT OF FILTER CULTURE BY EXTENT OF USE AND TIME IN SITU
Type of equipment used

There was a difference in the percentage of positive cultures obtained according to the type of equipment used, as shown in Figure 4.44.

**FIGURE 4.44: BAR CHART OF TYPE OF ATTACHED EQUIPMENT AND RESULT OF FILTER CULTURE**

Cost of filtration.

The use of the first hundred filters gave a cost saving of £629 in consumables (excluding transducer sets), in the manner in which they were used, based on the Unit policy of changing administration sets containing drugs 24 hourly, and with fluid only, at 48 hours. The cost of a single filter at full price was £7.36. The difference was easily made up by the reduction in wastage of drugs, particularly in syringe drivers and burettes, which were previously discarded after exactly 24 hours regardless of the amount remaining. These calculations, and drug use, are given in Appendix 10.

**AIM III. INCIDENCE OF TIP AND HUB COLONISATION**

As reflected by culture of the filter male luer lock only one catheter hub exhibited bacterial growth at the time of filter removal. As no growth was present in the fluid leaving the filter, this was assumed to be due to contamination during attachment of the filter. Seventy percent of catheter tips were cultured: Two patients died with catheters in situ, one patient was transferred to another hospital, two catheters fell out, one was rewired, and three were not sent for culture. The temporal relationship of individual filters to the associated catheter is shown in Figure 4.45, pages 196 and 197. Of the 33 catheters included in the study, in only four cases was a filter present on every lumen of the catheter for the total duration of catheterisation. Of these four cases, two tips were cultured of which one was positive for bacterial growth and one negative.
Where filters were present on every lumen at line removal (10 cases) no lumen exhibited bacterial growth. Eight tips were cultured of which four were positive and four negative.

The rate of positive tip culture was compared with that of ICU patients in the previous study. As pulmonary artery catheters and long-lines were not included in the previous study, these were excluded from the analysis, as was the one single lumen catheter cultured. Only one of these 10 excluded catheters (10%) had a positive tip culture, compared to 10 of 16 cultured triple-lumen catheters (62%) This was not significantly different to that observed in the previous study as shown below (Table:4.48). Patients in both groups were comparable in terms of age, and severity of illness.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>TOTAL TRIPLE-LUMEN CATHETERS (ICU)</th>
<th>NUMBER CULTURED</th>
<th>GROWTH ON TIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO FILTERS</td>
<td>27</td>
<td>20 (74%)</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>SOME FILTERS</td>
<td>23</td>
<td>16 (70%)</td>
<td>10 (62%)</td>
</tr>
</tbody>
</table>

The average time in situ of a catheter with a positive tip culture was 6.5 days (median 7, range 1-11) compared with 6.9 days (median 5, range 3-11) in the previous study, with negative tips in situ an average of 5.8 days (median 4 days, range 3-11) as opposed to 4.0 days in the previous study. These differences are not significant.
DISCUSSION

AIM I. THE EFFECTIVENESS OF THE FILTER IN PREVENTING PASSAGE OF BACTERIA

No bacterial growth was detected in fluid leaving the filter suggesting that the filters were effective in preventing the passage of bacteria. Organisms were detected on the patient side of the filter on only one occasion (with sterile filter fluid), presumably due to manipulation of the catheter distal to the filter.

AIM II. FURTHER EXAMINATION OF INTRALUMINAL CONTAMINATION

The isolation of organisms from the three-way taps and inlet port of the filter on only five occasions raised the possibility that the rate of intraluminal contamination had decreased. This would represent a six-fold reduction in the rate of intraluminal contamination since the previous study. Comparison of nursing practice between the two studies revealed only one change in practice: In the previous study, three-way taps were not changed unless faulty and remained in situ until the line was removed. Eighteen taps (25%) were found to be contaminated with an average time in situ of 7 days. In this study taps were attached to filters and thus changed with them after a maximum of 4 days (average 2.7) which may have been insufficient time for detectable levels of contamination to occur, although small numbers of organisms may have passed through the taps and been retained inside the filters. This was confirmed by culture of the membrane of the second 52 filters which demonstrated organisms present in 38% of filters. All but one patient and 79% of catheters were associated with one or more positive filters. This compares with a rate of intraluminal contamination of the catheter hubs of 30% in the previous study. Other authors have recorded similar results: Cheeseborough and Finch (1984) showed that in-use contamination of IV fluids may exceed 30% when additives were injected on the ward. Rates of positive culture of in-line filters in a variety of settings have been reported in the range of 2.8 to 29.1% (Gill 1984). In an Intensive Care Unit, and using a similar method, Quercia et al. (1986) detected a contamination level of 14% after only 24 hours use, with at least one contaminated filter from each patient during the study period. This compares closely to 3/18 (16.6%) of filters removed after 24 hours in this study. Geiss (1988) demonstrated...
a 35% contamination rate in a surgical ICU when filters were changed every 72 hours. Catheter tips were not cultured in either study, and the relationship between intraluminal contamination and hub colonisation is unknown. This is the first study to examine filters changed at 96 hours: the high (38%) rate of contamination observed in this study supports the observation of 30% colonisation of triple-lumen catheter hubs in the previous study, and suggests that the two are related.

Filter use

Contamination of the filter was linked to the length of time the filter was in situ, growth being present within 50% of filters in situ for three or more days. The effect of increasing amount of attached equipment and duration of filter use appeared both correlated with each other and with a positive filter culture (Figure 4.43, page 190) This was not due to increasing disconnection of the filter: with the exception of bolus injections, the number of disconnections at the level of the filter did not necessarily increase with time as administration sets were only changed with the filter unless no longer required or replaced by a different treatment. This suggests that organisms contaminating the system during manipulations may need time to grow to detectable levels. This is new finding: whilst in agreement with the finding of Maki et al. (1973) that the risk of an administration system becoming contaminated during use was closely related to the duration of infusion with growth being more likely after 48 hours, this was previously presumed to be due to cumulative contamination. The number of disconnections upstream to change syringes or infusion bags or to top up burettes would generally increase with time. The presence of bacterial growth in 30% of filters not disconnected since attachment to the catheter suggests that contamination is occurring upstream during these procedures. The difference in associated filter contamination rate observed between the different types of infusion set in use (Figure 4.44 page 191) does not reach statistical significance, but reflects what one would intuitively expect from the distance from the filter and the degree of manipulation required, that is to say, a lower rate of contamination with standard infusion bags with attached administration set, and a higher rate with burette administration sets, the use of syringe drivers, and the administration of bolus injections. This was not an aim of the study, and would need to be confirmed by a much larger sample, but adds to information provided by
previous studies: Newman, Dempsey and Walker (1975) found continuous use of giving sets for 4 days led to contamination of 27% of in-line filters (changed 24 hourly), irrespective of the type of container, with the apparent source the hands of staff changing infusion containers. In contrast, McAllister, Buchanan and Skolaut (1974) demonstrated that using the minibag (swabbing port, adding drug and inserting set spike) resulted in a significantly lower rate of contamination of infusion fluid than inline burettes (swabbing port and injecting drug) for intermittent infusions (0.7% vs 7.1% for one procedure). Previously, Duma et al. (1971) traced 4 cases of sepsicaemia to contaminated burette administration sets, which are repeatedly punctured to top-up drugs, and found that 35% of burettes used in the hospital became contaminated during use. Regarding bolus injection, Brismar et al. (1984) used two filters, one in the infusion line and one on the stopcock side port of 44 TPN catheters used only for amino-acid and glucose solutions, and IV injections. Despite similar rates of disconnection (mean 2.1 injections and 2.3 breaks in infusion line per 24 hours), growth was observed in 14% stopcock filters, versus 3% infusion filters ($p<0.01$), also confirming that growth in a stopcock is carried into administration system. The use of in-line filters has been demonstrated by this study to be a suitable method for further examining in-use contamination, which has implications for, for example, the frequency of changing administration sets, which in turn effects the cost equation in implementing the use of in-line filters. In this study, 15.8% filters exhibited bacterial growth when removed on day 1, 40% day 2, 62% day 3, and 47% day 4.

Both filters that were broken exhibited bacterial growth. Splitting of the filter is a safety measure which results from a pressure rise due to the precipitation of incompatible solutions. Antibiotic administration via the filter did not always prevent microbial growth however all cases of negative filters with high rates of disconnection to give bolus injections were being used for antibiotic administration.
LIMITATIONS OF THE STUDY

AIM III. TIP AND HUB COLONISATION

A limitation of the study was that the results were unable to demonstrate an effect of filtration on the rate of tip colonisation due to the haphazard use of the filters combined with the need to remove them for the administration of TPN, fat emulsions (such as propofol, a frequently used sedative within the Intensive Care Unit) and blood products and, it was perceived, for the accurate use of a central venous pressure manometer. Only four of eighteen patients in this study did not receive blood products, TPN or propofol during their stay in ICU, of which only one catheter was inserted in ICU (2 A/E and 1 Theatre). The average proportion of catheters with a filter throughout the duration of catheterisation was 39%, compared to 56% in the study by Quercia et al. (1986) in a surgical ICU. However in this study, filters were not available in Theatre, A/E, or on the wards thus only catheters both inserted and removed in ICU are comparable. On 5 occasions filters were not attached until day 2, and in 3 instances day three of catheterisation. Tip colonisation of triple-lumen catheters may thus have occurred before filter attachment, via a lumen not possessing a filter or via the catheter insertion site. As it was anticipated that this was a possibility, it was decided not to culture skin sites and catheters on removal as in the previous study. In the ten catheters demonstrated to have no intraluminal contamination at line removal, five tips (50%) exhibited microbial growth which may have arisen from the catheter insertion site or haematogenously from a distant focus. This compares with 60% in the previous study. The development of tip contamination did not appear to have been delayed by the use of filters in the manner in which they were used although there was a tendency for negative tips to have remained negative for longer. As the procedures for care of the insertion site were unchanged from the previous study, it is possible that tips that would otherwise have been colonised due to intraluminal colonisation were later colonised via the insertion site, both being demonstrated to occur, often by the same organism, in the previous study, although with less clinical consequences in extraluminal colonisation.

A second limitation of the study was that it was not possible to quantify growth within the filter to determine the level of contamination with time, however it was anticipated that the filters would have remained in place for the full 96 hours as recommended. In addition, due to the small sample size and the use of drugs in differing
combinations, it was not possible to determine the effect of different types of infusion in this study. This effect of differing solutions on bacterial growth will be examined in study IV (chapter 5).

**SUMMARY**

In-line filters were effective in preventing passage of organisms from contamination of the administration system which remained a significant problem, 38% of filters exhibiting bacterial growth which appears to be cumulative with time and increasing use. Change of three-way taps at 3 day intervals prevented contamination reaching detectable levels. The rate of colonisation of the catheter tip was unchanged, presumably due to colonisation from lumens without filters, during the administration of blood products, total parenteral nutrition and Propofol, and from the catheter insertion site. Nevertheless, pathogenic organisms such as *K. pneumoniae* and *S. aureus* were prevented from reaching the patient, the importance of which was shown by the demonstration of an intraluminal source of five cases of microbiologically documented bacteraemia in the previous study. The effect of common infusion fluids on bacterial growth will be examined in the next study.

- Filters were *in situ* an average of approximately 3 days (range 1-5). All filters were effective in preventing passage of bacteria.

- Organisms were isolated from the membrane of 20 filters cultured (38%). These were predominately CNS but included *S. aureus*, *E. faecalis* and *K. pneumoniae*.

- Filters positive for growth were *in situ* significantly longer than negative filters. The proportion of positive filters rose with increasing use and time *in situ*, suggesting contamination was cumulative and/or time is required for growth to detectable levels.
• 10/30 filters (33%) not disconnected after initial attachment exhibited growth indicating contamination occurred upstream in changing infusion bags or syringes, or puncturing burettes.

• Changing three-way taps on average every three days reduced the rate of positive tap culture from 25% in the previous study, to 5%.

• In an Intensive Care Unit population, it was not possible to maintain a filter on every lumen throughout the period of catheterisation. Nevertheless, the use of filters was cost effective.

• Where filters were present until catheter removal, no lumen exhibited bacterial growth, thus in-line filters are effective in preventing intraluminal colonisation. This has not previously been demonstrated.
CHAPTER FIVE: IN-VITRO MULTIPLICATION OF MICRO-ORGANISMS IN INTRAVENOUS SOLUTIONS

INTRODUCTION

Study Two (Chapter Three) identified contamination of the administration system as a major cause of colonisation of the catheter tip and septicaemia in patients with a central venous catheter. Whilst intrinsic contamination of IV fluid is now extremely rare due to stringent control of the manufacturing process, extrinsic contamination of in-use fluids and administration systems is well documented as previously discussed in Chapters Two and Three, with airborne and touch contamination identified as the major hazards (Holmes and Allwood 1979). Studies examining growth of organisms in IV solutions have therefore concentrated on organisms present in the environment of the hospital ward (Lamikanra and Sofekun 1988) or pharmacy clean room, predominately normal skin flora (Whyte et al.). In addition, previous studies have focused on the fate of these external contaminants in the infusion container rather than the outcome of human pathogens introduced from the patients skin, for example into three-way taps or extension sets. No study has examined growth in drug-containing infusions.

Whilst nursing and medical practice in the form of additives to, and disconnections of the IV system are the cause of contamination, other factors such as the type of organism, inoculum size and type of fluid infused may determine the effect of this contamination on colonisation of the central venous catheter and the development of infection.

BACKGROUND TO THE STUDY

Saprophytic, free-living organisms are well adapted to survival in a wide range of pH and temperature with low nutrient availability, but are generally of low pathogenicity, whereas most human pathogens require a relatively narrow range of pH.
and temperature and are nutritionally demanding. However, Michaels and Rubner (1953) proposed that the nature of the infusion fluid itself was the single most important selective factor in determining why certain organisms contaminate intravenous administration systems more frequently than others. Meers et al. (1973) found that the number of invading organisms was an important determinant of whether an infection developed.

Study Two identified a high level of intraluminal contamination of lines used for TPN administration despite low rates of disconnection, which one may hypothesise is due to the ability of dextrose/amino-acid/lipid admixtures to support growth. The recent use of 3-in-1 parenteral nutrition solutions has clinical benefits in preventing over-rapid infusion of lipid, reducing the incidence of hyperglycaemia associated with separate infusion of glucose, and optimising the utilisation of nitrogen by infusion concurrently with an energy source (Taylor and Goodinson-McLaren 1992). In addition, the potential for extrinsic contamination may be reduced by the decreased number of manipulations of the delivery system. Nevertheless, these benefits may be negated if the solution provides a better growth medium. Evidence is conflicting and may vary with the formulation used. There was therefore a need to examine the ability to support growth of the formulation of TPN employed in the author’s clinical situation.

In addition, the ability of many commonly used IV solutions to support microbial growth has not been established. Comparison between reported studies in the literature is also difficult due to the use of different incubation temperatures and times, different inoculum sizes, starved versus unstarved cells, and the use of unwashed cells from liquid culture allowing carry-over of nutrients to the test solutions. Furthermore, the majority of studies do not cite the pH or osmolality of their solutions despite the considerable variability occurring between suppliers. The ability to support growth of the frequently employed drug-containing infusions within an Intensive Care Unit has not been determined.
The aim of this study was to determine the ability of eleven intravenous solutions, frequently administered in an Intensive Care Unit but not previously investigated, to support the growth in vitro of three organisms commonly associated with infection of central venous catheters.

**TEST SOLUTIONS**

Eleven solutions were examined as shown in Table 5.49.

**TABLE 5.49: LIST OF SOLUTIONS EXAMINED**

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total parenteral nutrition (Kabi 3)*</td>
</tr>
<tr>
<td>2</td>
<td>Human albumin solution 4.5%</td>
</tr>
<tr>
<td>3</td>
<td>Propofol 10mg/ml (in Intralipid 10%)**</td>
</tr>
<tr>
<td>4</td>
<td>Hetastarch solution 6% in NaCl 0.9%</td>
</tr>
<tr>
<td>5</td>
<td>0.9% sodium chloride</td>
</tr>
<tr>
<td>6</td>
<td>0.9% sodium chloride + sodium heparin 2 units/ml</td>
</tr>
<tr>
<td>7</td>
<td>0.9% sodium chloride + Actrapid insulin 1 unit/ml</td>
</tr>
<tr>
<td>8</td>
<td>0.9% NaCl + dopamine hydrochloride 200mg in 125ml.</td>
</tr>
<tr>
<td>9</td>
<td>5% aqueous dextrose solution</td>
</tr>
<tr>
<td>10</td>
<td>5% dextrose + dopamine hydrochloride 200mg in 125ml.</td>
</tr>
<tr>
<td>11</td>
<td>Frusemide 10mg/ml</td>
</tr>
<tr>
<td></td>
<td>* Vamin 9 glucose 1000 ml ** Soya bean oil 100g</td>
</tr>
<tr>
<td></td>
<td>Glucose 10% 1000 ml ** Egg phospholipid 12g</td>
</tr>
<tr>
<td></td>
<td>Intralipid 20%** 500 ml ** Glycerol 22.5g</td>
</tr>
<tr>
<td></td>
<td>KCl 15%, Addiphos 20 ml **</td>
</tr>
</tbody>
</table>

The constituents, pH and osmolality of the test solutions were as follows:(Table 5.50)
**TABLE 5.50: COMPOSITION OF TEST SOLUTIONS**

<table>
<thead>
<tr>
<th>SOLUTION</th>
<th>FORMULA</th>
<th>TYPE OF COMPOUND</th>
<th>OSMOLALITY</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>NaCl</td>
<td>Electrolyte</td>
<td>290</td>
<td>5.63</td>
</tr>
<tr>
<td>NaCl + Dopamine</td>
<td>3,4,hydroxyphenyl-ethylamine</td>
<td>Catecholamine</td>
<td>&quot;</td>
<td>5.39</td>
</tr>
<tr>
<td>NaCl + Heparin</td>
<td>Heparin sodium</td>
<td>Glucosaminoglycan</td>
<td>&quot;</td>
<td>5.70</td>
</tr>
<tr>
<td>NaCl + Insulin</td>
<td>C257H383N65O77S6</td>
<td>Amino acids</td>
<td>&quot;</td>
<td>5.64</td>
</tr>
<tr>
<td>(glycerol preservative)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td>C6H12O6,H2O</td>
<td>Monosaccharide</td>
<td>265</td>
<td>4.35</td>
</tr>
<tr>
<td>Dextrose + Dopamine</td>
<td>&quot;</td>
<td></td>
<td>4.35</td>
<td></td>
</tr>
<tr>
<td>Frusemide</td>
<td>4-chloro-2-furfurylamino-sulphamylbenzoic</td>
<td>Organic acid</td>
<td>&quot;</td>
<td>9.00</td>
</tr>
<tr>
<td></td>
<td>C12H11ClN2O5S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hetastarch</td>
<td>hydroxyethyl starch</td>
<td>Polysaccharide</td>
<td>310</td>
<td>5.50</td>
</tr>
<tr>
<td>Human albumin</td>
<td></td>
<td>Amino acids</td>
<td>295</td>
<td>7.5</td>
</tr>
<tr>
<td>Propofol (in Intralipid)</td>
<td>2,6,bis(1-methylethyl)-phenol C12H18O</td>
<td>Phenol</td>
<td>300</td>
<td>8.24</td>
</tr>
<tr>
<td>Parenteral nutrition</td>
<td>&quot;Vamin 9 glucose&quot; - KCl, KH2PO4</td>
<td>L-amino acids</td>
<td>938</td>
<td>5.80</td>
</tr>
<tr>
<td></td>
<td>&quot;Intralipid&quot; -</td>
<td>monosaccharide</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>electrolytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>phosphatidyl esters</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>carbohydrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>triglycerides</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>alcohols</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**STUDY DESIGN**

**CHOICE OF ORGANISMS**

The three organisms chosen for this study were those most commonly isolated from central venous catheter tips in a previous study by the author (Chapter Three) where *S. epidermidis* accounted for 44% of isolates followed by other Staphylococcal species, thirdly *E. faecalis* 19%, and fourthly *K. pneumoniae* 3%. Similar results have
been obtained by other authors: In two studies of ICU patients, Haslett et al. (1988) cultured *S. epidermidis* most frequently (28%) from catheter tips, followed by other Staphylococci, then thirdly *E. faecalis* (13%), with 3% of cultures *Klebsiella pneumoniae*. Richet et al. (1990) found that 48% of tips grew *S. epidermidis*, with *E. faecalis* the fourth most frequent isolate (4%) with *K. pneumoniae* again accounting for 3% of positive cultures.

The ability of an organism to grow in an infusion fluid is dependent upon the pH, osmolality and nutrient availability of the solution (Akpan et al. 1989) and temperature, inoculum size and the physiological state of the bacterium (Hoffman et al. 1982). All three organisms examined were mesophilic, and able to grow at temperatures of 20 - 40°C with an optimum of 37°C. With regard to hydrogen ion concentration, all were neutrophilic, and thus able to maintain an internal pH of approximately 7.5 over an external range of 5.5-8.5. The majority of bacteria are extremely osmotically tolerant, with a higher concentration of osmotically active solutes inside the cell than outside, and have evolved complex transport systems and osmotic pressure regulating mechanisms to maintain constant osmotic conditions within the cell.

*E. faecalis* will grow at a wide range of pH and is tolerant of alkali, growing at pH 4.8 - 11.0 (Novich 1955), and of a 6.5% solution of sodium chloride. It is an aerotolerant anaerobe, unable to synthesise ATP by respiratory means, and is homofermentative with the production of lactic acid. It has limited synthetic ability requiring enriched media for growth. Growth factor requirements include B vitamins, amino acids and purine and pyrimidine bases (Wilson and Miles 1968).

*S. epidermidis* has an optimum pH of 7.4-7.6 but will tolerate acid to pH 4.0-5.0 and a 12% solution of sodium chloride. It is facultatively anaerobic producing energy via respiration or fermentation of sugars to lactic acid. Under aerobic conditions it will grow on complex media in the absence of carbohydrates. Its nutritional requirements include amino acids, B vitamins and organic sulphur (Mandlestam and McQuillen 1968).

*K. pneumoniae* will tolerate a wide range of pH growing at a maximum rate and constant generation time between pH 6.45-8.3 (Dean and Hinshelwood 1966). It is facultatively anaerobic and can utilise many simple organic compounds for respiration including organic acids, amino acids and carbohydrates. Under anaerobic conditions it is
dependent on a fermentable carbohydrate, with the production of butanediol. No growth factors are required.

**EXPERIMENTAL CONDITIONS**

Experimental conditions were chosen to reflect the clinical situation as far as possible. Solutions were tested at concentrations employed in the Intensive Care Unit. Only one batch of each solution was used to ensure that variables such as pH and osmolality were identical for each organism, concentration and temperature tested.

Organisms were taken from quarter strength nutrient agar to provide a degree of nutrient depletion. Use of a colony from solid media prevents transfer of nutrients from broth culture to the test solution and has been shown to give results comparable to the use of washed cells (Guynn et al. 1973). A low inoculum was used to simulate levels of contamination *in vivo*. Fan et al. (1988) in quantitative culture of catheter hubs found organisms in the range 80–2x10^7 colony forming units per hub, with a median of 1000. In addition, a concentration of 100 organisms per ml is recognised by the American National Committee on Large Volume Parenterals (1978) as the size of inoculum that might result from accidental touch contamination. An incubation time of 24 hours represented the interval between change of administration sets in practice and incubation was performed at both room (22°C) and body temperature (37°C).

A viable count was chosen as a measure as only live organisms are capable of causing infection. The Miles and Misra technique (Miles and Misra 1938) of serial dilutions and drop counts was identified as the most accurate for organisms able to grow well on opaque media and enabled verification that colonies were of the original species inoculated with no contaminants.

**SOURCES OF ERROR**

It was recognised that the method was subject to preanalytic, analytic and postanalytic variability in the preparation of samples, experimental procedure and counting and transcribing results with the largest errors potentially occurring in the preparation of serial dilutions and subsequent counting of colonies.
As inaccuracies in the volume content of pipettes has been shown to average to zero (Hedges 1967), a fresh randomly selected pipette was used for each dilution and count. One pipette only was used to inoculate test solutions to give a constant volume. A microtitre pipette was used as it did not rely on a visual reading and was accurate to volumes of 1μl. Dilutions were chosen to give visible and quantifiable colonies at two successive dilutions, a factor of 100 apart. Each dilution was sampled ten times to minimise random error and to increase the sensitivity such that a threshold of 10 colonies/ml could be detected. The original number of colonies was calculated using the formula; \( \text{original number} = \frac{\text{mean}}{(\text{volume} \times \text{dilution})} \).

**VALIDITY OF PLATE COUNT**

It was recognised that a plate count does not give an absolute count but measures the viable bacteria able to grow on a given medium at specific conditions of incubation. This difference was minimised by providing optimum conditions for growth. To maximise the probability that each colony arose from a single cell rather than a clump or chain each sample was vortex mixed to separate cells by dispersion and produce macroscopically distinct colonies.

**RELIABILITY**

Colony counting was found by "Student" (W.S.Gosset) (1907) to follow the Poisson distribution of particles randomly distributed in space, whereby the mean colony count per plate is an estimate of the mean number of bacteria per dilution of the original specimen. Results of successive counts may be expected to follow a Poisson distribution if the experimental technique is good, whereas with imperfect methods, counts will follow distributions with different means, as inadequate mixing or unequal volumes produce heterogeneity in the observed counts (Armitage and Berry 1994). Reliability of results can thus be assessed with the Poisson heterogeneity test\(^1\). Test-retest reliability was assessed during piloting of the method as described below.

\[ x^2 = \sum \frac{(x - \bar{x})^2}{\bar{x}} \]
PILOT STUDY

A pilot study was performed to test the accuracy of the dilution method, ensure discrete colonies were obtained and to calculate the size of the initial inoculum and the number of dilutions required to reproducibly provide the test inoculum of $10^2$ and $10^3$ organisms. Once defined by trial and error, five sets of duplicate measurements were performed for each organism to assess test-retest and intrarater reliability. A variation of the Levy-Jennings “duplicate measurements control chart” (Dekeyser and Coniff-Pugh 1990) for ensuring laboratory quality control was plotted using the differences between paired samplings as shown in Figure 5.46. All results fell within two standard deviations of the mean and thus were well within the limits of acceptable reliability (Dekeyser and Coniff-Pugh 1990). Where high or low variability was noted within a dilution or between serial dilutions the Poisson Heterogeneity test was applied. Where counts at two dilutions were acceptable, the larger number were counted where possible, the standard error being approximately equal to the square root of the number of colonies counted (Miles and Misra 1938).

FIGURE 5.46: DIFFERENCES IN COUNT BETWEEN PAIRED SAMPLES
Correlation coefficients (Pearson) for the two sets of five measurements for each of the three organisms were +0.80, +0.69 and +0.83, thus the technique was reproducible. It was however costly in media and plates required and labour intensive in filling and sterilising bottles, pipetting drops and counting organisms. It was calculated that to sample each solution at 4 hour intervals for 24 hours covering the 3 organisms, 2 concentrations and 2 temperatures would have required a minimum of 1800 bijoux bottles, 1700 agar plates and 2500 pipette tips. More importantly, there would be a delay of up to 2.5 hours between sampling the first and the last solutions.

In view of these problems and the potential for error, the microbiology department was contacted for advice. Impedance measurement was suggested although it had not been used for this application before. The experiment was therefore amended to allow a trial of the “Bactometer Microbial Monitoring System”.

**IMPEDANCE MEASUREMENT**

Measurement was performed in a "Bactometer" microbial monitoring system (MTC, New Jersey). Solutions were placed in wells containing metal electrodes and an alternating voltage applied. The resulting current, plotted in graph form, is dependent on the impedance of the system, where:

\[
impedance = \sqrt{(1/conductance)^2 + (1/2\pi \text{ frequency} \times \text{ capacitance})^2}
\]

Microbial metabolism generally causes a decrease in impedance as uncharged or weakly charged substrates are broken down to highly charged end products, for example glucose to lactic acid, causing a change in current which during the logarithmic growth phase is proportional to the number of viable cells once a detection threshold is reached (Eden and Eden 1984). The specific advantage of this system is that it reflects numbers of live bacteria rather than a total particle count with for example, a Coulter counter or photometer. Frequent measurements are made by computer, eliminating sampling error. Furthermore, bacterial generation times can be calculated from the graphs which would allow an accurate and sensitive direct comparison of growth rates between organisms and solutions.
VALIDITY OF IMPEDANCE MEASUREMENT

Calculation of generation times is dependent upon a period of detectable acceleration in the impedance curve. Characteristics of an ideal curve are shown in Figure 5.47 (page 211). The detection time at which acceleration is visible (acceleration point) is dependent upon microbial characteristics such as concentration, metabolism and generation time, and physical conditions of electrode type, media and temperature. Two concentrations of bacteria were inoculated a factor of 10 apart, and the exact number subsequently counted. Temperature was thermostatically controlled and digitally displayed throughout the experiment. The display was checked with a thermometer before and after the experiment. Two control wells were used - one was left empty, the other contained 5% dextrose but was uninoculated. Both produced straight line graphs. The uninoculated well served as a control for temperature changes, evaporation or degradation of the medium during incubation.

RELIABILITY OF IMPEDANCE MEASUREMENT

The reliability of the method is dependent on the quality of curve obtained with the specific organism/medium combination tested. An initial stabilisation period is required which is affected by factors such as the volume of the sample and the temperature difference between sample and incubator. Following this period a baseline is established prior to the onset of the acceleration phase. This must possess minimal drift to permit accurate determination of the detection time. The slope of the curve needs to be sufficiently steep for the computer to be able to detect a difference between acceleration and drift. The maximum level, or total percentage change, determines the reliability with which changes may be detected, with values of 10% or greater required for accurate detection. Curves characterised by minimal drift and maximal acceleration produce more distinctive deflections and minimise differences in calculated detection times.
METHODOLOGY

Three organisms were tested, two Gram-positive and one Gram-negative - *S. epidermidis*, *E. faecalis* and *K. pneumoniae* respectively, which were clinical isolates cultured from colonised central venous catheter tips during an earlier investigation of central line infection by the author, and identified as previously described (Lee 1996). Solutions of a spectrum of drugs frequently given in ICU were prepared aseptically using strengths and dilutents used in clinical practice as listed in Tables 5.49 and 5.50 (pages 203 and 204). Components were commercially manufactured and prepared aseptically. Pure cultures of test organisms were incubated at 37°C overnight on 1/4 strength nutrient agar. A single colony of each organism was transferred into either 0.9% sodium chloride or 5% dextrose solution according to the composition of the substance to be tested and serially diluted in that solution as shown in Figure 5.48 (page 213). A volume of 0.1ml of each organism was inoculated into 0.9ml of each
test solution to give concentrations of approximately 100 and 1000 organisms per ml. Inoculated test solutions were incubated for 24 hours under stationary conditions of incubation, at both the ambient temperature of the Intensive Care Unit (22°C) and body temperature (37°C).

Growth was assessed experimentally in two ways as represented diagramatically in Figures 5.48 and 5.49 (pages 213 and 214): Firstly, the size of the initial inocula and the final number of organisms present after 24 hours was measured by counting colony forming units (cfu) after serial dilution in peptone water using the Miles and Misra technique: After vortex mixing to reduce clump formation, 10 x 10^2 ml drops of the test solution (10^-2 ml) and dilutions of 1:100 (10^-4 ml) and 1:10,000 (10^-6 ml) were pipetted onto nutrient agar plates and incubated overnight at 37°C. Individual colonies could be counted up to concentrations of approximately 200 cfu/10^2 ml with confluent growth observed at approximately 500 cfu/ml giving a detection range of 10^-5 x 10^8 cfu/ml. Secondly, rate of growth of organisms was observed by impedance measurement in a "Bactometer" microbial monitoring system (MTC, New Jersey): 0.1 ml of each organism at both concentrations was added to 0.9 ml of each test solution and repeated to allow incubation at 22°C and 37°C. This gave 128 solutions of 1 ml which were pipetted into wells of the "Bactometer" containing metal electrodes. Before and after incubation the concentration of organisms was counted manually as above.
FIGURE 5.48: ESTIMATION OF GROWTH BY SERIAL DILUTION

1 colony

10 x 10 μl plated on agar

1000 colonies/ml

100 colonies/ml
FIGURE 5.49: ESTIMATION OF GROWTH BY IMPEDANCE MEASUREMENT

11 SOLUTIONS

BACTOMETER @ 22°C

BACTOMETER @ 37°C

$10^2$ + $10^3$

KLEB
STREP
STAPH

64 WELLS

64 WELLS

$990 \mu l$

$10 \mu l$

$x 128$
REPRESENTATIVENESS OF SOLUTIONS

It was recognised that variability in composition may occur in supply from the manufacturer or in the preparation of solutions which would reduce the generalisibility of the results, as discussed below, but it was decided that it was the relative risks between organisms and solutions that were the important factors. To ascertain the extent to which generalisations could be made from the growth results, manufacturers were contacted and random batches of solutions tested to determine the representativeness of the test solutions of the range of batches produced by the manufacturer.

Osmolality

No manufacturer contacted tested osmolality as part of their quality control programmes. All stated that osmolality was near constant as reliant on, and calculated from, the solute content of the solution which was closely regulated.

As micro-organisms are extremely tolerant of changes in osmotic pressure, variations in osmolality would not vary outwith the optimum range in the majority of clinically employed solutions.

Hydrogen ion concentration

The possibility of the pH of the solution varying around the limits of the optimum range for growth was of concern. All three organisms were neutrophilic and able to maintain a constant internal pH within an external pH range of 5.5 to 8.5. Three solutions were found to be near the limits of this range: TPN, 5% dextrose and, unexpectedly, 0.9% sodium chloride. The pH was tested with a pH meter of resolution 0.01 pH and an accuracy of +/- 0.02. The meter had an automatic temperature compensation, and a dual point calibration was performed at pH 4.01 and 7.01.

Total parenteral nutrition (kabivitrum 3)

The pH of TPN was stated by the manufacturing pharmacist to be dependent on variation in the pH of the dextrose solution, but buffered to a large extent by the
amino acids. A pH of 5.4-5.8 was required to maintain stability of the emulsions, with 5.4 as the lowest theoretical value possible.

The pH of the test solution was 5.8, with the pH of other batches not able to vary significantly outwith the optimum growth range.

5% dextrose solution

The licence limit for the solution is pH 4.0-6.0. Data provided by the manufacturer from their random testing of 150 batches found a range of 4.21 to 5.87, with a mean of 4.98. These values were at the time of manufacture. The pH was found to fall exponentially with time with a drop of 0.29 in the first three months. The test solution was five months old and had a pH of 4.35. Five batches were randomly tested from the ICU. None of the batches on the shelves were found to be less than 3 months old (range 3-5 months). The pH varied from 4.32 - 4.41 with a mean of 4.34. The test solution was thus a representative sample, although outwith the optimum growth range. There is however, theoretically, a chance of a batch having a pH greater than 5.5 if used within the first three months of manufacture (p<0.1, one tailed, normal distribution).

0.9% sodium chloride

The licence limits for sodium chloride are 5.0-7.0. The mean value of the product from the manufacturer concerned was given as pH 5.5. This was not expected to vary with time as the components were already in their simplest form. Ten batches were randomly sampled from the ICU, with a range of 5.00-5.70 and a mean of 5.14. The pH of the test solution was 5.63. Results of growth in 0.9% NaCl are thus likely to reflect the maximum expected growth and be applicable to 75% of the licensing limit.
RESULTS

RESULTS OF GROWTH IN TEST SOLUTIONS

The concentrations of initial inoculae (cfu/ml) were as follows:

- **Klebsiella pneumoniae**
  - Initial concentration: $1.0 \times 10^3$
  - Additional concentration: $5.0 \times 10^2$

- **Staphylococcus epidermidis**
  - Initial concentration: $0.98 \times 10^3$
  - Additional concentration: $1.80 \times 10^2$

- **Enterococcus faecalis**
  - Initial concentration: $0.59 \times 10^3$
  - Additional concentration: $0.66 \times 10^2$

The number of viable colony forming units obtained after 24 hours incubation in each solution from an initial inoculum of approximately 1000 organisms are shown in Table 5.51 and Table 5.52 (page 218). As the viable count fell in the majority of cases, the results using the higher initial inoculum are given.

Table 5.51: Drug/Electrolyte Solutions. Growth (CFU/ml) after 24 Hours at 22°C and 37°C

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>KLEB 1x10^3</th>
<th>STAPH 1x10^2</th>
<th>STREP 6x10^3</th>
<th>KLEB 1x10^3</th>
<th>STAPH 1x10^2</th>
<th>STREP 6x10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HETASTARCH</td>
<td>8x10^2</td>
<td>&lt;10</td>
<td>60</td>
<td>1x10^3</td>
<td>2x10^2</td>
<td>3x10^2</td>
</tr>
<tr>
<td>0.9% SODIUM CHLORIDE</td>
<td>10</td>
<td>&lt;10</td>
<td>20</td>
<td>20</td>
<td>30</td>
<td>1x10^2</td>
</tr>
<tr>
<td>0.9% SODIUM CHLORIDE+HEPARIN</td>
<td>7x10^2</td>
<td>&lt;10</td>
<td>40</td>
<td>2x10^3</td>
<td>90</td>
<td>1x10^2</td>
</tr>
<tr>
<td>0.9% SODIUM CHLORIDE+INSULIN</td>
<td>&lt;10</td>
<td>10</td>
<td>50</td>
<td>2x10^2</td>
<td>90</td>
<td>1x10^2</td>
</tr>
<tr>
<td>0.9%SODIUM CHLORIDE+DOPAMINE</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>ND</td>
<td>40</td>
<td>&lt;10</td>
<td>ND</td>
</tr>
<tr>
<td>5% AQUEOUS DEXTROSE</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>10</td>
<td>30</td>
<td>&lt;10</td>
<td>3x10^2</td>
</tr>
<tr>
<td>5% DEXTROSE+DOPAMINE</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>10</td>
<td>&lt;10</td>
<td>2x10^2</td>
</tr>
<tr>
<td>FRUSEMIDE 10MG/ML</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>30</td>
<td>2x10^2</td>
<td>1x10^2</td>
<td>1x10^2</td>
</tr>
</tbody>
</table>

2 Concentrations, pH and osmolality as in Tables 5.49 and 5.50. ND = not done

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217
GROWTH IN DRUG/ELECTROLYTE SOLUTIONS (TABLE 5.51)

- The results demonstrate that there was no growth above the inoculation level in any of the clear fluids. In the majority of cases the number of viable organisms decreased. At 22°C, this was by a magnitude of $2\log_{10}$ with the exception of 0.9% sodium chloride with heparin (2 units/ml) where there was little change in the numbers of *Klebsiella pneumoniae* and *Enterococcus faecalis*. Growth at 37°C was generally improved by a factor of 10.

- A concentration of $10^2$ - $10^3$ cfu/ml was maintained in Hetastarch, 0.9% sodium chloride with insulin (1 unit/ml) and Frusemide solution at 37°C.

- *E. faecalis* persisted at a count of approximately 100 organisms in all solutions at 37°C.

**Table 5.52: Complex Solutions**

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>KLEB 1x10³</th>
<th>STAPH 1x10³</th>
<th>STREP 6x10²</th>
<th>KLEB 1x10³</th>
<th>STAPH 1x10³</th>
<th>STREP 6x10²</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL PARENTERAL NUTRITION (Kabi 3)</td>
<td>1x10⁴</td>
<td>&lt;10</td>
<td>5x10⁶</td>
<td>3x10⁷</td>
<td>1x10⁵</td>
<td>2x10⁸</td>
</tr>
<tr>
<td>4.5% HUMAN ALBUMEN SOLUTION</td>
<td>2x10⁵</td>
<td>2x10²</td>
<td>1x10⁵</td>
<td>3x10⁷</td>
<td>1x10⁶</td>
<td>5x10⁷</td>
</tr>
<tr>
<td>PROPOFOL</td>
<td>4x10⁷</td>
<td>&lt;10</td>
<td>2x10⁶</td>
<td>1x10⁸</td>
<td>1x10⁵</td>
<td>2x10⁸</td>
</tr>
</tbody>
</table>

GROWTH IN COMPLEX SOLUTIONS (TABLE 5.52)

- There was no growth of *S. epidermidis* at either temperature in any solution except Human albumin solution (HAS) in which significant growth of all organisms was obtained with a count of $10^6$ - $10^7$ cfu/ml at 37°C. Examples of impedance curves of growth in HAS are shown in Figure 5.51 (page 220).

- *K. pneumoniae* and *E. faecalis* grew well in TPN and Propofol at both temperatures but particularly at 37°C with a final concentration of $10^7$ - $10^8$ cfu/ml. Examples of impedance graphs observed for growth in TPN are shown in Figure 5.50.
FIGURE 5.50: IMPEDANCE IN TPN SOLUTION AT 37.4°C

Klebsiella x 100
Staphylococcus x 100
Enterococcus x 100

Klebsiella x 1000
Staphylococcus x 1000
Enterococcus x 1000
Generation times ($tg$) were calculated from the above curves (Figures 5.50 and 5.51) of two serial dilutions using the formula

$$tg = \frac{\Delta \text{detection time} \times \log 2}{\log \text{conc.} 1 - \log \text{conc.} 2}$$

### TABLE 5.53: CALCULATION OF GENERATION TIMES FROM IMPEDANCE CURVES

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>SOLUTION</th>
<th>DETECTION TIME (HOURS)</th>
<th>DOUBLING TIME (MINS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. epidermidis</em></td>
<td>HAS 37°C</td>
<td>12.9</td>
<td>74</td>
</tr>
<tr>
<td>1.80x10^2</td>
<td></td>
<td>9.9</td>
<td>74</td>
</tr>
<tr>
<td>0.98x10^3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>TPN 37°C</td>
<td>10.3</td>
<td>19</td>
</tr>
<tr>
<td>0.66x10^2</td>
<td></td>
<td>9.4</td>
<td>19</td>
</tr>
<tr>
<td>0.59x10^3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.66x10^2</td>
<td>HAS 37°C</td>
<td>9.9</td>
<td>38</td>
</tr>
<tr>
<td>0.59x10^3</td>
<td></td>
<td>8.0</td>
<td>38</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>TPN 37°C</td>
<td>12.4</td>
<td>22</td>
</tr>
<tr>
<td>5.0x10^2</td>
<td></td>
<td>11.6</td>
<td>22</td>
</tr>
<tr>
<td>1.0x10^3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Unfortunately, impedance measurements are not of use in observing a fall in number of viable cells with a low inoculum as the detection threshold is not reached. However some changes, generally a decrease in impedance were evident on cell death, particularly with sodium chloride solution which had a high initial conductivity (base level) due to cell lysis with release of complex molecules. An example of cell death is shown below (Figure 5.52).

FIGURE 5.52: IMPEDANCE IN 0.9% SODIUM CHLORIDE + DOPAMINE AT 37.4°C
DISCUSSION

The lack of available data on the growth of organisms causing catheter-related infections in drug-containing solutions has been noted. The ability of some Klebsiella, Staphylococcal and Streptococcal species to survive in common, simple IV solutions has been examined under various conditions, and whilst it is appreciated that there are large phenotypic differences within each genus, species other than those examined in this study, but within the same Tribe, will be discussed for reference due to the lack of data for more direct comparison:

DRUG/ELECTROLYTE SOLUTIONS

Growth was poorest in 5% aqueous dextrose solution (pH 4.4, osmolality 265 mOsm/kg) particularly with the addition of Dopamine (pH 4.2). Sodium chloride 0.9% solution (pH 5.6, 290 mOsm/kg) was more conducive to growth, again reduced by the addition of Dopamine (pH 5.4), but increased by the addition of Insulin (pH 5.6) or Heparin (pH 5.7). In contrast with the findings of Guynn et al. (1973), E. faecalis, a Gram-positive organism, exhibited a comparable degree of growth to the Gram-negative K. pneumoniae. Grow in drug solutions has not previously been examined, but these new findings are supported by earlier studies of higher inoculae in clear fluids. Guynn et al. (1973) looked at several species of Gram-negative and Gram-positive organisms including K. pneumoniae and S. epidermidis (10^4-10^5 organisms/ml) in 0.9% sodium chloride and 5% aqueous dextrose solutions at 23 and 37°C. Viable counts of Gram-negative bacilli generally increased by 2 log 10 in 48 hours whilst those of Gram-positive cocci remained stationary or declined. They postulated that Gram-negative bacilli are more resistant to hypoosmolar conditions due to their lower internal pressure.

Lamikanra and Sofekun (1988) inoculated 10^7 S. epidermidis/ml into 0.9% sodium chloride and 5% aqueous dextrose solutions and found that the viable count reached zero within 8 hours at 28°C with no recovery by 48 hours. In particular the presence of dextrose coupled with a low pH was found to be detrimental to growth. Gelbart et al. (1973) examined the growth of K. pneumoniae and S. aureus (10^4 cfu/ml) in 5% aqueous dextrose solution at 37°C. Whilst K. pneumoniae had maintained its
inoculum level after 24 hours, counts of *S. aureus* fell sharply within 6 hours, attributed to the requirements of *Staphylococcus* for specific amino-acids and vitamins.

The increase in survival of all 3 organisms in the presence of Heparin sodium (a glucosaminoglycan) is of concern due to its use over a 72 hour period with arterial cannula pressure monitoring systems. This has not previously been examined in a comparative study, however Whyte, Niven and Bell (1989) examined growth of 16 species of organisms in 24 small volume parenteral injections: None supported the growth of *S. epidermidis*. Two fluids supported the growth of *K. pneumonia* of which one was heparin sodium solution.

The low level of growth at room temperature observed in this study is in accordance with findings of fluids sampled in clinical use. Despite contamination rates of 6-35% with multiple organisms, only Klebsiellae were found in concentrations greater than 10 organisms/ml. Maki and Martin (1975) proposed that Klebsiellae possessed a selective ability to proliferate in glucose-containing IV fluids at room temperature because they were facultatively psychrophilic. Conversely, Guynn et al. (1973) found that whereas pH and availability of nutrients played a major role in determining survival and growth, temperature was less important: growth of *K. pneumoniae* and death of *S. epidermidis* were merely slowed at 27°C versus 37°C. These findings were extended by this study to conditions simulating clinical practice. The expected higher rate of growth of *K. pneumoniae* versus *S. epidermidis* was not generally seen in this study possibly due to the use of a lower inoculum size and shorter incubation time than in the above studies, reducing the potential for growth. A long lag phase is frequently associated with a small inoculum. In addition, a large change in environmental nutrient availability and osmolality has been shown to cause the death of a large proportion of an inoculum and a marked increase in the lag phase of those surviving, often with a significantly reduced growth phase (Mandlestam and McQuillen 1968). Guynn et al (1973) observed a lag phase of approximately 72 hours with *S. epidermidis* in 5% dextrose solution. To reduce this effect a long incubation time or repeated subculture in minimal media may be employed. In this study, growth of organisms on 1/4 strength nutrient agar provided a degree of nutrient depletion whilst
maintaining clinical relevance, however, it is recognised that the growth profiles observed may have changed with a longer incubation. In an experiment testing methods of detecting contamination of commercial IV solutions, Pappalardo et al. (1988) inoculated 1 litre of 5% dextrose and 0.9% NaCl with 10 and 100 cfu of 9 organisms including *S. epidermidis, Serratia marcescens* and *Enterobacter cloacae*. Up to 50% of organisms were recoverable by filtration after 10 minutes, but by 24 hours after contamination, *S. epidermidis* was not detectable and counts of the Klebsiellae fell progressively in both solutions. This is in agreement with the findings of this study. Meers et al. (1973) investigating the "Devonport Incident" in which 5 patients died due to contamination of 5% dextrose solution at manufacture, inoculated 5% dextrose solution with 7 cfu/ml *K. aerogenes* and allowed a longer incubation. A maximum count of $10^7$ cfu/ml was obtained after 3 weeks.

Growth of *E. faecalis* was greater than may be expected of a Gram-positive organism, generally exceeding that of *K. pneumoniae*. This has not previously been demonstrated. It appears that it may be well suited to growth in intravenous solutions, perhaps due to its wide pH and temperature tolerance, and because of its now frequent isolation in sepsis associated with intravenous therapy, deserves further investigation. These new findings are supported by those of Achaempong (1990) who looked at growth of a related Streptococcal species, *S. pyogenes* ($10^3$ cfu/ml) in 5% aqueous dextrose and 0.9% sodium chloride solutions at 24-26°C. Viability was reduced at $11 \times 10^2$ and $8.7 \times 10^2$ cfu/ml respectively after 4 hours, but increased to $8 \times 10^5$ and $5.2 \times 10^5$ cfu/ml after 3 days.

**COMPLEX SOLUTIONS**

Total Parenteral Nutrition generally comprises a nitrogen source with extra calories from glucose and lipid solutions. Vitamin and mineral supplementation may be added. Early studies examined microbial growth in single constituents. Lipid solutions were found to be good growth media and early USA Centers for Disease Control guidelines recommended a 12 hour maximum infusion time for lipid solutions (Simmons et al. 1982).
Propofol (carrier solution 10% "Intralipid", pH 8.0, 260mOsm/l) has been demonstrated to support rapid growth of *S. aureus* and *E. faecalis*, and growth with endotoxin production in some species of Enterobacteriaceae (Arduino et al 1991). Both *K. pneumoniae* and *E. faecalis* grew well in Propofol in this study, which is in agreement with the findings of Melly *et al.* (1975) of bacterial growth in lipid emulsions. Glucose, generally provided as a 50% dextrose solution has been demonstrated to cause rapid death of both Gram-positive and Gram-negative organisms. The first nitrogen sources were protein (casein or fibrin) hydrolysate solutions which were found to be highly selective for fungi over bacteria (Gelbart *et al.* 1973). The introduction of crystalline amino-acid solutions (with 50% dextrose) was hailed as a partial solution to the problem of infection by retarding the growth of both fungi and bacteria. Gelbart *et al.* (1973), found that crystalline amino-acid solutions retarded growth of *K. pneumoniae* and caused death of *S. aureus* over at 24 hour period at 37°C. They postulated that this was due to their high osmolality, however starved cells were used in a stationary phase of growth.

In 1982, the American Food and Drug Administration approved the use of the 3 in 1 admixtures of amino-acids, dextrose and lipids, which are in common use today (D’Angio *et al.* 1987) Comparison of results between studies is difficult due to the relatively recent introduction of the one bag system and the number of different formulations available, and results appear contradictory. Three studies have compared 3 in 1 admixtures (TNA) with conventional TPN (without lipid): D’Angio *et al.* (1987) found that *S. epidermidis* was unable to grow in any admixture (at room temperature and 10-100 cfu/ml). Growth of *K. pneumoniae* was retarded in TPN but proliferate in TNA. Growth was improved in peripheral as opposed to central formulations presumably due to their lower osmolality. Melly *et al.* (1985) found that *S. aureus* failed to multiply in TPN solutions containing lipid. Conversely, Scheckelhoff *et al.* (1986), whilst observing the non-survival of *S. epidermidis* in 10% and 20% lipid emulsions, found that growth of *S. epidermidis* and *E. faecalis* was greater in TNA solutions than standard TPN, but notably a large inoculum (10⁵ cfu/ml), high temperature (37°C) and 48 hour incubation were employed with a total rather than viable count. Scott *et al.* (1985) found that whilst *S. epidermidis* (50cfu/ml) grew poorly in TPN in the absence...
of lipid, growth was improved in its presence although it was noted that lipid containing solutions were of lower osmolality. Nevertheless, a level of only $10^4$ cfu/ml was obtained in "Kabi 3" (with vitamin and mineral supplementation), after 24 hours at 25°C, with a generation time of 209 minutes. *K. aerogenes* grew readily in all TPN solutions tested with a generation time of 69 minutes in "Kabi 3". Thus, whilst rapid growth of *K. pneumoniae* is supported by the literature, and growth of *E. faecalis* has been observed in one study, evidence regarding growth of *S. epidermidis* is conflicting due to differences in methodology and in the formulations used primarily due to the large variety of amino-acid solutions available. The results of this study demonstrate for the first time that *S. epidermidis* was unable to multiply in “Kabi 3” (without vitamin and mineral supplementation) whereas rapid growth of *E. faecalis* was observed. It may however not be possible to generalise from the results to other TNAs: The presence in a solution of the non-essential amino acid proline (as in “Kabi 3”) has been shown to dramatically increase the ability of bacteria to grow in solutions of high osmolality (Ingram 1987). Conversely, glycine, glutamic acid and aspartic acid (non-essential, also in “Kabi 3”) have been proposed as inhibitory substances (Scheckelhoff 1986). In addition, certain additives such as albumin and vitamins can enhance the ability to support growth (Scheckelhoff 1986). All three organisms in this study exhibited rapid growth in Human albumin solution. Inconsistent findings amongst studies on the effect of amino-acids on growth may also suggest that the tolerance of individual organisms is highly variable and specific for each species (Scott et al. 1985) Variations may occur in the use of type cultures rather than clinical isolates, and in the use of sub-cultured as opposed to wild strains. For example, Llop et al. (1993) demonstrated that outbreak strains of *S. saprophyticus* could proliferate in TPN containing lipid at 25 and 37°C. *S. hominis* was demonstrated in the author’s prospective study (Chapter Three) to have grown to significant levels in a contaminated TNA bag leading to bacteraemia.

The results of this study and review of the literature would suggest that each TPN/TNA solution needs to be assessed for it’s ability to support growth using species and strains of organism prevalent in clinical practice, and the effect of individual additives evaluated for their impact on growth.
LIMITATIONS OF THE STUDY

Impedance measurement was found to be of benefit in comparing solutions supporting bacterial growth. Unfortunately, no results were obtained with Propofol solution due to the lipid emulsion coating the electrodes, and growth curves obtained with TPN were difficult to interpret due to the formation of gas bubbles. This was unexpected in the case of E. faecalis which does not produce gas during fermentation, and may have been due to reaction of by-products within the admixture. The resulting random fluctuations in the curve may mean that the calculated doubling times (K. pneumoniae 22 mins, E. faecalis 19 mins) in TPN were not accurate. Mason (1935) documented doubling times of <20 minutes for Klebsiellae and 25-35 minutes for Staphylococci and Streptococci under optimum conditions. The problem of gas formation could be reduced by the addition of a surfactant or by the measurement of capacitance rather than impedance. The results obtained with growth in Human albumin solution suggest that this method can provide useful information in comparing the ability of intravenous solutions to support bacterial growth.

With further experimentation, impedance measurement may provide an effective means of rapidly and reliably comparing the effect of different TPN components, combinations and additives on different species and strains of bacteria. The technique could also be used to examine growth in clear fluids over a longer time period or with a higher inoculum.

SUMMARY

The results of this study and review of the literature would suggest that each TPN/TNA solution needs to be assessed for its ability to support growth using species and strains of organism prevalent in clinical practice, and the effect of individual additives evaluated for their impact on growth. This can be achieved by impedance measurement.

Propofol and Human albumin solution were demonstrated to require the same level of asepsis as in preparation of parenteral nutrition. This has not as yet been appreciated in clinical practice. Conversely, solutions such as dopamine in 5% dextrose
are unlikely to support growth despite high levels of contamination. Many drugs may be safely diluted in either dextrose or saline solutions. In this study, 5% dextrose solution was found to be a more inhibitory diluent than 0.9% NaCl despite its glucose content. Addition of very small amounts of heparin (a glucosaminoglycan) or insulin (a protein) to sodium chloride solution significantly increased its ability to support growth. This has not previously been recognised. The increase in survival of all 3 organisms in the presence of Heparin sodium is of concern due to its use over a 72 hour period with arterial cannula pressure monitoring systems. This may be of clinical significance.

In summary, this study demonstrated the following new information:

- The Total Nutrient Admixture examined ("Kabi 3") provided a very good medium for growth. Comparable results were obtained with Propofol and Human albumin solutions.
- Solutions with a low pH are inhibitory to growth. 5% glucose is a more inhibitory diluent than 0.9% sodium chloride.
- Addition of very small amounts of nutrient containing drugs such as Heparin or Insulin to electrolyte solutions markedly increases their ability to support bacterial growth.
- *E. faecalis* may be selected as a cause of catheter-related sepsis by its previously unrecognised ability to survive in commonly used intravenous solutions at room temperature.
CHAPTER SIX: FINAL DISCUSSION

Infection is the most frequent life-threatening complication of central venous catheterisation, with a 3.8-14 fold increase in mortality, and an increase in hospital stay of 2-3 weeks, at a cost of greater than $6000 per episode of septicaemia (Maki et al. 1988, Maki 1990, 1991). Infections involving invasive medical devices such as intravascular catheters are proposed to be the most preventable of nosocomial infections (Stamm 1978), however they have been observed to derive from a complex interaction between the microsurface of the foreign body, the host and the pathogen (Mermel and Maki 1994). Three stages are necessary for the development of clinical, catheter-related infection: attachment of micro-organisms to the catheter, persistence and multiplication, and progression from colonisation to infection (Goldman 1990). These are influenced by intrinsic variables affecting the susceptibility of the host, and extrinsic variables relating to the choice and maintenance of the catheter. Once the catheter is inserted, care of the insertion site, the catheter and any attached equipment is primarily the responsibility of the nursing staff. Prevention of infection requires that the potential routes of infection are recognised, the risk assessed, and appropriate preventive measures implemented, both in Infection Control, and individualised nursing care practice.

Many of the extrinsic variables influencing central venous catheter infection have been recognised yet there is little consensus on the optimal methods of care. A major reason for this is a continuing debate over the predominant route of infection. The catheter insertion site has long been recognised as a potential route of infection via the subcutaneous tunnel or along the external surface of the catheter. Nevertheless there is no established standard regimen for frequency of dressing change, cleansing agent or dressing employed. In particular, there are many new transparent plastic film dressings recently available whose risks/effects have not been fully evaluated. Whilst contamination of infusion fluids and delivery systems is also well established, since stricter manufacturing controls and regular change of equipment were instituted, the significance of the intraluminal route of infection had appeared to diminish until the introduction of multi-lumen catheters and reports of intraluminal infection in patients
receiving TPN.

Administration of TPN is a recognised risk factor for infection due to its being a good medium for microbial growth, and frequent administration to immunosuppressed hosts. The aetiology of infection at a microbiological level is unclear. The reasons for an increased risk of infection reported in many studies of triple-lumen catheters is also unclear, and may be due either to the larger entry site and need for an incision at insertion, or to the multiple access points afforded by the delivery system.

Whilst very many studies of central venous catheter-related infection have been performed, the majority have changed practice and attempted to demonstrate a change in the infection rate, or have retrospectively analysed the results. It is difficult to compare or generalise from these results due to differences in (or lack of information on) patient groups, severity of illness, type of catheter, catheter usage and catheter care regimens. Even controlled trials have produced conflicting results due to the influence of these confounding variables, and differences in methodology, in particular definition of infection, sampling and culture techniques, and level of classification of organisms. Many studies fail to examine both routes of infection and few examine the problem from a nursing perspective. Very little information is available on the development of intraluminal colonisation. This chapter will discuss the results of this thesis, a series of studies in one centre examining the impact of variables on risk factors and their modification, in the wider context of the problems of past studies and areas for future research.

CONTRIBUTION OF EXTRINSIC FACTORS TO TIP COLONISATION

The focus of studies one and two was to identify routes of infection and identify the contribution of extrinsic factors to tip colonisation which could be modified in nursing practice to reduce the risk of infection.

The problems perpetuating the debate over the predominant route of infection were discussed on page 41. These are primarily differences in definition of infection, particularly outcome chosen i.e. clinical infection/septicaemia or catheter infection/colonisation, failure to sample or adequately culture to exclude the other route, failure to discriminate to an adequate level to ensure isolates at two sites are
identical, and failure to link the outcome to preceding aetiology i.e. site care and catheter usage. Following a pilot study which identified high risk patient groups (Study One), Study Two was designed to rectify these methodological problems, and provide new information on the aetiology of tip colonisation.

Previous studies examining the route of infection in TPN catheters have been small (less than 50 catheters) and only three have speciated CNS with conflicting results (table 1.5, page 45). Of the four previous studies examining triple-lumen catheters, only two cultured both the catheter hub and insertion site, and none used a quantitative tip culture method to detect intraluminal colonisation or speciated CNS. Where stated, care of the insertion site and administration system varied widely between studies (table 1.6, page 46).

Study Two overcame these previous methodological problems of sampling, culture and classification, finding a reliable and valid culture and typing method for discrimination of organisms within individual patients, the results of which highlighted errors that would have occurred with a less stringent method i.e. organisms falsely similar, and the need for intraluminal culture. This method was shown to be feasible for a clinical laboratory.

The findings are summarised in Chapter Seven. As illustrated in tables 3.34 and 3.35, page 143, rather than a predominant route of infection, both the intra- and extraluminal routes of tip colonisation were demonstrated to occur simultaneously in a single catheter, or to change with consecutive catheters in a single patient. A balance in risk was demonstrated which was shown to be affected by host factors, the type of organism present, cross-infection, the solution being administered through the sets, and insertion site care. For example, administration of TPN and use of multilumen catheters was demonstrated to increase the risk of intraluminal colonisation, and the extended use of plastic film dressings, extraluminal colonisation. This study demonstrated that presence of organisms other than CNS at the insertion site, and moderate or heavy growth of CNS were associated with tip colonisation. A high level of intraluminal colonisation was found which was previously unrecognised. All colonised hubs were associated with a colonised tip, although not necessarily of the same organism, suggesting hub colonisation acted as an indicator of the sterility of the administration system with perhaps repeated contamination and transient hub
Many areas for improvement in our clinical practice were highlighted, although when examining the results in the wider context it must be recognised that several of the limitations of previous studies identified in the literature review apply to these results: In particular, the sample size was small and limited to specific patient groups in a local setting. The results, for example the equal frequency of infection by both routes in patients with a triple-lumen catheter or receiving TPN, and an intraluminal source of bacteraemia in TPN patients, reflected conditions in clinical practice. However, accurate documentation and recording of patient and catheterisation variables, catheter usage and site care allows some generalisation of the results to similar settings, and allows duplication of the study by other authors to confirm these results. Nevertheless, the balance of risk shown to exist between the two routes of infection and demonstrated to be influenced by practice, will, therefore, be altered by changes in, for example, the type of catheter, patient group, severity of illness, and with different catheter maintenance. The effect of the contribution of these individual variables needs to be assessed by repeating the study with a larger sample size and in different settings. Nevertheless, new findings were demonstrated that are important independent of the environment. Both routes of tip colonisation were demonstrated to be able to occur simultaneously by different pathogens, with changing patterns within an individual over time. An intraluminal source of bacteraemia was demonstrated in short term TPN catheters, extending the findings of Sitges-Serra et al., and in contrast to their findings, occurred even in the presence of high skin colonisation. This requires further investigation as the factors influencing development of clinical infection from skin/hub colonisation are currently unknown. Raad et al. (1993) by SEM found no difference in extent of biofilm or degree of colonisation in catheters associated with clinical infection and concluded that colonisation is a common, perhaps universal event but rarely results in infection. Noting that whilst all catheters were colonised by SEM but only 11% could be cultured, they suggest that infection and possibly a positive tip culture may require planktonic organisms. Sitges-Serra and Linares (1988) in reviewing the relationship between positive semi-quantitative culture and bacteraemia, suggest that some time elapses between contamination able to produce a positive culture, and subsequent
bacteraemia. Infection appears to be associated with larger numbers of organisms on tip culture, but the factors hindering/potentiating this have not been determined. Intuitively, they would include the immune competence of the host, virulence of the organism and growth medium. There is some evidence to substantiate this: Arnow et al. (1993), examining consequences of intravascular sepsis noted that most patients with CRS caused by multiple pathogens had rapidly fatal underlying disease. Segura et al. (1990) inoculated P. aeruginosa into the catheter hub (in a rabbit model) which rapidly caused severe infection with all hubs and tips colonised, and bacteraemia in 5/9 cases. In contrast, with S. epidermidis after 15 days, only 5/8 hubs and 2 tips were colonised with no bacteraemia. Salzman et al. (1993) found that 5/7 hubs yielding Candida led to fungaemia, whereas none of 31 cultures of Propionibacterium acnes led to infection. Colonisation of the catheter hub by an organism given a suitable growth medium may allow high levels of bacterial growth in an area outwith host defence mechanisms. In an in vitro model, Merlino et al. (1988) colonised catheters by incubation for 15 hours from an inoculum of approximately 5000 cfu then used them for administration of TPN. If applicable to the clinical situation, the results suggest that in staphylococcal catheter-related sepsis, 1000-10000 cfu are infused into the patient per minute at an infusion rate of 1ml/min reaching $10^{6.5}$ at the start of infusion if the catheter is previously non-infused for a sufficient period. This may explain the high association of hub colonisation with bacteraemia observed in this study and is an important area for further research.

**INTRALUMINAL CONTAMINATION AND TIP COLONISATION**

Studies Three and Four were exploratory studies to add to the little information available on intraluminal contamination and tip colonisation. Study Three looked at the use of IV filters and again identified high levels of intraluminal contamination in our clinical practice, rising with time in situ, and showed in-line filters were effective in preventing passage of micro-organisms, and cost effective. Limitations of the study were the haphazard use of filters, need for removal for administration of fat emulsions, blood etc., and their lack of availability outwith the Intensive Care Unit. To overcome these problems, the study needs to be repeated with a much larger sample size, and the level of contamination determined in other centres.
Use and culture of filters provides a simple way for individual units to determine if a problem exists with intraluminal contamination in their practice, and to evaluate the effectiveness of any change in practice. The study also demonstrated for the first time that the use of in-line IV filters can prevent intraluminal colonisation of the catheter. A large controlled trial (with standardised skin care) is required to determine the effect of this on tip colonisation and clinical infection. In addition, filters should be used in \textit{in vitro} simulations to determine the effect of different types of catheter, administration equipment and preventative measures such as routine administration set change.

Other measures aimed at preventing intra-luminal colonisation are available. Halpin et al. (1991), in 47 TPN catheters, found a significant reduction in catheter-related sepsis when the catheter hub was enclosed in a Betadine shield. Inoue et al. (1992) in 230 TPN catheters (used for multiple access), demonstrated that the rate of catheter-related sepsis was significantly lower in a group using a new connection system (latex end injection port attached to the hub with a needle device attached to the infusion set) compared to a three-way tap and/or piggyback system. The effect of these systems is likely to be due to a mixture of protection of the connection from contact with underlying skin, removal of three-way taps and stopcocks, and hindering disconnection of the catheter for non-designated purposes. Whilst this may be very effective in TPN administration, where admixtures are prepared and connected to an administration set under a laminar flow hood in pharmacy, the results of this study suggest that in addition to manipulation of the hub/administration set junction, a significant level of contamination occurs upstream, from routine bag/syringe changes or top-up of infusions. This contamination is frequently unnoticed when sampling infusion fluid, and the clinical relevance of repeated low levels of contamination is not known. However the 38% level of contamination detected in the filters (including 30% where filters were not disconnected/opened during use) is comparable to the 31% contamination of triple-lumen catheter hubs detected in the previous study, and correlated with tip colonisation and septicaemia.

Further work is needed on the dynamics of intraluminal colonisation, such as the effect of solution infused and infusion rate on the outcome of contaminants introduced into the administration system. Whilst the incidence of growth within IV
filters in Chapter Four was of a similar level to the incidence of hub colonisation demonstrated in the previous study, many organisms found on the tip of triple-lumen catheters could not be demonstrated on the catheter hub/three-way tap at the time of catheter removal supporting earlier suggestions that hub colonisation may be transient or related to the organism involved.

The relationship between organisms passing through the administration set or three-way taps with subsequent hub colonisation is not known. In an *in vitro* model, Locci *et al.* (1981) found that increased perfusion time or increased inoculum increased adherence to intravenous cannulae. Segura *et al.* (1990) examined intraluminal contamination in an *in vivo* model and proposed that the consequences may differ according to the motility, adherence and virulence of the organism involved; whilst *Ps. aeruginosa* rapidly colonised the tip, they postulated that Staphylococci required repeated contamination and sufficient multiplication time to achieve a large enough inoculum. In clinical practice, Sitges-Serra *et al.* (1990) proposed that TPN contaminated with a low inoculum may be able to colonise the catheter hub and result in sepsis several days later. Indeed Snydman *et al.* (1982) observed that in TPN patients, "line violations" for example administering a drug through the line, resulted in infection three days later.

In relation to change of administration sets it is possible that four administration sets changed 24 hourly each with a 15% rate of contamination are equivalent to 60% contamination of a single set after 4 days. In practice there is the danger that albeit small numbers of organisms, adhere to the tubing or within the luer attachments and multiply to larger numbers with increasing time. In the study by Quercia *et al.*, 11% of the filter sets not containing a filter membrane exhibited bacterial growth, after 24 hours, due to adherence to the plastic casing. Frequent change of giving sets may prevent significant multiplication of organisms, however similar dynamics of adherence are liable to affect colonisation of the catheter hub and lumen, which of course cannot be changed during the period of catheterisation. *In vitro* simulations reflecting catheter usage in the clinical situation are required to determine the outcome, and assess the significance of, intraluminal contamination of intravenous administration systems, the differences between types of equipment, and the effectiveness of measures such as frequent change of giving sets on the development of tip colonisation. The use and culture of in-line
filters would provide an effective measure of in-use microbial contamination \textit{in vitro} and \textit{in vivo}.

\textbf{BACTERIAL GROWTH}

Study Four was examined bacterial growth in a small sample of drugs and limited range of organisms and therefore the results cannot be generalised to different concentrations or strains. However, it was demonstrated that adding drugs such as insulin or heparin to electrolyte solutions was equivalent to adding nutrients and increased bacterial growth. Further \textit{in vitro} work is required in this area, and impedance measurement may be of benefit. \textit{E. faecalis} was found to grow exceptionally well for a Gram-positive organism and this needs further investigation. Unfortunately the strain used for the experiment, chosen as being the predominant clinical isolate from Study Two, was subsequently found to be a high-level gentamicin resistant epidemic strain and may therefore be atypical, and there is a need to repeat the experiment with several different strains of \textit{E. faecalis}.

In addition to \textit{in vitro} experimentation, clinical studies are required to correlate the differences in formulations and corresponding growth rates with clinical symptoms. Whilst \textit{in vitro} experimentation can provide information on relative growth rates, additional factors may influence bacterial growth/survival \textit{in vivo}. Whilst this study demonstrated minimal growth in clear fluids over 24 hours, growth may occur with a longer time-span, in standing columns of fluid in burettes or pressure-monitoring systems, or within stopcocks and extension sets. Maki \textit{et al.} (1973) found that once organisms were introduced into an administration system they could persist for days despite regular changes of the infusion bag and high rates of flow. Even small numbers of contaminating organisms may grow on reaching a clot associated with a catheter (Holmes and Allwood 1979) or after adherence to the catheter itself (Marrie and Costerton 1984). In laboratory models, perfusion of a catheter with organisms in solution rapidly leads to catheter colonisation (Bayston 1984). Once adherent, bacteria are able to grow on catheter surfaces even in the absence of externally supplied nutrients by utilizing catheter components, with demonstrable catheter surface erosion \textit{in vitro} (Norwood 1991). In addition, in experiments of growth in fluids, Guynn \textit{et al.} (1973) proposed that contamination of fluids by organic matter or organisms during production.
even if killed by sterilization, provides a source of molecular carbon, sulphur and nitrogen. This is supported by the original study by Favero (1971) demonstrating growth of *Pseudomonas aeruginosa* in sterile distilled water. In addition, atmospheric carbon-dioxide and ammonia may contribute to growth as suggested by the study of Biggar and Nelson (1943). Furthermore, the addition of minute quantities of blood to dextrose solutions has been shown to buffer the fluid and provide nutrients for organisms otherwise incapable of growth (Ansel and Gigadent 1971) and it has been suggested that blood components may diffuse slowly from the catheter into fluid in the administration set (Achaempong 1990).

Nevertheless, it is generally true that the number of invading organisms is the most important factor in determining whether an infection develops (Meers and Calder 1973), and thus those organisms with the ability to proliferate in infusion fluids are more likely to cause nosocomial infection than those that do not (Lamikanra and Sofekun 1988). As previously stated, growth of *E. faecalis* was greater than may be expected of a Gram-positive organism, generally exceeding that of *K. pneumoniae*, and it appears that *E. faecalis* may be well suited to growth in intravenous solutions, perhaps due to its wide pH and temperature tolerance, and, because of its now frequent isolation in sepsis associated with intravenous therapy, deserves further investigation.

**SUMMARY**

By addressing gaps in the literature and in the methodology of previous studies, this thesis has provided new findings which can be verified and extended by similar research in a variety of additional settings, and which highlight important areas for further research. At a local level, many areas for improvement in clinical practice were highlighted which will reduce the risk of catheter-related infection. These are summarised in the concluding chapter.
CHAPTER SEVEN: SUMMARY OF RESULTS AND IMPLICATIONS FOR PRACTICE

This thesis had two concurrent overall aims, to address local clinical problems, and to provide new knowledge of the aetiology of tip colonisation at a microbiological level. Both of these aims were achieved: The retrospective survey highlighted two areas of concern, with high levels of tip colonisation demonstrated in patients with triple-lumen catheters, and patients receiving Total Parenteral Nutrition. One third of patients on TPN developed clinical septicaemia. This was unrecognised prior to the survey, and was confirmed by the prospective study.

The prospective study successfully used culture and typing methods that enabled a source for these infections to be determined, and combined with accurate recording of patient and practice variables, provided new information on the microbiological factors leading to tip colonisation. New information on the epidemiology of tip colonisation was provided, which was found to be complex, with multiple organisms involved at different sites. In triple-lumen catheters, there was found to be no predominant route of tip colonisation. Indeed, this study is the first to document concurrent tip colonisation in a single patient by both the intra- and extraluminal routes, by pathogenic organisms (K. pneumoniae and E. faecalis, figure 3.33, page 143). In a further patient, an organism found on the catheter hub and tip of a first catheter was demonstrated on the insertion site and tip in a subsequent catheter (figure 3.34, page 143). These findings suggest a balance which may change over time, and an association between flora at the insertion site and within the catheter hub was demonstrated, with skin colonisation a risk factor for hub colonisation by either direct contact with colonised skin or via contaminated hands of staff. This balance between the extra- and intraluminal routes of tip colonisation was strongly related to nursing/medical practice: Whilst the level of growth on the skin and progression to clinical infection is affected by host susceptibility, the risk of colonisation is increased by the use of plastic film dressings with inadequate skin disinfection, and an increasing interval between dressing changes. Skin colonisation increased with duration of catheterisation in a setting where dressings were not routinely disturbed before catheter removal. The concerns raised in the literature over the use of
transparent dressings were thus confirmed. The likelihood of an identical organism on
the tip and insertion site also increased with time, with an average of 8 days. In
contrast, hub colonisation was not related to duration of catheterisation, as shown by
comparing positive and negative lumens of the same triple-lumen catheter, but was
significantly related to the number of breaks in the system. This has not previously
been demonstrated. Hub colonisation is independent of host susceptibility, and is
entirely reflective of nursing/medical practice, with unexpectedly high levels of
intraluminal contamination found to occur during routine practice. Whilst intraluminal
colonisation may occur less frequently than extraluminal colonisation, it appears from
this study to be associated more frequently with bacteraemia. Preventing intraluminal
colonisation was shown to be achievable by the use of in-line filters.

The influence of practice on tip colonisation in TPN catheters was less clear,
where, despite equally high levels of skin colonisation, and comparatively low
numbers of breaks to the catheter/administration system, 92% of catheters exhibited
intraluminal colonisation, and an intraluminal route of bacteraemia/clinical infection
was demonstrated. These results therefore agree with the findings of the team of
Linares, Sitges-Serra and Segura, and extend the results to short term TPN catheters,
in the presence, unlike their previous studies, of significant skin colonisation. One
episode of bacteraemia was found to be due to a contaminated bag of TPN from the
addition of supplements on the ward, compounded by the overly long hang times
observed in the absence of automatic pumps.

The TPN solution in use (Kabi 3, without additives) was found to facilitate
rapid bacterial growth in vitro. Other high risk solutions were identified such as
Propofol and Human albumin solution, which whilst this would be expected from
their similar constituents to TPN, do not receive the same level of aseptic precautions
in clinical practice. An unexpected, but significant finding was the increase in the
ability to support bacterial growth observed of simple intravenous solutions when very
small amounts of additives such protein solutions or those possessing lipid carriers
were added. Information on the growth of skin flora, rather than environmental
contaminants in common drug containing IV solutions used in the ICU was not
previously available. An important finding was the previously unrecognised ability of
E. faecalis to grow to the same or greater extent as Klebsiella pneumoniae. Together
with the observation that the presence of *E. faecalis* on the skin led to tip colonisation in 75% of cases, this may explain the recent emergence of *E. faecalis* as one of the most common pathogens in catheter-related infection.

**IMPLICATIONS FOR PRACTICE**

The results demonstrate that rather than one predominant route of infection, there is a balance between the risk of intraluminal and extraluminal colonisation which is modified by clinical practice. To prevent infection, measures to reduce the risk of infection by both routes need to be employed. Patients with triple-lumen catheters, or receiving TPN were identified as a high risk group. In the short-term catheters studied, both the intraluminal and extraluminal routes of infection were found to operate in these patients, even within the same patient at one time or with subsequent catheters. The studies demonstrated the potential for improvement in both these areas, i.e. care of the insertion site, and maintenance of the administration system, and the need for prevention of cross infection between patients was demonstrated. Recommendations for changes in practice arising from these results are summarised below.

In addition to modifying practice in the care of high risk groups, surveillance cultures may be employed. This study demonstrated that presence of organisms other than CNS at the insertion site, and moderate or heavy growth of CNS were associated with tip colonisation. A high level of intraluminal colonisation was found which was previously unrecognised. All colonised hubs were associated with a colonised tip, although not necessarily of the same organism. Swabbing of the insertion site at dressing change, and of the catheter hub at change of the administration set would thus be of value in identifying patients at high risk of catheter-related infection.

As a result of these studies, the following recommendations can be made:
Catheter insertion /insertion site care

- There is a need to standardise the procedure for insertion of central venous catheters.
- Chlorhexidine solutions should be used for insertion and subsequent site care.
- Transparent dressings should not be left in situ for the duration of catheterisation.
- Routine regular redressing of insertion sites should be implemented.
- Details of catheter insertion and subsequent site care should be documented.
- The detection of non-skin organisms such as *E. faecalis* at the insertion site is a risk factor for subsequent infection. Surveillance cultures should be performed in high-risk patients.
- The need for continued central venous catheterisation should be assessed prior to leaving ICU.

Care of the administration set/triple-lumen catheters

- Catheters should be disconnected as infrequently as possible.
- Aseptic technique should be used for all disconnections to the catheter/administration system.
- In-line filters should be used where possible, particularly the lumen used for bolus injections or where high numbers of disconnections are anticipated.
- The catheter hub/three way taps should be protected from contact with skin flora or respiratory secretions.
- Where their use is unavoidable, three-way taps should be changed with the administration set or at least 72 hourly.
- The same level of asepsis as for TPN should be used for Propofol, Human albumin solution and other lipid/amino acid solutions. These precautions should apply to all lumens of a triple-lumen catheter.
- The catheter should be removed or changed to a single-lumen catheter as soon as possible.
• Demonstration of a contaminated catheter hub, e.g. by surveillance culture, is an indication of a colonised catheter tip.

TPN administration
• Triple-lumen catheters should not routinely be used for administration of TPN.
• The catheter should not be used for other purposes.
• Each TNA should be assessed for its ability to support growth of clinically prevalent organisms.
• Additives to the bag should be made in pharmacy.
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• The hang time of a bag of TPN should be restricted to 24 hours via a pump.
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APPENDIX ONE: VARIABLES RECORDED IN RETROSPECTIVE STUDY

**Patient variables**
- Hospital number
- Sex
- Age
- Date of admission
- Hospital speciality
- Ward
- Consultant
- Reason for admission
- Underlying disease
- Operative procedures
- Antibiotic therapy
- Immunosuppressive therapy
- Change of ward or ICU admission (dates)
- Apache II score
- Pyrexia >37°C
- Microbiology results

**Catheterisation variables**
- Catheter number
- Date of insertion
- Days between admission and insertion
- Anatomical site of insertion
- Tunnelled or non-tunnelled
- Type of catheter
- Size of catheter
- Place of insertion
- Reason for insertion
- Use for TPN

All comments relating to central venous catheter, or possible infective process (any site)

- Date of catheter removal
- Reason for removal
- Days *in situ*
- Result of tip culture
- Final diagnosis or post-mortem results
- Outcome of admission
APPENDIX TWO: STAPHYLOCOCCAL SPECIES

STAPHYLOCOCCAL SPECIES

Coagulase positive
S. aureus
S. intermedius
S. delphini

Coagulase variable
S. hyicus

Coagulase-negative
S. arlettae
S. auricularis
S. capitis
S. caprae
S. carnosus
S. caseolyticus (formerly Micrococcus)
S. chromogenes
S. cohnii
S. epidermidis
S. equorum
S. gallinarum
S. haemolyticus
S. hominis
S. kloosii
S. lentus
S. lugdunensis
S. saccharolyticus (formerly Peptococcus)
S. saprophyticus
S. schleiferi
S. simulans
S. warneri
S. xylosus
APPENDIX THREE: CODING OF REASON FOR CATHETER INSERTION

PRESENTING CONDITIONS (Number of patients)

"Access"
no peripheral access (4)
cardiac arrest (12)
pacing (1)
centrally administered drugs - dopamine (1)

amiodarone (4)

"Fluid balance"
dehydration - obstruction (1)
vomiting (2) and diarrhoea (1)
diabetes (1)
hypotension - cause unknown (2)
post-operative (1)
trauma (2)
hypovolaemia
gastrointestinal bleed - haematemesis (6)
-per rectum (2)
-varices (1)
shock
septic (6)
hypervolaemia
renal failure (4)
cardiac failure (4)
haemolytic anaemia (1)
pancreatitis (10)

"Access and fluid balance"
Major operation
oesophagogastrrectomy (8)
Whipples or Hartmanns procedure (5)
other abdominal (8)
total cystectomy (1)
aortobifemoral bypass (6)
abdominal aortic aneurysm repair (9)
other (1)
Intensive care
unconscious (5)
ventilation (5)
other (3)

shock (5) / haemorrhage (3) requiring ICU admission

"Total parenteral nutrition"
Administration of intravenous feeding solutions requiring delivery into a central vein (18)
APPENDIX FOUR: PRE-EXISTING INFECTION AT OTHER BODY SITES

<table>
<thead>
<tr>
<th>INFECTION*</th>
<th>ORGANISM</th>
<th>TIP RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>urinary tract, chest</td>
<td>No growth</td>
<td></td>
</tr>
<tr>
<td>infected renal haematoma</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>faecal peritonitis</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>pneumonia with DIC</td>
<td>S. epidermidis</td>
<td>No growth</td>
</tr>
<tr>
<td>septicaemic shock</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>pneumonia with DIC</td>
<td><em>S.pneumoniae</em></td>
<td>none</td>
</tr>
<tr>
<td>septicaemia</td>
<td><em>E.coli</em></td>
<td>No growth</td>
</tr>
<tr>
<td>chest</td>
<td><em>E. coli</em></td>
<td></td>
</tr>
<tr>
<td>cholangitis, septic shock</td>
<td>K.pneumoniae</td>
<td>No growth</td>
</tr>
<tr>
<td>appendicitis, pneumonia</td>
<td><em>S.epidermidis</em></td>
<td>None</td>
</tr>
<tr>
<td>septicaemia</td>
<td></td>
<td>No growth</td>
</tr>
<tr>
<td>cholangitis, bronchopneumonia</td>
<td><em>S.aureus</em></td>
<td></td>
</tr>
<tr>
<td>bronchopneumonia, sepsis</td>
<td><em>E.faecalis</em></td>
<td></td>
</tr>
<tr>
<td>cholangitis, septic shock</td>
<td><em>Proteus sp.</em></td>
<td></td>
</tr>
<tr>
<td>septicaemia</td>
<td><em>Proteus sp.</em></td>
<td>S.epidermidis</td>
</tr>
<tr>
<td>chest</td>
<td><em>E.faecalis</em></td>
<td>Yeasts</td>
</tr>
<tr>
<td>faecal peritonitis</td>
<td>No growth</td>
<td></td>
</tr>
<tr>
<td>septicaemia</td>
<td>(1) No growth</td>
<td></td>
</tr>
<tr>
<td>pneumonia</td>
<td>(2) S.epidermidis</td>
<td></td>
</tr>
<tr>
<td>peritonitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>perforation, septicaemia</td>
<td>(1) No growth</td>
<td></td>
</tr>
<tr>
<td>septicaemia (from catheter)</td>
<td>(2) S.epidermidis</td>
<td>None</td>
</tr>
<tr>
<td>septicaemia</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>cellulitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chest</td>
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</tr>
<tr>
<td>perforation, septicaemia</td>
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<tr>
<td>chest</td>
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<td></td>
</tr>
<tr>
<td>meningitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUO, malaria, gastroenteritis</td>
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* as documented in medical notes
## APPENDIX FIVE: MICROMETHODS

### API STAPH

<table>
<thead>
<tr>
<th>TESTS</th>
<th>SUBSTRATE</th>
<th>REACTIONS/ENZYMES</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>POSITIVE</td>
</tr>
<tr>
<td>0</td>
<td>No Substrate</td>
<td>Negative control</td>
<td>-</td>
</tr>
<tr>
<td>GLU</td>
<td>D-Glucose</td>
<td>(Positive control)</td>
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</tr>
<tr>
<td>FRU</td>
<td>D-Fructose</td>
<td>Acidification due to the breakdown of the carbohydrate</td>
<td>Yellow</td>
</tr>
<tr>
<td>MNE</td>
<td>D-Mannose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAL</td>
<td>Maltose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAC</td>
<td>Lactose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRE</td>
<td>D-Trehalose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAN</td>
<td>D-Mannitol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XLT</td>
<td>Xylitol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEL</td>
<td>D-Melibiose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIT</td>
<td>Potassium Nitrate</td>
<td>Reduction of nitrate to nitrite</td>
<td>NIT 1+NIT 2-10MIN</td>
</tr>
<tr>
<td>PAL</td>
<td>a-methyl phosphate</td>
<td>Alkaline phosphatase</td>
<td>ZYM A+ZYM B-10 MIN</td>
</tr>
<tr>
<td>VP</td>
<td>Sodium pyruvate</td>
<td>Production of acetyl-methyl-carbinol</td>
<td>VP 1+VP 2-10MIN</td>
</tr>
<tr>
<td>RAF</td>
<td>Raffinose</td>
<td>Acidification due to the breakdown of the carbohydrate</td>
<td>Yellow</td>
</tr>
<tr>
<td>XYL</td>
<td>Xylose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAC</td>
<td>Saccharose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDG</td>
<td>a-methyl glucoside</td>
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<td></td>
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<tr>
<td>NAG</td>
<td>N-acetyl-glucosamine</td>
<td></td>
<td></td>
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<tr>
<td>ADH</td>
<td>Arginine</td>
<td>Arginine dihydrolase</td>
<td>Red</td>
</tr>
<tr>
<td>URE</td>
<td>Urea</td>
<td>Urease</td>
<td>Red-violet</td>
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</table>

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### APPENDIX FIVE CONTINUED:

#### API 20E

<table>
<thead>
<tr>
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<th>SUBSTRATES</th>
<th>REACTIONS /ENZYMES</th>
<th>RESULTS</th>
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248
APPENDIX SIX: CULTURE MEDIA

ISO-SENSITEST AGAR

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CLED MEDIUM

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**BLOOD AGAR BASE**

Formula  
Protease peptone  15.0  
Liver digest  2.5  
Yeast extract  5.0  
Sodium chloride  5.0  
Agar  12.0  

**NUTRIENT AGAR**

Formula  
“Lab lemco” powder  1.0  
Yeast extract  2.0  
Peptone  5.0  
Sodium chloride  5.0  
Agar  15.0  

**TRYPTONE SOYA BROTH**

Formula  
Pancreatic digest of casein  17.0  
Papaic digest of Soybean meal  3.0  
Sodium chloride  5.0  
Dibasic potassium phosphate  2.5  
Dextrose  2.5
## APPENDIX SEVEN: ORGANISMS ISOLATED ON FILTER CULTURE

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- = No Growth  
+ = Growth
## APPENDIX NINE: TYPE AND DURATION OF USE OF FILTERS

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<td>DOPAMINE (CONT)</td>
<td>EPOPROSTENOL</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>BURETTE</td>
<td>CVP</td>
<td>4</td>
</tr>
<tr>
<td>(91) 4</td>
<td>SYRINGE</td>
<td>BAG</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>INSULIN</td>
<td>DIGOXIN</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>BAG</td>
<td>POTASSIUM CHLORIDE</td>
<td>2</td>
</tr>
<tr>
<td>(92) 4</td>
<td>BAG</td>
<td>SYRINGE</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>CVP</td>
<td>DOPAMINE</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>BAG</td>
<td>EPOPROSTENOL</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>SYRINGE</td>
<td>DOPAMINE</td>
<td>3</td>
</tr>
<tr>
<td>(93) 2</td>
<td>BURETTE</td>
<td>SYRINGE</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>GLYCERYL TRINITRATE</td>
<td>MIDAZOLAM</td>
<td>1</td>
</tr>
<tr>
<td>(94) 3</td>
<td>SYRINGE</td>
<td>BAG</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>EPOPROSTENOL</td>
<td>DIGOXIN</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>SYRINGE</td>
<td>POTASSIUM CHLORIDE (CONT)</td>
<td>2</td>
</tr>
<tr>
<td>(95) 3</td>
<td>SYRINGE</td>
<td>BAG</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>EPOPROSTENOL (SWITCHED)</td>
<td>POTASSIUM CHLORIDE (CONT)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>SYRINGE</td>
<td>CVP</td>
<td>4</td>
</tr>
<tr>
<td>(96) 2</td>
<td>BURETTE</td>
<td>SYRINGE</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>DIGOXIN (CONT)</td>
<td>CVP</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>BAG</td>
<td>EPOPROSTENOL</td>
<td>2</td>
</tr>
<tr>
<td>(97) 4</td>
<td>BURETTE</td>
<td>SYRINGE</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>DOPAMINE</td>
<td>CVP</td>
<td>3</td>
</tr>
<tr>
<td>(98) 3</td>
<td>SYRINGE</td>
<td>BAG</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>CVP</td>
<td>MIDAZOLAM</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>SYRINGE</td>
<td>BAG</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>DOPAMINE</td>
<td>ADRENALINE (CONT)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>BURETTE</td>
<td>CVP</td>
<td>3</td>
</tr>
<tr>
<td>(99) 3</td>
<td>BURETTE</td>
<td>SYRINGE</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>ADRENALINE (CONT)</td>
<td>DOPAMINE</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>BAG</td>
<td>INSULIN</td>
<td>2</td>
</tr>
<tr>
<td>(100) 1</td>
<td>SYRINGE</td>
<td>BAG</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>SYRINGE</td>
<td>SYRINGE</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>ATRACURIIUM</td>
<td>ALFENTANYL</td>
<td>1</td>
</tr>
</tbody>
</table>
APPENDIX TEN: ASSESSMENT OF THE FINANCIAL IMPLICATIONS OF FILTERS

<table>
<thead>
<tr>
<th>giving set</th>
<th>total used with filter</th>
<th>number needed without filter</th>
<th>number of sets saved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male-female</td>
<td>43</td>
<td>126</td>
<td>83</td>
</tr>
<tr>
<td>60 drop</td>
<td>54</td>
<td>175</td>
<td>121</td>
</tr>
<tr>
<td>20 drop</td>
<td>23</td>
<td>51</td>
<td>28</td>
</tr>
</tbody>
</table>

Filter
ELD96 £7.36 x 100 = £736.00

Consumables
Male-female Lectrocath £1.93 + 50ml BD syringe £0.32 x83 = £186.75
60 drop Baxter Buretrol £2.80 + 500ml 5% dextrose/0.9% NaCl £0.70 x121= £423.50
20 drop Baxter RM £0.67 x28 = £18.76
£629.01

Drugs
estimating burettes/syringes discarded when on average half full daily versus every four days:
savings e.g. dopamine 37/2 x £3.77 = £69.75
glyceryl trinitrate 6/2 x £21.33 = £63.99
alfentanil 13/2 x £13.25 = £86.13
vecuronium 6/2 x £25.38 = £76.14
epoprostenol 3/2 x £103.90 = £155.85
£451.86
APPENDIX ELEVEN: STATISTICAL TESTS

Statistical guidance was provided by Mr. Peter Williams, Department of Mathematics, University of Surrey. The following tests were utilised:

**DESCRIPTIVE STATISTICS**

**Measures of location**
Mean, mode, median, upper and lower quartiles.

**Measures of variability**
Range, inter-quartile range (box-whisker plots) and standard deviation.

**STATISTICS OF INFEERENCE**

**Samples of continuous data**
Two-sample Student’s t-test to compare sample means,
Chi-squared comparison of proportions, and for small samples, Fisher’s exact test of association.

**Samples of non-continuous data**
Mann-Whitney $U$ test

**FORMULAE**

The Mann-Whitney $U$ test and Fisher’s exact test were performed using SPSS (SPSS inc. 1993, Chicago), using the general formulae below. Chi-squared tests, t-tests and standard deviation were performed using SPSS, or Microsoft Excel (Microsoft corporation, USA, version 5.0), using the attached algorithms.

**MANN-WHITNEY U TEST**

\[ z = \frac{T-n_1(n_1+ n_2+ 1)/2}{\{ n_1 n_2(n_1+ n_2+ 1)/12\} } \]

**FISHER’S EXACT TEST**

\[ p = \frac{m!n!r!s!}{N!a!b!c!d!} \]
CHITEST
See Also

Returns the test for independence. CHITEST returns the value from the chi-squared ($\chi^2$) distribution for the statistic and the appropriate degrees of freedom. You can use $\chi^2$ tests to determine if hypothesized results are verified by an experiment.

Syntax

CHITEST(actual_range, expected_range)

Actual_range is the range of data that contains observations to test against expected values.

Expected_range is the range of data that contains the ratio of the product of row totals and column totals to the grand total.

Remarks

- If actual_range and expected_range have a different number of data points, CHITEST returns the #N/A error value.
- The $\chi^2$ test first calculates a $\chi^2$ statistic and then sums the differences of actual values from the expected values. The equation for this function is $\text{CHITEST} = P(X > \chi^2)$, where:

$$X^2 = \sum_{i=1}^{r} \sum_{j=1}^{c} \frac{(A_{ij} - E_{ij})^2}{E_{ij}}$$

and where:

- $A_{ij}$ = actual frequency in the $i$-th row, $j$-th column
- $E_{ij}$ = expected frequency in the $i$-th row, $j$-th column
- $r$ = number of rows
- $c$ = number of columns

CHITEST returns the probability for a $\chi^2$ statistic and degrees of freedom, df, where df = $(r - 1)(c - 1)$.

Example

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Actual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>3</td>
<td>Agree</td>
<td>58</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>Neutral</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>Disagree</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Expected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>9</td>
<td>Agree</td>
<td>45.35</td>
<td>47.65</td>
</tr>
<tr>
<td>10</td>
<td>Neutral</td>
<td>17.56</td>
<td>19.44</td>
</tr>
<tr>
<td>11</td>
<td>Disagree</td>
<td>16.09</td>
<td>16.91</td>
</tr>
</tbody>
</table>

The $\chi^2$ statistic for the data above is 16.16957 with 2 degrees of freedom.

CHITEST(B3:C5, B9:C11) equals 0.000308
t-Test: Two-Sample Assuming Unequal Variances analysis tool

Performs a two-sample student's t-test. This t-test form assumes that the variances of both ranges of data are unequal; it is referred to as a heteroscedastic t-test. You can use a t-test to determine whether two sample means are equal. Use this test when the groups under study are distinct. Use a paired test when there is one group before and after a treatment. For more information about dialog box options, click ».

The formula used to determine the test statistic value $t$ is:

$$t' = \frac{\bar{x} - \bar{y} - \Delta_0}{\sqrt{\frac{s_1^2}{m} + \frac{s_2^2}{n}}}$$

The following formula is used to approximate the degrees of freedom. Because the result of the calculation is usually not an integer, use the nearest integer to obtain a critical value from the $t$ table.

$$df = \frac{\left(\frac{s_1^2}{m} + \frac{s_2^2}{n}\right)^2}{\frac{(s_1^2/m)^2}{m-1} + \frac{(s_2^2/n)^2}{n-1}}$$
STDEV
See Also

Estimates standard deviation based on a sample. The standard deviation is a measure of how widely values are dispersed from the average value (the mean).

Syntax

STDEV(number1, number2,...)

Number1, number2,... are 1 to 30 number arguments corresponding to a sample of a population. You can also use a single array or a reference to an array instead of arguments separated by commas.

Remarks

- STDEV assumes that its arguments are a sample of the population. If your data represents the entire population, you should compute the standard deviation using STDEVP.
- The standard deviation is calculated using the "nonbiased" or "n-1" method.
- STDEV uses the following formula:

\[ \sqrt{ \frac{n \sum x^2 - (\sum x)^2}{n(n-1)} } \]

Example

Suppose 10 tools stamped from the same machine during a production run are collected as a random sample and measured for breaking strength. The sample values (1345, 1301, 1368, 1322, 1310, 1370, 1318, 1350, 1303, 1299) are stored in A2:E3, respectively. STDEV estimates the standard deviation of breaking strengths for all the tools.

STDEV(A2:E3) equals 27.46
INTRALUMINAL CONTAMINATION OF TRIPLE LUMEN CATHETERS

Karen Lee MSc. BN.
Intensive Care Unit, Frimley Park Hospital, Surrey
and Department of Nursing & Midwifery,
University of Surrey
INTRODUCTION

Central venous catheter infection may occur extraluminally via the skin of the insertion site, or intraluminally secondary to contamination of the catheter hub or infusate.Whilst intraluminal contamination may be prevented from reaching the patient by the use of in-line microbial filters, they are not widely used as an infection control measure, although their use has been suggested to be of benefit in high risk areas such as Intensive Care.

This study highlights the problem of intraluminal contamination of triple lumen catheters (TLC’s) demonstrated by a study aimed at determining the predominant route of catheter related infection in the critically ill patient.

TIP CULTURE

Catheter tips and hubs were cultured from 50 adult surgical patients using semi-quantitative and quantitative methods. The results showed a high incidence of bacterial growth on tip culture of triple lumen catheters of 59% compared to 11% of single lumen catheters (SLC’s). Triple lumen catheters were found to be associated with a higher severity of illness (by APACHE II scoring), duration of catheterisation and rate of disconnection of the catheter for bag/line changes and bolus injections.

<table>
<thead>
<tr>
<th>CATHETER TYPE</th>
<th>APACHE SCORE</th>
<th>DAYS IN SITU</th>
<th>DISCONNECTIONS/DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+VE</td>
<td>-VE</td>
<td>+VE</td>
</tr>
<tr>
<td>Single lumen</td>
<td>9</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Triple lumen</td>
<td>18</td>
<td>13</td>
<td>7</td>
</tr>
</tbody>
</table>

HUB CULTURE

Growth was obtained on swabbing the hubs of 10% SLC lumens and 31% TLC lumens, and from 20% of attached three-way taps. In both of the two cases of clinical septicaemia in patients with a TLC an identical organism (by biotype, antibiogram and phage type) was isolated from the three-way tap, catheter hub and tip.

Growth on hub culture was assumed to be reflective of practice and independent of patient variables such as severity of illness. The proportion of hubs exhibiting growth increased with an increase in the number of disconnections (fig 1.0). This relationship remained when individual lumens were matched for time in situ.

Very high rates of disconnection (maximum 26/day) and total numbers (maximum 203 in 10 days) were observed in TLC’s. The mean total number of disconnections was 40 for lumens exhibiting growth as opposed to 20 for lumens with no growth, with a mean of 88 disconnections for positive tips versus 37 for negative tips. This was a highly significant difference (Mann Whitney U test p<0.005).
FILTER CULTURE

Subsequently, a trial of in-line IV filters (Pall ELD 96) was undertaken in the Intensive Care Unit. These filters possess a 0.2µm positively charged membrane to retain microorganisms, endotoxin and particles over a maximum of 4 days.

Forty eight filters were cultured from TLC’s at a mean of 2.6 days/filter. Organisms were cultured from the membrane of 41% of filters and included K. pneumoniae, S. aureus and E. faecalis.

The frequency of growth on culture increased with the time the filter was in situ (fig 2.0) with 50% of filters removed on day three or four exhibiting growth. One or more contaminated filter was obtained from 79% of catheters.

No growth was obtained from the catheter hubs confirming the effectiveness of the filters.
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