Enterohaemorrhagic and other Shiga toxin producing *Escherichia coli* (STEC); where are we now regarding diagnostics and control strategies?

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Abstract

Escherichia coli comprises a highly diverse group of Gram negative bacteria and is a common member of the intestinal microflora of humans and animals. Generally, such colonisation is asymptomatic, however, some *E. coli* strains have evolved to become pathogenic and thus cause clinical disease in susceptible hosts. One pathotype, the Shiga toxigenic *E. coli* (STEC) comprising strains expressing a Shiga-like toxin is an important foodborne pathogen. A subset of STEC are the enterohaemorrhagic *E. coli* (EHEC) which can cause serious human disease, including haemolytic uraemic syndrome (HUS). The diagnosis of EHEC infections, and the surveillance of STEC in the food chain and the environment, require accurate, cost-effective and timely tests. In this review we describe and evaluate tests now in routine use, as well as up-coming test technologies for pathogen detection, including loop-mediated isothermal amplification (LAMP) and whole genome sequencing (WGS). We have considered the need for improved diagnostic tools in current strategies for the control and prevention of these pathogens in humans, the food chain and the environment. We conclude, that although significant progress has been made, STEC still remains an important zoonotic issue worldwide. Substantial reductions in the public health burden due to this infection will require a multi-pronged approach, including ongoing surveillance with high resolution diagnostic techniques currently being developed and integrated into the routine investigations of public health laboratories. However, additional research requirements may be needed before such high resolution diagnostic tools can be used to enable the development of appropriate interventions, such as vaccines and decontamination strategies.

1. **Introduction**

Escherichia coli comprises a highly diverse group of Gram negative bacteria with a wide range of habitats, physiological characteristics and pathogenic associations for animals and humans. *E. coli* is a common member of the intestinal microflora of humans and animals. Generally such colonisation is asymptomatic, however, some *E. coli* strains have evolved to become pathogenic and thus cause clinical disease.

When colonising the human or animal intestinal tract such strains usually cause diarrhoea, but may also transit the intestinal mucosa to result in systemic infections. Other pathogenic *E. coli* strains, such as the uropathogenic *E. coli* (UPEC) and avian pathogenic *E. coli* (APEC) have evolved to colonise extraintestinal sites. In the case of UPEC and APEC the primary site of infection are the urogenital tract of humans and the respiratory tract of birds, respectively. Such strains are termed Extraintestinal *E. coli* (ExPEC).

The nomenclature for *E. coli* strains is extremely complex and confounded by historical terminology. Unfortunately with newly acquired data from technology such as whole genome sequencing, this terminology is becoming even more confusing.

In humans, diarrhoeic strains of *E. coli* (DEC) have been divided into at least 5 pathotypes (Enteropathogenic *E. coli* (EPEC), Shiga toxigenic *E. coli* (STEC) (of which Enterohaemorrhagic *E. coli* (EHEC) are a subset), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETEC), and Enteroinvasive *E. coli* (EIEC)), a classification reflecting the pathology induced during infection and to some extent the virulence factors they express. The enterohaemorrhagic *E. coli* (EHEC) have been of particular concern as a zoonotic and foodborne infection because of the low infectious dose (<100 organisms) and serious nature of the disease, especially in young children, the immunocompromised and the elderly (Karmali, 1989).

All EHEC strains express a Shiga toxin-like activity and are, therefore, a subset of an *E. coli* pathotype known as STEC (Shiga toxigenic *E. coli*). Historically such strains are also known as Verotoxigenic *E. coli* (VTEC) reflecting the ability of this toxin to induce morphological changes in Vero tissue culture cells (an African green monkey kidney cell line) *in vitro* (Konowalchuk, Speirs & Stavric, 1977), which in the past was commonly used as a diagnostic test. For the purpose of this review STEC and VTEC are treated as synonymous and STEC will be the preferred terminology used. Colonisation with STEC strains can cause a spectrum of outcomes in humans, including mild to severe diarrhoea, however, the subset of STEC strains termed EHEC are specifically associated with causing human haemorrhagic uraemic syndrome (HUS). The EHEC strain *E. coli* O157:H7 is the most-well recognised and is used as a representative of the STEC pathotype. STEC strains have also been associated with diseases in livestock, for example, oedema disease in pigs and swollen head syndrome in poultry (Marques, Peiris, Cryz & O'Brien, 1987).

The objective of this chapter is to review EHEC and other STEC strains with particular focus on those characteristics relevant to their detection in human disease and in livestock reservoirs and the food chain, in order to reduce the public health burden of the disease in humans. There are many literature reviews of the clinical aspects of this infection, so to avoid repetition and where relevant, we will only reference these.

As with many zoonotic foodborne bacterial infections, disease associated with STEC is primarily observed in humans, while the livestock reservoirs remain largely unaffected by colonisation with this bacterial agent. In consequence, approaches to STEC detection have tended to focus on the need for specific and sensitive tests to diagnosis the disease in humans. However, in order to undertake the surveillance necessary to control and prevent disease transmission to humans from the animal reservoirs, appropriate methods are needed to detect the pathogen in all potential sources and routes including animals, foods and the environment.

There have been numerous reviews of *E. coli* O157:H7 pathogenesis, epidemiology and control, and more recently, additional general STEC reviews. The current review has been undertaken within the remit of the DISCONTOOLS project (http://www.discontools.eu). DISCONTOOLS was originally developed under the EU funded FP7 programme and aims to meet the challenges of future food supplies by developing prioritisation methodology and gap analysis for new or improved diagnostics, vaccines and pharmaceuticals for target diseases. Here we aim to highlight current research challenges for the investigation, diagnosis, prevention and control of STEC (see Table 1). In particular we will focus on the role that novel and innovative detection and diagnostic techniques can play in meeting these challenges. Where relevant we will refer to other more comprehensive literature, but in order to describe current challenges and assess the value of available tools it will be necessary to provide a brief overview of the properties of these organisms and their infections. Primarily, we will focus STEC in the European environment consistent with the DISCONTOOLS aims and objectives. We will use available European data to illustrate statements and support opinions but other data will be included where relevant.

2. Disease and pathogenicity:

Most of our knowledge about the pathogenicity of STEC is derived from research on strains of *E. coli* O157:H7 as this is the strain most commonly isolated from HUS patients and is, therefore, considered representative of EHEC strains. The known pathogenicity mechanisms of EHEC strains have been extensively reviewed elsewhere (for example Stevens and Frankel, 2014), however, the representativeness of laboratory *E. coli* O157:H7 strains of other STEC, or even of all O157:H7 strains, is debatable. It is well-recognised that the attenuation of laboratory strains of enteric pathogens through repeated passage can have significant effects on pathophysiological properties (Newell, Manning, Goldberg, Morgan, & Wassenaar, 2016).

In humans the clinical presentation of gut colonisation with *E. coli* O157:H7 ranges from asymptomatic carriage, through non-bloody diarrhoea to haemorrhagic colitis and haemorrhagic uraemic syndrome (HUS) (Karmali, Petric, Lim, Fleming & Steele, 1983). HUS can lead to thrombocytopaenia, haemolytic anaemia and ultimately kidney failure.

The pathogenicity of *E. coli* O157:H7 starts with colonisation of the intestinal mucosa following ingestion of contaminated food or water. The infectious dose is extremely low (<100 organisms) apparently reflecting the bacterium's ability to withstand the acidic environment of the stomach (Foster, 2004). Whether the dose response is similar for all STEC strains is unknown. Certainly, epidemiological evidence indicates significant variability in the incidence rates during O157:H7 outbreaks. Such variability could have substantial effects on the outputs of risk assessment models (Teunis, Ogden, & Strachan, 2008; Newell et al., 2016). Moreover, recent studies have focused on how STEC interfaces with the normal microbiota and how the normal microflora may influence susceptibility to infection (Bian et al., 2016).

Efficient intestinal colonisation requires bacterial motility to reach the intestinal mucosa and then attachment of the bacterium to host enterocytes for effective systemic delivery of bacterial toxins. EHEC strains are generally considered non-invasive pathogens but this may not be true for all STEC strains (Rogers, Thorpe, Paton & Paton, 2012).

Bacterial motility is mediated by active flagella, which are considered essential accessory virulence factors for many enteric pathogens, including STEC (Yang et al., 2013) but non-motile *E. coli* strains can also be pathogenic and of course non-pathogenic *E. coli* are often motile. So presence of flagella is not a definitive virulence factor. Nevertheless, flagellin is encoded by the *fliC* gene and because certain flagellin antigen types dominate within pathogroups of *E. coli,* flagella may have additional roles in virulence including adherence and innate immunity (Smith et al., 2003), and host association.

E. coli expresses a number of fimbrial and non-fimbrial adhesins, which are involved in attachment to a greater or lesser extent (La Ragione, Cooley & Woodward, 2000). Much of our knowledge of *E. coli* fimbrial adhesins has been gained from research on EPEC strains (Cleary et al., 2004) and EPEC adhesins that have been associated with intestinal colonization include bundle-forming pili (BFP), EspA filaments and intimin. Other fimbrial types documented for STEC include AAF/I and LPF (McWilliams & Torres, 2014; Nagy, Xu, Bauchan, Shelton, & Nou, 2016; Ross, Rojas-Lopez, Cieza, McWilliams, & Torres, 2015).

However, For EHEC strains attachment appears to be primarily mediated by the outer membrane protein adhesin, intimin, encoded by the *eae* gene localised within a ~35kb chromosomal pathogenicity island termed the Locus of Enterocyte Effacement (LEE). The presence of the LEE locus and *eae* gene have been considered indicative of EHEC (EFSA, 2013), but LEE- and *eae*-negative STEC can also cause severe disease including HUS (Franz et al., 2015). In some *E. coli* pathotypes, such as

EAEC, adherence and long-term gut colonisation are also associated with the capacity to form biofilms and biofilm formation may also influence the pathogenesis of STEC (Villegas et al., 2013).

The attachment process involves the induction of characteristic attaching and effacing (AE) lesions in the luminal surface of the host intestinal cells (Donnenberg & Kaper, 1991). AE lesion formation requires Tir (Translocated intimin receptor). This receptor is translocated into the host cell by a type 3-secretion system (T3SS) (Jarvis et al., 1995), which provides the complex structure required to span the host cell membranes and enable the entrance of bacterial effectors into the host cell cytoplasm. Bacterial attachment is then associated with host cell F-actin condensation, the induction of a pedestal and microvillus effacement. This intimate attachment allows the effective systemic delivery of the *E. coli* toxin(s).

In LEE-negative STEC strains alternative mechanisms for gut colonization are employed. In some non-LEE STEC strains Long Polar Fimbriae (Lpf), encoded by *lpfA* genes, appear to enable adhesion and colonisation (Galli Torres & Rivas, 2010) while in other strains, such as O113:H21, which do not produce adherence factors (Feng et al., 2014), *in vitro* invasion of intestinal epithelial cells is largely dependent on flagellin expression (Rogers et al., 2012). Thus, the absence of LEE and *eae* in STEC strains cannot be used to exclude the potential to cause severe human disease (Franz et al., 2015).

In 1997 Konowalchuk et al. demonstrated that that a subset of *E. coli* produced potent toxins, which were heat label and unlike previously identified *E. coli* heat-labile enterotoxins (LTs). These toxins caused Vero cells to round up and die and were antigenically cross-reactive with the toxin (Stx) produced by *Shigella dysenteriae 1* (O'Brien & LaVeck, 1983). In consequence the toxin was named Shiga toxin 1 (Stx1). Subsequently, a similar toxin Stx2 with distinguishable antigenicity and toxicity was identified. Several subtypes of these two toxins (Stx1 a, c and d and Stx2 a, b, c, d, e, f and g) have now been described with varying disease associations in humans (Scheutz et al., 2012; Franz et al., 2015) and colonisation properties in cattle (Dallman, et al., 2015a). Some EHEC strains express multiple Stx subtypes (Shaaban et al., 2016).

The genes for Stx, and various effector proteins of the T3SS, are encoded on integrated bacteriophages (prophages) distributed around the EHEC genome. Prophages are also involved in the regulation of Shiga toxin production. Such prophages have played an important role in the evolution of EHEC (Kruger and Lucchesi, 2015; Shaaban et al., 2016), and the emergence of new STEC strains. The considerable diversity of *stx* genes, and variations in their *stx* prophage environment affect the virulence of STEC strains.

Because the genome of *E. coli* is plastic and prophage uptake is a common event, Stx expression is not confined to EHEC. Stx-producing enteroaggregative *E. coli* (EAEC) have characteristics of both pathotypes and can induce severe disease, including HUS, as demonstrated by the outbreak of *E. coli* 104:H4 in Germany in 2011 (Frank et al., 2011). These strains may not possess the *eae* gene, but have genes associated with enteroaggregative adhesion by which they can be identified, such as *aggR*, which is located on a plasmid and regulates the aggregative adherence fimbriae pathogenicity island (AFF PAI). Because plasmid loss can occur the EAEC pathotype is also identified by the presence of *aaiC*, which is chromosomally located and encodes a secreted protein of EAEC (EFSA, 2013).

The Stx toxins are transported across the intestinal barrier to access the systemic circulation and reach susceptible tissues expressing the glycosphingolipid cell receptor Gb3. The structure and activity of the Stx toxins are well known (Melton-Celsa, 2014). In humans the endothelial cells of the kidney are the major targets causing inhibition of protein synthesis and triggering apoptosis. The damage to the glomerular structure activates platelets, resulting in a coagulation cascade and initiating an inflammatory response. Localised thrombi formation in the glomerulus can cause thrombocytopaenia purpura and haemolytic anaemia. The resulting impairment of the renal vasculature is characteristic of HUS (Obrig & Karpman, 2012). In addition, Stx can also bind to Gb3 on the surface of cells of the

central nervous system causing neurological symptoms including epileptic seizures (Nathanson et al., 2010).

Because the distribution of Gb3 receptors throughout the body varies with host species, susceptibility to the toxic effects of Stx also varies from one species to another. For example the tolerance of cattle to STEC colonisation is ascribed to the lack, or different distribution, of Gb3 receptors in their gastrointestinal tract and internal organs (Pruimboom-Brees et al., 2000; Hoey et al., 2002).

Increasingly, evidence indicates that infection with STEC strains results in varying clinical outcomes, presumably due to a combination of differences in patient susceptibility and strain virulence properties. With the increasing availability of whole genome sequences of STEC strains and data mining using pipelines and predictive algorithms, investigations suggest that only a small proportion of even O157 strains, perhaps as few as 10%, have the potential to cause human disease. Furthermore, this virulence potential is likely associated with the abundance of genes encoding prophage proteins (Lupolova, Dallman, Matthews, Bono & Gally 2016). The ability to rapidly and accurately distinguish between those human STEC infections which will result in severe as opposed to mild symptoms is a major clinical requirement so that appropriate and timely patient treatment can be provided. Unfortunately, to date, no clear association between the presence or absence of known virulence factors and the severity of infection has been identified (Franz et al., 2015). Although Stx2-producing strains appear more virulent than Stx1-producing strains (Beutin et al., 2008), and certain Stx subsets have a greater disease potential than others (Shaaban et al., 2016), clearly other virulence factors, such as adhesins, are also involved (Franz et al., 2015). Therefore, it appears that strain virulence is determined by sets of multiple genes and that the gene content of such sets can vary. Defining the essential gene content for virulence and persistence is now a primary research need in order to provide accurate predictive hazard assessment. Of course, improved knowledge of the pathogenic diversity and mechanisms of non-O157, as well as O157 STEC strains, will be an underpinning requirement to the development such tools.

3. Epidemiology, source attribution and risk assessment

The efficient monitoring and surveillance of all human foodborne infections is considered crucial to the implementation of effective interventions. Methods of epidemiological investigation are constantly evolving. In particular new techniques, which will be addressed later, to detect foodborne pathogens and discriminate between strains are having a significant impact on source identification and risk assessment.

In European countries there has been a long history of the epidemiological investigation and source tracing of foodborne bacterial disease, data from which is now collected in comprehensive and well analysed annual reports from the European Union (EU) highly suitable for illustrating epidemiological characteristics of these infections. For *E. coli* where non-pathogenic strains are common in multiple hosts and the environment, and potentially highly pathogenic strains need to be correctly identified especially in clinical isolates, subtyping has been an essential tool in epidemiological investigations. Historically, in Public Health laboratories world-wide, the then existing skills and expertise in serotyping encouraged the wide-spread adoption of this subtyping technique for *E. coli.* There are two serotyping schemes for *E. coli* based on the O (lipopolysaccharide (LPS)) and H (flagellar) antigens and these tend to be used in parallel. Both use antisera in simple agglutination assays. To date over 180 *E. coli* O-serogroups and over 50 H-serotypes have been identified (Ingle et al., 2016) and there are more than 100 serotypes of STEC associated with human disease (Ferdous et al., 2016). Surveillance of STEC infections is often undertaken using serotyping (full or partial) only or using methods which only recover and identify O157:H7 strains.

Since the O104:H4 outbreak in Germany in 2011 (Frank et al., 2011) there has been increasing pressure on public health services to strengthen and broaden STEC surveillance in animals, humans and food. In particular, there is a call for enhanced HUS surveillance in children under 5, especially for non-O157 sero-pathotypes (Severi et al., 2016).

Human infections with STEC strains are reported worldwide, both as sporadic and outbreak-associated cases. The notification of human STEC infections is mandatory in most the EU member states. In 2015, 5901 cases of STEC infection were confirmed in the EU, (EFSA, 2016). The EU incidence of disease has now been reported for over 15 years. In addition, isolated STEC strains are routinely serotyped using the O typing scheme. Such longitudinal surveillance indicates that STEC disease is dynamic with incidence changing over time and geographical location (EFSA, 2016) and season (Garvey et al., 2016a). Moreover, the strains causing the disease are also changing over time (EFSA, 2016), suggesting evolving differences in sources and transmission vehicles (Adams et al*.,* 2016; Óhaiseadha, Hynds, Fallon & O'Dwyer, 2017). Thus there is a clear ongoing requirement for the active surveillance of STEC disease and infecting strains, with standard approaches to identify emerging trends and enable historical comparison.

Using a combination of serotype, the reported frequency of infection, severity of disease and association with outbreaks Karmali et al*.* (2003) were able to divided STEC into seropathotypes A-D which has informed epidemiological studies and supported risk assessment. However, with the exception of *E. coli* O157:H7, which is a relatively stable clone (Liesegang et al*.*, 2000), the relationship between serotype and disease potential is generally poor (Franz et al., 2015). In the investigation of EU infections the identification of HUS-associated strains can apparently be improved by adding health outcome and laboratory confirmation to the epidemiological analysis (Messens et al*.*, 2015). Nevertheless, the most useful future epidemiological tools for strain differentiation will require the inclusion of appropriate virulence factors using genetic screening approaches. However, in addition to *stx* gene types and subtypes, the combinations of virulence factors required to generate a pathogenic organism are, as yet, unknown.

The sources of STEC infection causing disease in humans have been extensively investigated. STEC can colonise a wide range of animals and the majority of this bacterial population probably reside in asymptomatically colonised animal hosts. Using classical epidemiological studies, including outbreak and case-control analyses, and molecular epidemiological approaches, source attribution studies identify ruminants, especially cattle, as a major reservoir and the food chain as a significant transmission route (Pennington, 2010; Mughini-Gras et al., 2017) as well as person-to-person spread (Garvey, Carroll, McNamara, & McKeown, 2016b).

The primary site of colonisation in cattle is the terminal rectum where the bacteria form attaching and effacing lesions on the apical epithelial surface, but colonisation is generally asymptomatic (Naylor et al., 2003), although histopathological effects are observed, so the organism is not a commensal in this environment (Naylor et al., 2007). Cattle faeces containing excreted organisms contaminate hides and subsequently meat during slaughter and processing, but can also contaminate milk (Severi et al., 2016).

Other ruminant and non-ruminant livestock, including sheep, goats, pigs and chickens, can also be asymptomatically colonised with, and excrete, STEC especially *E. coli* O157 (Best et al., 2006, 2009; La Ragione et al., 2005; Wales et al., 2005), though the pathobiology may vary from that in cattle (La Ragione, Best, Woodward & Wales, 2009). All such colonisation may contribute to contamination of farm environments, including ground water, constituting a significant risk factor of human disease, particularly for rural communities (Óhaiseadha et al., 2017).

There have been multiple studies on the risk factors for cattle colonisation and excretion, including environmental factors such as season, rainfall, temperature, latitude of farm and relative humidity;

animal factors such as age, breed, colostrum deprivation in calves and stress levels; and farm husbandry factors such as feed, group size, new animals incorporated into the herd, housing, water supply, and positive animals in the proximity (Jaros et al., 2016; Rugbjerg, Nielsen & Andersen, 2003; Smith, Pollitt & Paiba, 2016; Venegas-Vargas et al., 2016; Widgren et al., 2016; Williams, Ward, Dhungyel & Hall, 2015).

Some cattle are recognised to be "Super shedders", which can substantially increase the risk of herd colonisation and environmental contamination (Chase-Topping, Gally, Low, Matthews & Woolhouse, 2008). The super shedding status of cattle is related to degree of colonisation of the lymphoid follicle-dense mucosal region close to the recto-anal junction (Cobbold et al., 2007; Low et al., 2005).

There are few national surveys of STEC in livestock, however, throughout 2003 a national survey of *E. coli* O157 carriage by animals at slaughter was undertaken in Great Britain. This survey indicated that carriage was 4.7% in cattle, 0.7% in sheep and 0.3% in pigs (Milnes et al., 2008). STEC detected in food and animals is now recorded annually by EU member states and technical guidelines have been issued to enable the standardisation of sampling and STEC detection (EFSA, 2016). These guidelines recommend that detection methods used should recover all STEC strains and that recovered strains should be serotyped to enable comparative surveillance with human isolates. The EU Summary Report on Zoonoses, Zoonotic Agents and Food-borne Outbreaks now records STEC recovered from all ruminants, including goats, sheep and deer, as well as pigs, cats, dogs and solipeds. This data demonstrates that cattle are not the only potential reservoir of infection. Overall 6.8% of all animals tested (n=6881) were STEC positive. The highest prevalence was in sheep and goats (18.5%) compared to 8.3% in cattle and 8.3% in pigs. For those isolates where typing methods were applied serogroup O157 constituted less than half of the STEC reported in sheep (5.6%) and cattle (2.7%).

Food monitoring in the EU supports the role of bovine and other ruminant meats as sources of human infection with 1.6% of bovine meat and 12.1% of sheep/goat meats contaminated with STEC in 2015 (EFSA, 2016). Monitoring also detected contamination in 1.8% of samples of raw cow's milk. Although the proportion of samples of foods contaminated with serogroup O157 was substantially lower, most of the STEC strains recovered were associated with human disease in the EU.

For some time there has been increasing awareness worldwide that fresh produce, such as fruits, vegetables and seeds, can also be vehicles of transmission for STEC infections. For example, the outbreak in Germany and France in 2011, involving about 4000 cases with over 50 deaths, was epidemiologically-linked to sprouting seeds (Frank et al., 2011). Fresh products are frequently grown in soils fertilised or contaminated with animal waste and/or irrigated with brown water, which can lead to plant contamination on harvesting (Mishra, Pang, Buchanan, Schaffner & Pradhan, 2017). STEC is capable of an endophytic lifestyle (Wright et al., 2013) enabling attachment to, and survival in, plant material and even protection from post-harvest disinfection processes. The organism survives well in the rhizosphere of vegetable plants and can colonise plants both externally and internally. Internalisation and phylosphere survival are dependent on the inoculum, plant age, plant type and the presence of insects as well as the bacterial capacity to express various virulence factors (Ongeng et al., 2015).

The detailed epidemiological investigations undertaken on outbreaks over the last 30 years have allowed the sources and routes of transmission of O157 STEC human infections to be generally well understood. However, the increasing surveillance called for O157 and, most recently, non-O157 strains (Ferdous et al., 2016), is demonstrating the widespread presence of STEC in livestock, foods, water and the environment. In order to develop and implement appropriate risk management strategies the relative importance of these strains in human disease needs to be determined. In particular the capacity to subtype strains and differentiate between levels of virulence as predictors of severity of human disease is an urgent requirement. To date phenotypic and molecular epidemiological techniques have had limited success at addressing these questions. However, the

recent adoption of whole genome sequencing (WGS) accompanied by developments in pan-genomic pipelines and, in particular, the use of machine learning algorithms, for example the Support Vector Machine (SVM), (Lupolova, Dallman, Matthews, Bono & Gally, 2016) are beginning to provide novel information about the proportion of strains which can cause human disease (see section 7.2.2.3). Unfortunately, such approaches are currently highly dependent on expert bioinformaticists/ mathematicians/statisticians, who use dedicated and jargon-ladened language largely uninterpretable by non-expert clinicians and clinical microbiologists. Therefore, there is an evident need for compromise to ensure best benefit of such technological advances. The bioinformaticists need to learn to talk at an acceptable level to, and work with, their less expert colleagues. On the other hand non-experts need appropriate level education resources such as simple learning texts (for example Ussery, Wassenaar and Borini, 2009), but which need to be frequently up-dated to keep up with the enormous speed of development in this scientific field.

4. Socio-economic impact including zoonotic aspects

STEC strains can colonise a wide range of hosts but are considered primarily a foodborne disease problem in humans causing both outbreaks and sporadic cases. Majowicz et al. (2014) have recently estimated the global public health burden of infections with STEC strains to include 2,801,000 cases of acute illness, 3890 cases of HUS, 270 cases of end stage renal disease (ESRD) and 230 deaths per year. HUS is one of the most serious sequelae of STEC infection and is a particular problem in the young. In 2015 HUS was reported in 65% of infected children under the age of 5 years and 20% of infected children aged 5-14 years old in the EU (EFSA, 2016).

Despite such case numbers, analyses from the Netherlands (van Lier et al., 2016) indicates that the relative importance of *E. coli* O157:H7 as a foodborne disease, in terms of disability-adjusted life years (DALYS), is significantly lower than that of toxoplasmosis, campylobacteriosis, salmonellosis or norovirus infections. Similarly the foodborne diseases salmonellosis and campylobacteriosis are considered of a greater public health burden than O157:H7 in the United Kingdom (O'Brien et al., 2016). Nevertheless, STEC infection remains an important cause of morbidity and mortality worldwide.

In 2015 5901 cases of STEC infection were reported in the EU (EFSA, 2016), of which 41% were due to O157, but the disease is considered to be significantly under-reported. The estimated disease multiplier for STEC O157 and non-O157 are 51.2 and 209.6 respectively (EFSA, 2013). Over one third (36%) of cases of STEC infection were reported as hospitalised, with an overall case fatality rate reported at 0.2% but the outcome of such infections are not always recorded. The risk of hospitalisation varies with the serogroup of the infecting strain. STEC O157 infection has a higher risk of hospitalisation (68%) than all non-O157 infections (less than 1%), excepting serogroup O104 (46%) (Preußel, Höhle, Stark, & Werber 2013). However, infections with both O157 and O104 strains have similar mortality risks, which are about 10 fold more than infections with strains of other serogroups.

As with many foodborne infections, the cost of the STEC disease is largely borne by the public health system because gut colonisation is usually asymptomatic in livestock. The costs to public health in terms of clinical care and outbreak investigation can be huge (Pennington, 2010). However, as a consequence of epidemiological investigations, sources of infection are now often identified and can result in legal action. Moreover, product recall can be a substantial cost to the industry. For example, in an outbreak in Odwalla, USA associated with unpasteurised apple juice, product recall costs alone were $6.5 million (Pennington, 2010).

Prioritization of the use of public health resources is a major problem for most countries. The data and tools required to undertake the comparative estimation of burdens of acute foodborne diseases have

only recently been developed (Newell et al., 2010). These tools can now provide relative indicators of global and national social-economic costs using approaches largely led by the World Health Organization's Foodborne Disease Burden Epidemiology Reference Group (FERG) (Havelaar et al., 2015). Such approaches now need to be applied to at national levels to enable governments and regulatory authorities to set targets for the control and prevention of STEC infections.

5. Prevention and control

The direct prevention and control of human STEC infections is difficult because of the diversity of infecting strains and the wide variety of sources and routes of transmission for these pathogens. Attempts to prevent or treat human colonisation using probiotics or dietary supplements or modifications have had little evidence of success and at worse may be detrimental (Thorpe et al., 2017) to the patient. Thus the management of human STEC infection currently remains restricted to supportive clinical care.

The consensus opinion is that the public health burden of STEC infection is best reduced by controlling carriage of the organisms in livestock and preventing pathogen dissemination in foods, water and the environment.

The risk of human disease from STEC in the environment accrues from strain characteristics, exposure, including the number of viable organisms present, and host susceptibility. The monitoring and routine surveillance of animals and foods is, therefore, an important strategy in foodborne STEC infections. In the EU, the Directive 2003/99/EC establishes criteria for the collection of "relevant, and where applicable, comparable data on verotoxigenic *Escherichia coli* (VTEC) in animals and foodstuffs". Technical specifications have been introduced, to provide harmonised data collection for the monitoring and reporting of STEC in foods and environmental samples, based on the use of ISO/TS 13136:2012. The technical approach recommended depends on the detection, by real-time PCR, of the major STEC virulence genes (*stx1/stx2* and *eae*) and genes associated with serogroups O157, O111, O26, O103 and O145. Any foods found to contain one or both of the *stx* genes are then processed and cultured to attempt to isolate STEC strains. In 2009 a similar method was recommended for bovine hides and sheep fleeces (EFSA, 2009). Most recently, following the O104:H4 outbreak in Germany, the EU expanded the harmonised monitoring to sprouted seeds (EFSA, 2016). Current surveillance strategies detect the presence of potential STEC hazards, but for accurate risk assessment of contamination, quantitative methods to determine the number of organisms present and level of exposure should be undertaken.

The layered strategy of PCR, followed by culture, substantially improves the surveillance of all STEC strains in foods and animals. However, unfortunately many of the strains detected by PCRs for the *stx*$_1$/*stx*$_2$, *eae*, and O-antigen genes alone, especially in food and environmental samples, would constitute a low risk of human disease. Recent recommendations suggest that the additional genes *aagR* and *aaiC* are included in PCRs to ensure detection of Stx-producing EAEC strains (EFSA, 2013) With the introduction of this much broader based approach in Europe the prevalence of non-O157 strains in foods and animals is now becoming apparent, even though the role of such strains in human disease has yet to be established. Nevertheless, the ability to identify strains that will cause serious disease, for example EHEC strains, and distinguish these from the large numbers of non-virulent strains in the same habitats, remains a fundamental surveillance issue. Interestingly, Delannoy, Beutin & Fach (2013) have shown that the combination of the type 111 effector genes *espK* with either *espV* or *ureD* or *Z2098* can effectively discriminate EHEC from non-EHEC strains and that such a targeted approach for the monitoring of meat contamination will reduce the number of false positives (Delannoy et al., 2016).

The main function of surveillance is to provide an evidence base for the implementation of interventions to reduce the public health burden and to monitor the effectiveness of such interventions. The infectious dose for EHEC is in the order of only hundreds of cells and such a small infectious dose constitutes a significant challenge for risk management. Hazard Analysis Critical Control Points (HACCP) is the major tool used by the food industry to prevent and control microbiological hazards in the food chain. HACCP aims to ensure good personal hygiene, separation of uncooked from cooked foods and the use of suitable storage and cooking temperatures where appropriate. However, despite the introduction of substantial regulation and training, human failure still occasionally results in significant outbreaks (Pennington, 2010). Thus risk management of STEC strains in foods remains a challenge. In the USA *E. coli* O157 contamination of ground beef is not tolerated and results in immediate recall. In the EU, Article 14 of Regulation (EC) No 178/2002 prohibits food being placed on the market if it is unsafe. Food is deemed to be unsafe if it is considered to be injurious to health or unfit for human consumption. Currently an EC guidance document is being drafted to assess the risk and determine the risk management strategies for STEC-positive food samples
(http://www.origingi.com/images/stories/PDFs/English/Lobby/Regulatory_issues/Draft_VTEC_guidance_document_on_application_Art_14_GFL_REV_3-3.pdf).

To date most interventions have been targeted at bovines, even though small ruminants are also commonly colonised. The contribution of “Super shedder” cattle to the risk of carcass and environmental contamination, as well as the risk of horizontal transmission within a herd, has highlighted the need for focussed interventions (Arthur et al., 2009; Arthur et al., 2010; Cobbold et al., 2007; Low et al., 2005; Matthews et al., 2006; Omisakin, MacRae, Ogden & Strachan, 2003). "Super shedders" shed at least 10^4 CFU/g in their faeces, constitute about 2% of cattle herds and generate the highest public health risk. Super shedding appears to be a reflection of host properties, such as site of colonisation rather than the STEC strains shed (Arthur et al., 2013), but may not be a constant state in individual cattle (Munns et al., 2015).

Finding ways of preventing or reducing STEC colonisation, particularly in cattle, remains a huge research challenge despite considerable effort to date. In 2012 Thomas and Elliott systematically reviewed interventions for diarrhoea-associated HUS. Interventions aimed at reducing animal carriage assessed included biosecurity/farm practices, vaccination, diet, bacteriophages, probiotics and other feed additives. This systematic review concluded that "vaccination and improved farming and feeding practices, including dietary manipulation (e.g. probiotics and sodium chlorate feed additives), cohorting of animals and dry bedding and soil solarization reduce STEC carriage and faecal shedding in animals, and hence the risk of STEC transmission to humans." (Thomas & Elliott, 2012). In a subsequent systematic review of potential interventions (Wisener et al., 2015), the probiotics *Lactobacillus acidophilus* (NP51) and *Propionibacterium freudenreichii* (NP24) fed pre-harvest were found to significantly reduce faecal *E. coli* O157 shedding in beef cattle during field trials. Such evidence supports suggestions that the diversity and composition of the cattle gut microbiome are important factors in controlling pathogen carriage (Munns et al., 2015). However, variation in feeds and administration of probiotics had little, if any, positive effect on "Super shedders" in other studies (Spencer, Besser, Cobbold & French, 2015). Thus the relationship between gut microbiome, diet and STEC carriage requires considerable further investigation.

Faecal contamination of meat usually occurs during animal slaughter. Poor slaughter practices, abattoir hygiene and animal handling practices all contribute to the risk of contamination. Understanding the routes of contamination has enabled guidelines to be established to minimise meat contamination by pathogens including STEC (ftp://ftp.fao.org/docrep/fao/007/y5454e/y5454e.pdf; http://www.fao.org/fileadmin/user_upload/agns/pdf/FAO_E.Coli_FCC_2011.06.231.pdf).

Cattle hides are a particular source of carcass contamination and the levels of STEC on hides at slaughter are a good predictor of meat contamination after processing (Wheeler, Kalchayanand & Bosilevac, 2014). Interestingly the diversity of the commensal microbial population of cattle hides appears to be important in determining the risk of contamination with STEC strains (Chopyk et al., 2016). The food industry has developed a number of interventions targeted at hide decontamination in the abattoir, especially the use of rectal ties, de-hairing and improved methods of hide removal, and antimicrobial treatments, including washing with water, selected chemicals and organic acids and bacteriophages (Loretz, Stephan & Zweifel, 2011; Wheeler et al., 2014).

STEC successfully survive and persist in multiple, frequently hostile, environments during food production and processing as well as in the wider livestock environment. This success is dependent on an ability to rapidly sense and respond to specific environmental hazards. To date multiple stress response mechanisms have been described (Vidovic & Korber, 2016), which enable STEC such as *E. coli* O157:H7 to respond to acid, heat, cold and oxidative stressors, and are potentially associated with an increased virulence. Variations occur in both the range of mechanisms utilised by individual strains to respond to environmental stresses and the effectiveness of such responses. Understanding the molecular basis of such responses is important so that effective strategies to control STEC can be put in place, particularly during food production. This is especially important given that some STEC strains can develop tolerance to disinfectants widely used in the farming industry as well as to organic acids used as decontaminants during meat processing (Lajhar, Brownlie & Barlow, 2017).

Although available interventions can reduce STEC contamination in meat products (Pollari et al., 2017), the public health burden remains high, leading to suggestions that complementary control and prevention methods to reduce environmental contamination from colonised ruminants is required (Smith, 2014). Vaccination is one obvious complementary strategy, which would reduce STEC carriage by cattle on-farms and thereby reduce environmental contamination. Considerable attention has been given to the development of such vaccines over the last 2 decades. The review by Smith (2014) provides a comprehensive description of the various laboratory-based studies and field trials on vaccination against *E. coli* O157 to that date. Despite the absence of disease, cattle elicit both innate and acquired immune responses to STEC colonisation. A number of STEC O157 antigens have been identified as potential vaccine candidates and subunit vaccines. To date two vaccines have been developed and licensed; Econiche (Econiche Corp, Belleville, Canada), is based on type III secreted proteins (T3SPs) and Epitopix (Willmar, USA) comprises cell membrane extracts prepared from iron restricted cultures (Corbishley et al., 2016). Although both vaccines induce antibody responses effective in significantly reducing colonisation they are only partially protective (Snedeker, Campbell, & Sargeant, 2012).

Considerable research effort is going into the development of new vaccines by identifying alternative vaccine candidates, such as flagella, the EhaB autotransporter protein and proteins associated with T3SS-mediated adherence (Mahajan et al., 2009; McNeilly et al., 2010; Wells et al., 2009). In addition, efforts have been undertaken to improve vaccine efficiency, such as by including vaccine candidates that induce a cellular response (Corbishley et al., 2016). The on-going mining of WGS data (Lupolova, Dallman, Matthews, Bono & Gally 2016) may allow vaccine development to focus on vaccine candidates expressed only by virulent STEC.

Over the last 3 decades, many potential interventions have been investigated and used to reduce human STEC infection at various levels in the food chain. However, the burden of disease remains high and there is some evidence that implemented interventions may be associated with shifts in both infecting strain subtypes and in sources of infection. For example (Adams et al., 2016) investigating *E. coli* O157:H7 infections in the UK during 1983-2012 observed no difference in incidence despite the multiple interventions implemented but did observed a shift in subtype (PT2 to PT21/28) and a change in observed sources from contaminated meat/milk to petting farms and school/nursery outbreaks.

6. **Detection, diagnosis and subtyping**

E. coli are easily cultured on general media (such as MacConkey agar) at 37^{o}C under aerobic conditions. Enterobacteriaceae are simply identified using commercial biochemical test strips. The traditional diagnostic approach involves the culture of faecal samples on chromogenic agar selective for *E. coli* O157, which is generally sorbitol negative, using media such as MacConkey-Sorbitol agar or CHROMagar O157. However, the identification of sorbitol-fermenting STEC O157:H7 strains, and the increasing awareness of non-O157 STEC strains causing disease, means that the use of sorbitol fermentation is not a reliable phenotype for STEC screening (Gouali, Ruckly, Carles, Lejay-Collin and Weill, 2013). Moreover, culture takes up to 5 days to provide results (if enrichment is required), which in a clinical setting could constrain treatment and endanger lives. More recently clinical laboratories have adopted Polymerase Chain Reaction (PCR) techniques with primers for specific targets including the *stx* genes and usually with subsequent culture. This approach generates a positive result for all STEC within 1-2 days.

Being able to reliably predict the severity of an STEC infection could have a significant influence on treatment and impact on clinical outcome. It has long been recognised that infection with certain STEC seropathotypes could have more serious outcomes (Karmali et al., 2003). However, retrospective studies have concluded this classification neither defines STEC pathogenicity nor includes all potential pathogenic STEC strains (EFSA, 2013; Messens et al., 2014). Nevertheless, strains that represent the highest risk of severe disease appear to largely belong to serogroups O157, O26, O103, O145, O111 and O1O4 and are positive for the genes *eae* or *aaiC/aggR*, in addition to *stx2*. Most recently positivity for the stx2a gene has been described as an indicator of severe disease potential (Byrne et al., 2014). Thus diagnostic PCR tests should include primers for at least this minimum set of virulence factors. However, the full complement of factors important in severe infections has yet to be established. Moreover, because *stx* gene-containing phages may exist in faeces and environmental samples, such as food and water, using PCR as the primary indicator of STEC infection or environmental contamination may generate positive results, which have no clinical relevance or risk to humans (Martinez-Castillo & Muniesa, 2014).

Serology can also be used to diagnose infection. Both innate and adaptive immune responses are elicited by STEC-infected individuals. Serological investigations of outbreak subjects have demonstrated that humoral and mucosal IgG, IgM and IgA antibodies specifically directed against STEC antigens are detectable in serum and saliva (Chart & Cheasty, 2008), consistent with intestinal bacterial pathogen colonisation. However, the kinetics, isotype, specificity and protective capacity of such antibody responses in humans remain largely un-investigated. Despite this, serology has been recommended for diagnostic purposes particularly in children (Desin, Townsend & Potter, 2015) even in the absence of detectable bacteria or Stx in faeces (Chart, Cheasty, Cope, Gross & Rowe, 1991).

Antibody specificity is directed against a range of STEC antigens including LPS O antigens, flagella, Stx1 and Stx2, and intimin. The anti-O antigen response is directed against both conserved epitopes in the core LPS and serotype-specific epitopes. Thus serodiagnosis can apparently be used to detect both putative STEC infection and the serogroup of the infecting strain (Chart & Cheasty, 2008).

The differentiation of *E. coli* isolates, firstly into different pathotypes and then into subtypes, should enable appropriate clinical treatment to be initiated quickly, provide some indication of prognosis and, in the case of outbreaks, inform strategies for control and intervention. Because of its public health prominence, surveillance for STEC infections is particularly important to detect outbreaks and to differentiate strains with the potential to cause severe symptoms.

The most definitive approach for the diagnosis of STEC infection is the detection of Stx expression in isolated *E. coli* strains or directly in faecal material. Stx detection is still occasionally undertaken by observation of the effect of bacterial supernatant on Vero cells (Verotoxigenic effect) but this is labour and resource intensive and nowadays immunoassays are more frequently used. However, most laboratories prefer to detect the presence of the *stx1* or *stx2* genes, usually by PCR, rather than the expression of the toxin.

To ease routine diagnosis, biotypic culture-based methods have been developed for *E. coli* 0157:H7, which characteristically lacks B-glucuronidase activity (GUD-) and is unable to ferment sorbitol (SOR-) (Paton & Paton, 1998). Based on such specific metabolic characteristics culture media, such as sorbitol MacConkey (SMAC) agar and CHROMagar STEC medium (de Boer et al., 2015), are routinely used in public health laboratories for preliminary diagnosis of *E. coli* O157:H7 strains. With increasing availability of whole genome sequences of *E. coli* strains genome scale metabolic models, initially confined to commensal strains, are now being extended to pathogenic strains (Baumler, Peplinski, Reed, Glasner & Perna 2011). Such models will contribute to an even greater understanding of the biochemistry and physiology of STEC and could aid the development of improved culture-based detection methods

The subtyping of STEC strains enables the (1) tracing of sources and routes of transmission of human infections, (2) the identification and monitoring, both temporally and geographically, of specific strains with important phenotypic characteristics and (3) the development of strategies to control organisms within the food chain.

The population structure of *E. coli* is heterogeneous and many subtyping methods have been applied to this species and its pathotypes. The current criteria for an effective bacterial subtyping method includes cost, speed, accessibility, typeability, discriminatory power, reproducibility, epidemiological relevance and phylogenetic relevance. Subtyping methods applied to STEC strains currently include the phenotypic approaches of serotyping and phage typing and the genotypic approaches, such as virulotyping, pulsed field gel electrophoresis (PFGE), multi-locus sequence typing (MLST), microarrays and, most recently, WGS. Different subtyping methods may be advantageous for different purposes, for example where reagents are already available and time is not critical but high throughput is required, simple serotyping will have advantages over sophisticated genotypic methods but where high discriminatory power is critical, say for outbreak investigation, then WGS is probably now the method of choice.

The relationship between the serotype and pathotypes of STEC is well recognised (Karmali et al., 2003). Although it is now widely accepted that this correlation is imperfect, most public health laboratories rely on serotyping. However, the number of O-non-typeable strains reported in surveillance studies, suggests that the collection of anti-O-antisera is insufficient to cover the whole *E. coli* population. For example, in 2015, 11.9% of STEC strains isolated in the EU and submitted for typing were untypeable, while a further 1.2% were designated O-rough. Reports of the increasing prevalence of O-untypeable strains isolated (EFSA, 2016) and the evolution of the *E. coli* population causing shifts in the serotype, raises questions about the continued usefulness of this phenotypic subtyping method (Ingle et al., 2016). H antigen typing also has limitations. Often H antigen typing is not undertaken because of lack of sera or the presence of non-motile strains (Beutin & Strauch, 2007). Nevertheless, the wealth of historical information on STEC strains is serotype-based and the simplicity of the agglutination assay enables its worldwide application, and should ensure its continued use at least while antisera remains widely available.

Phage typing of STEC also facilitates public health surveillance and outbreak investigations and has been used to categorize sporadic cases. Certain phage types are more likely to occupy specific niches and are associated with specific age groups and disease severity (Cowley et al., 2015). STEC phage-

typing is based on the use of 16 bacteriophages including 14 T4 phages and 2 T7 phages A phage infection profile, indicated by the level of lysis achieved with each phage (Ahmed, Bopp, Borczyk and Kasatiya, 1987), is generated for each strain. Approximately 80% of all STEC O157 strains typed are phage type (PT) 8, 21/28, 2, 4 or 32 in the UK (Gastrointestinal bacteria reference unit (GBRU)). (Cowley et al., 2015). Certain PTs are more likely to be associated with human infection but so far there is little understanding of the basis for this.

With the advent of Next-Generation Sequencing, forgoing time-consuming Sanger-based methods and improving read lengths of second generation methods, the delivery of the DNA sequence of fragments of pathogen genomes, or even whole genomes has become a cheap, rapid and routine occurrence. This has enabled the mining and exploitation of DNA sequences for genomic pathogen detection, identification and characterisation.

MLST, based on the partial sequences of 7 housekeeping genes, has been a useful genetic tool for understanding the population structure of *E. coli*. Early studies demonstrated the clonality of some serogroups, such as *E. coli* O157:H7 (Noller et al., 2003) and the technique was subsequently used for epidemiological investigations. Five cryptic MLST-based clades were identified (Walk et al., 2009) but the pathotypes were distributed throughout the phylo groups, which is unsurprising as the *stx* genes were located in prophages and remain mobile elements.

Increasing awareness of the plasticity of the *E. coli* genome and evidence that STEC evolution is characterised by the acquisition of numerous virulence factors through horizontal gene transfer mediated primarily by bacteriophage transduction (Reid, Herbelin, Bumbaugh, Selander & Whittam, 2000; Vanaja et al., 2013; Whittam, Reid & Selander, 1998), with subsequent gene gain or loss through duplication or deletion (Kaper, Nataro & Mobley 2004), combined with the reduced cost and improved computer algorithms for comparison, has most recently shifted the approach to WGS typing, especially as *in silico* MLST could be undertaken with the same data (Lukjancenko, Wassenaar & Ussery, 2010). The potential of WGS for microbial subtyping has recently been reviewed (Perez-Losada et al., 2017) and the technological requirements for this will be addressed in Section 7.

Whole genome sequencing is now widely accessible and affordable. Early comparative genomic studies showed a surprising level of genetic diversity within the *E. coli* species (Lukjancenko et al., 2010). The core genome of *E. coli* comprises 1996 genes, which is only 37-49% of the genomes of individual strains (Holmes et al., 2015). This number of genes is expected to fall as more strains are sequenced. Strains from different *E. coli* pathotypes tend to be from different major phylogenetic clades, suggesting that virulence is endowed by the variable genes constituting the remainder of the pan-genome.

Whole genome sequences of strains within the STEC population are also genetically diverse and once again there are no absolute correlates with disease outcome. Nevertheless, recent phylogenetic studies have established that *E. coli* O157:H7 can be categorised into three main lineages and nine clades (Shaaban et al., 2016) with disease and geographical relationships. Although more severe human disease is associated with strains of lineage 1/11 clade 8 in the USA, most clinical isolates belong to lineage I clade 4/5 in the UK (Dallman et al., 2015b).

Whole genome sequencing can also provide *in silico* information about both O and H serotype by targeting genes involved in surface antigen synthesis, the *wzx/wzy* and *fliC* gene sequences respectively, (DebRoy et al., 2004; Ingle et al., 2016; Wang, Curd, Qu & Reeves 1998). On the basis of cost, speed and availability such molecular serotyping may rapidly replace conventional serotyping (Delannoy et al., 2017).

WGS can also provide additional information on virulence and antibiotic resistance genes (Ferdous et al., 2016). Moreover, WGS enables a rapid understanding of strain evolution via horizontal gene

transfer. For example, in the German STEC outbreak of 2011 there was a transduction of an EAEC O104:H4 strain with Stx phage (Rasko et al., 2011) to produce a novel virulent STEC strain. For epidemiological purposes it would seem that the WGS approach is useful for detecting time-restricted outbreaks but, because of the possibility of gene uptake or loss, not necessarily for the comparison of strains from different outbreaks or the tracing of infection sources unless deep analysis is undertaken.

Clearly WGS, providing a wealth of molecular information for clinical, epidemiological and research purposes is now the optimal approach to STEC strain characterisation. However, the approach raises a number of issues, which will need to be addressed. In particular, there is an urgent need to develop bioinformatics tools and expertise to interpret and utilise the information being produced in huge quantities (the mining of 'big data'). There are also concerns regarding the storage space required, ownership, security and ethics of the genomic data. Finally, consideration should be given to the implications for historical and current collections of STEC strains. Although, the long-term storage of strains is costly and time consuming, the live organism provides invaluable phenotypic information that cannot be replaced with just a genome sequence.

7. Rapid detection and diagnosis

Speed can be essential in the diagnosis of human STEC infection as generally antibiotic therapy is contraindicated if an infection is caused by an EHEC strain. However, improved test speed is often at the cost of sensitivity and specificity. A major issue for the development of rapid tests is that STECs predominantly resemble commensal *E. coli*, both phenotypically and genotypically. Furthermore, they are often only a minority population among patient faecal coliforms.

Rapid tests are also important for environmental specimens to enable epidemiological tracing during outbreaks and the monitoring of food contamination in the production line. In such samples viable STEC are often present in even smaller numbers. In addition not all STEC strains have the same capacity to cause severe disease or present a public health danger. Therefore, the problems facing microbiologists are twofold: first, they must established whether STEC are present in a given sample and second, they need to establish whether this is a substantial disease risk by isolating and/or identifying and characterising the STEC present in the sample.

To date the development of methods of STEC detection have largely focussed on *E. coli* O175:H7 and these have been recently reviewed by Wu, Hulme & An (2015). In this section we will focus on those techniques, which (sometimes with modification) enable the rapid detection of all STEC infections in humans, their carriage by animals and their contamination of foods. Apart from *E. coli* O157:H7 the serogroups considered important causes of STEC infection in humans are O26, O45, O103, O111, O121 and O145: together these are often referred to as the "Top 7 STECs".

7.1 Culture dependent methodologies

Culture remains the "Gold Standard" for the detection of STEC in clinical and environmental samples and, with the advent of numerous chromogenic agars and antibody-bead retrieval systems, it is still the method of choice for many diagnostic laboratories. However, culture does have a number of disadvantages. These include the ability to detect only viable organisms, the potential for normal flora to mask STEC growth, the relatively low limit of detection, the time required (24-48hrs) to obtain a definitive diagnosis and the additional procedures needed to obtain serotype, virulotype or antimicrobial resistance (AMR) information. Antimicrobial treatment is generally contraindicated for patients with EHEC infections, however when antimicrobials are required they should be appropriately targeted so that unnecessary selection of resistance in residual flora is avoided. The generation of

antimicrobial resistance in STEC might also be detrimental to the implementation of some novel control strategies for example the direct application of antimicrobials to the terminal rectum of colonised cattle (Naylor et al., 2007). Because culture is labour intensive, expensive and time consuming, there is an urgent requirement for rapid, economic tests.

7.2 Non-culture dependent methodologies

The effectiveness of rapid microbiological diagnostic and detection tests depend on a number of factors including the type of microbial target, its surrounding environment, the expertise of the laboratory, etc. For the purposes of this review such tests will be divided into immunologically-based or genetically-based methodologies.

7.2.1. Immunologically-based methods:

Immunomagnetic separation (IMS) significantly increases the speed and sensitivity of detection of STEC, particularly for bacterial isolation and culture from complex matrices such as faeces and ground beef. Although, the efficacy of all immunologically-based tests is highly dependent on the specificity, clonality, isotype and kinetics of the antibodies used, insufficient consideration is often given to this test component. For IMS, the detection of all major serogroups of STEC requires treating each sample individually with serogroup-specific beads. Such a labour-intensive and time-consuming procedure can now be modified by combining serogroup-specific beads without loss of sensitivity (Noll et al., 2016).

A number of antigen-capture indirect and direct enzyme-linked immunosorbent assays (ELISAs) are commercially available as test kits for a range of applications in STEC detection including food monitoring and organism detection in human or animal faecal material with or without prior enrichment. Some of these test kits claim quantitative as well as qualitative outputs. In recent years considerable research effort has gone into increasing test kit speed, ease of use and sensitivity by improving the enrichment and preparation of samples, for example by immunomagnetic bead recovery, and by amplifying the detection signal. The development of nanotechnology has been particularly successful for this purpose. For example, by combining immunomagnetic separation and functional nanoparticle-enhanced ELISA (FNP-ELISA), detection levels for *E. coli* O157:H7 of less than 1000 cfu/ml in foods is achievable (Shen et al., 2014) with a test time of approximately 3 hours. However, such a level of sensitivity is often too low for detection to be practical, for example in many foods. Sensitivity can be a problem with this approach, especially if there is cross reactivity or other *E. coli* are present which might overgrow low numbers of pathogenic bacteria.

The presence of Shiga toxin in faeces is time limited during infection, however, this approach, with or without culture, is often used in suspected cases of HUS to ensure the rapid application of appropriate treatment. Stx expression levels vary between strains but can be upregulated by exposure to mitomycin C or polymyxin B during enrichment. However, until recently the ability of immunosorbent assays to detect all forms of Stx1 and Stx2 has been a significant problem (Wu et al., 2015). In 2016 the development of new antibodies was described (He, Kong, Patfield, Skinner & Rasooly 2016) directed against all the known subtypes of both Stx1 and Stx2, which substantially increased the sensitivity of ELISA for STEC strains both on cultured isolates and in ground beef samples.

With the availability of suitable polyclonal and monoclonal antibodies, the commercial test kit platforms currently used for *E. coli* O157:H7 detection, should become available for the "top 7 STECs". Many such kits have been developed and commercialised and marketed largely on the basis of speed. However, the performance characteristics of such tests, especially in terms of sensitivity, varies enormously and is often substantially poorer than culture. Also many of the modern immunologically-based test platforms are highly specialised, tying laboratories into long-term investments which may

not be economic. Choice of platforms should take into account sample type, through put and technological competence available. Wu et al., 2015 have reviewed several of the recently developed immunoassay platforms adapted for detection of *E. coli* O157:H7, with improved sensitivity based on approaches such as peptide nucleic fluorescence *in situ* hybridisation and improved ease of used based on microfluidics. Most recently novel platforms developed have included electrochemical immunosensors (for example Güner, Çevik, Şenel & Alpsoy, 2017) and lateral flow immunoassays, which increasingly exploit nanotechnology to improve reporter sensitivity (for example (Cheng et al., 2017). Such technologies will enable portability into the field as well as robustness and ease of use for unskilled workers.

7.2.2 Genetically-based methods:

7.2.2.1. PCR and DNA hybridisation-based assays

Most of the methods currently used to detect STEC in faecal specimens or food, involve DNA-based techniques. Overall, PCR-based methods have been most widely applied. In these assays, short sections of the DNA specific for the *stx* genes are usually selected and specific oligonucleotides (primers) used to amplify specific sections of these genes (Bettelheim & Beutin, 2003). Some PCR assays have also aimed to detect the serotype (Anjum, Tucker, Sprigings, Woodward & Ehricht, 2006; Maurer et al., 1999; Paton & Paton, 1999). PCR is rapid, cheap and these days widely accepted and utilised in routine laboratories and, with careful primer choice, can be adapted for the detection of multiple DNA targets at one time. However, the reliance on PCR only for STEC diagnosis or detection, especially using primers for *stx* genes, is questionable (Martinez-Castillo & Muniesa, 2014) given that free *stx*-phages may be present in faeces and environmental samples to interfere with the results. Similar caveats apply to the reliance on the presence of *stx* genes in whole genome sequences. Modification of sample preparation could reduce such interference (Martinez-Castillo & Muniesa, 2014).

Although the simple presence of EHEC strains is sufficient evidence of food contamination, in some countries, for the accurate assessment of public health risk a quantitative approach is necessary. Enumeration by the Most Probable Number (MPN) method is both tedious and time consuming. Quantitative PCR (qPCR) methods have now been developed to enumerate STEC in samples, such as milk, providing rapid and accurate results (Mancusi & Trevisani, 2014).

DNA arrays, however, are most useful for the detection of large numbers of different DNA targets. Such arrays are based on the detection of DNA hybridisation to multiple target DNA sequences. The development of miniaturised arrays (Anjum et al., 2007) with wide panels of virulence and antimicrobial resistance (AMR) genes has enabled the rapid characterisation of *E. coli* strains, including STEC (Bekal et al., 2003; Bruant et al., 2006; Anjum et al., 2014; Szmolka, Anjum, La Ragione, Kaszanyitzky & Nagy, 2012). Such arrays provide a practical high through-put tool for detecting pathotypes and antibiotic resistance mechanisms.

PCR has been adapted by combination with ELISA (Fach, Perelle, Grout & Dilasser, 2003) to develop a detection method for STEC in clinical samples. The principle of the method is the incorporation, during the PCR amplification process, of digoxigenin-labelled dUTP and a biotin-labelled primer specific for the *stx* genes. The labelled PCR products, bound to streptavidin-coated wells of a microtitre tray through the biotin, are then detected by an ELISA technique. PCR-ELISA has the advantages of specificity and sensitivity over standard ELISA as well as removing the need for specific antibodies or purified antigens. PCR-ELISA also provides opportunities for automation and improved test times over standard PCR.

The development of real-time PCR techniques has been of significant advantage and these have been applied to detect a number of pathogens. Real-time PCR has been used to detect and quantify STEC strains of serogroups O157, O111 and O26 in both beef and bovine faecal specimens (Sharma, 2002). Reverse transcriptase PCR designed to detect viable STEC has also proved useful (McIngvale, Elhanafi & Drake, 2002). Quantitative PCR assays have also been widely employed to determine the levels of colonisation and/or contamination in various samples (Verstraete et al., 2014).

7.2.2.2. LAMP technology

Loop-mediated isothermal amplification (LAMP) is a novel isothermal nucleic acid amplification method with high specificity, efficiency, and speed. It has been employed for the detection and identification of bacteria, fungi, viruses, and parasites. The LAMP assay utilizes simple and inexpensive laboratory equipment and, as a result, has been investigated as a potential molecular point-of-care diagnostic tool. Detection methods, including the use of turbidity (based on magnesium pyrophosphate generation during the reaction), fluorescent dyes (calcein and SYBR-1) and complexometric detectors, such as hydroxynaphthol blue, have all been explored for the detection of LAMP reaction products. Recent advances in genome sequencing technology enable primers to be designed on pan-genomes generated from multiple strains, thus increasing specificity. LAMP can detect between 5 – 10 organisms in a swab sample and is therefore considered to have superior sensitivity to culture or PCR based diagnostics (Diribe et al., 2014; La Ragione, R.M., personal communication). LAMP assays have been designed on a number of targets, including; *rfbe* for Enterohemorrhage *E. coli,* Z3276 gene for the detection of *E. coli* O157:H7 and *stx1* with *stx2* for Shiga toxins. Recent advances have also combined LAMP with propidium monoazide (PMA) assays for the detection of viable, but non-culturable organisms (Yan et al., 2017; Ravan, Amandadi, & Sanadgol, 2016).

LAMP was originally developed by the Eiken Chemical Company (Japan). It relies on auto-cycling strand displacement DNA synthesis by a DNA polymerase with strand displacement activity (*Bst* polymerase). LAMP uses a set of four specially designed primers (two sets of inner and outer primers) that recognise six distinct sequences within the target DNA. LAMP reactions are initiated by the inner primers, which contain sequences of both the sense and antisense strands of the target DNA. The outer primers prime the initial DNA synthesis step, leading to the release of single stranded DNA. The released single stranded DNA is primed by the inner and outer primers and serves as a template for the synthesis of a stem loop DNA structure with self-priming capabilities. Generation of this stem loop structure is an essential part of the amplification process.

LAMP reactions are typically performed at 60^{o}C – 65^{o}C, the optimum temperature for the polymerase used, as well as the temperature at which double and single stranded DNA are in dynamic equilibrium (Mori, Nagamine, Tomita & Notomi, 2001). The single temperature required for LAMP reactions enables simple equipment, such as temperature controlled heat block or water bath to be used. Under ideal conditions, a few copies of DNA can be amplified to 10^9 copies in less than an hour (Notomi et al., 2000). A number of LAMP assays have been developed for the detection of STEC in clinical samples (Dong, Cho, Hahn & Cho, 2014; Wang, Jiang & Ge, 2012; Wang, Jiang, Yang, Prinyawiwatkul & Ge, 2012).

There are multiple techniques for the detection of LAMP amplicons, but for real-time analysis, the LAMP reactions can be performed on a real-time PCR thermocycler, using the following cycling conditions: A constant temperature of 65°C for a set period of time (time depends on specific assay), followed by a 60 second period with a stepwise increase of temperature from 65°C to 96°C. Fluorescence is measured in both periods, with the melting temperature of the amplicon calculated from the dissociation curve. A reaction is considered positive when the level of fluorescence crosses the cycle threshold (CT), calculated for example by the MX3000p analysis software (Agilent

Technologies, UK). Once validated using RT-PCR, the tests can also be performed on the Genie machine, suitable for any basic laboratory with very little training.

LAMP amplicons can also be detected using a Lateral Flow Device (LFD)**.** For LFD mediated detection of LAMP reactions, minor modifications to the "loop" primers are required. The loopF and loopB primers are labelled at their 5' ends with fluorescein isothiocyanate (FITC) and biotin (Btn), respectively. The LFD device contains latex beads conjugated with anti-Btn antibodies, a test line (T-line) embedded with anti-FITC antibodies and a control line, that captures the latex beads, embedded with anti-Btn. For a positive LAMP reaction, where both labelled primers are incorporated into the amplicon, the Btn in the amplicon binds to anti-Btn embedded in the latex beads. On reaching the test line, the anti-FITC embedded in the test line binds to the FITC portion of the amplicon, while free and mobilised beads are trapped on the control line. In the absence of the target DNA, where no amplicon is produced (a negative reaction), there is no co-localisation of the antigen-antibody complex formed by the amplicon and latex beads on the test line of the LFD. PCRD-2, is a commercial sandwich immunochromatographic LFD, which specifically captures double stranded amplicons that contain both FITC and biotin (Pocket Diagnostics, UK).

The efficiency and speed of LAMP technology means that tests can detect STEC infections and provide information on, for example AMR profiles, in less than 15 minutes, directly from a swab, with excellent specificity and sensitivity (Diribe et al., 2014; Diribe, Thomas, AbuOun, Fitzpatrick & La Ragione, 2015).

7.2.2.3. Direct Next Generation Sequencing (NGS) approaches

Improved DNA sequencing methods have transformed the field of genomics over the last decade. This has become possible due to the development of inexpensive sequencing technologies, which have now resulted in four generations of sequencing platforms (Cherukuri & Janga, 2016). These NGS technologies have now been adapted for the diagnostic laboratory environment and can be used on pure cultures as well as mixed cultures/samples (Dallman et al., 2015b; Schmidt et al., 2017).

There are a number of NGS technologies already in use in diagnostic microbiology laboratories but most require specialist staff especially for analysis of the data. However, the recent development of the Oxford Nanopore Technology's MiniION sequencing device offers a patient-side personalised medicine solution. This technology is based on protein nanopores that are set in an electronically resistant membrane. An ionic current is sent through the nanopore by setting a voltage across the membrane. When a DNA strand is passed through the nanopore the current makes is possible to identify which nucleotides are passing through. This technology has already been applied to direct sequencing of pure and mixed bacterial cultures (Kilianski et al., 2015; Ma, Stachler, & Bibby, 2017) and has enormous advantages of robustness, portability and ease of use enabling application outside the laboratory - even in outer space (https://www.nasa.gov/mission_pages/station/research/news/dna_sequencing).

As mentioned above, the current technologies now allow for direct sequencing from cultures, thus providing accurate and detailed genomic data within hours and such direct whole genome approaches have already been applied to the diagnosis of STEC and outbreak investigation (Dallman et al., 2015a) and to the risk assessment of Stx-producing *E. coli* in cattle populations (Mainda et al., 2016).

It is easy to envision that in the near future doctors will take clinical samples such as faeces, robots will undertake DNA extraction, sequencing will only take a few minutes, and accessory software will assist applied bioinformatics to answer specific questions. Thus, within minutes, the presence of a pathogen will be confirmed and those genes, if any, which encode for its virulence and antimicrobial resistance will be identified. In combination with available patient data, the clinician can then swiftly

assess the risk of the infection to a specific patient and determine appropriate therapy. Similar technologies will be applicable to food safety and veterinary medicine.

With such technological advances molecular epidemiology is now able to mine pan-genome rather than core genome information for comparative purposes. The increased sensitivity for strain differentiation is enormous. However, the practical and philosophical problems associated with acquiring, handling and effectively interpreting such a wealth of data have also been, and continue to be, enormous, but have only recently been cosidered (Bertelli & Greub., 2013; Del Chierico et al., 2015; Kozyreva et al., 2017). There are two overarching problems which need to be addressed. Firstly, available microbial WGS data is immense, ever-growing and increasingly complex. The ownership, storage, security, quality control and ethics of use of such data needs to be discussed by the scientific community preferably as part of a public debate. Part of that debate should be about the impact of the major shift from traditional cultural to genomic approaches to infectious disease investigations in public health. With such a shift, economic restrictions are beginning to put pressure on the retention of new strains and historical strain collections. The assumption that WGS will provide all the information required and that all phenotypic properties will have little future value is at best naive and at worse foolish because bacteria will always evolve to surprise us.

The second major problem is the how to extract required and worthwhile information from this huge data pool. For the purposes of this review there appears to be one overall question: "Can we predict from WGS which STEC strains constitute a risk of serious human disease?" Of course within that question there are many others such as "Can we differentiate STEC strains of difference levels of virulence?" and "What is the distribution of virulence properties within strains of individual STEC serotype or from specified sources?". Such questions can only be answered if the available data is sufficiently comprehensive. There is a clear and urgent need for large databases of fully closed genomes of strains that are well described in terms of source, disease association, etc. It is important that such strains are collected from across the world, from multiple host species and environments and represent historical strain collections as well as ongoing surveillance. This will need considerable investment and the requirement for the collection and curation of WGS data on strains at the national and international levels.

Currently few laboratories have the expertise to interpret WGS data. Interrogating WGS databases to generate useful information has become a highly specialised skill of bioinformaticists who have developed multiple highly complex computer-based processes and tools, and a sophisticated jargon, to undertake this task. Bioinformatics pipelines, which are the logical ordering of multiple computing procedures automatically undertake all base calling analyses, sequence assembly of reads and gene identification. Such platforms, taking the raw WGS data and processing it for comparative genomics and data interpretation, are beginning to replace the tedious and time-consuming process of manual data handling (Kilianski et al., 2015). However, there are many such pipelines described and to date no standard workflow language has been adopted so that this area of expertise has become increasingly specialised. Frequently, there is limited understanding of how the data has been handled and the consequences of this on data interpretation. Increasingly such information restrictions, in addition to the barriers already existing between expert bioinformaticists and non-expert microbiologists/clinicians will limit the application of WGS to clinical decision making.

WGS is likely to be the future for routine STEC detection and diagnosis, especially during outbreak investigations, and one major advantage of this technology is its mobility. With the availability of miniaturized sequencing technologies, samples taken at the bedside or from the farm could be immediately sequenced and analysed with hand-held devices, which will then transmit the data, via improving wireless communications, such as 5G, to a central databank for strain comparison. Such a technological step change will almost certainly have a huge impact on future epidemiological investigations and enable interventions to be rapidly applied.

8. Conclusions

In conclusion, despite considerable progress in the detection and diagnosis of STEC a number of research issues remain to be addressed (Table 1).

Over the last 10 years or so the time required to identify, and even characterise the pathogen in clinical material and samples throughout the food chain has reduced from 5 days to a few minutes. Where available such facilities have dramatically improved the diagnosis and treatment of patients as well as improving epidemiological investigation and the management of microbiological hazards along the food chain.

In recent years the development of novel non-culture based technologies, with high efficiency, speed and ease of use, has even enabled the application of point of care tools raising the possibility of personalised diagnostics for infectious diseases such as STEC. Such technologies could easily be adapted to monitoring in food processing lines.

Nevertheless, the isolation and culture of organisms will always remain the "Gold Standard" in bacteriology and improved culture media and conditions, which will enable the recovery and rapid identification of non-O157 as well as O157 STEC are urgently required. The ongoing research using metabolic models will certainly contribute to such selective media.

The benefits of WGS using new generation technologies has provided a significant boost to the identification and characterisation of all *E. coli* and especially STEC. However, such rapidly evolving technologies have generated considerable new issues that need to be urgently addressed such as data security and interpretation strategies.

The development of rapid methods to monitor and characterise STEC strains in the food chain and environment has limited value unless it is used for the purposes of intervention. Recent studies indicate that interventions to reduce STEC entering the food chain have had limited success. Clearly there is an urgent need for more research on vaccines, especially in the major livestock reservoir, cattle. Unfortunately, the cost of vaccine development for livestock, where the benefit is to public health and not the food industry, is unlikely to command priority for funding. Nevertheless, our understanding of bovine immunity to STEC is increasing and the discovery of effective antigens, driven by WGS data mining, remains a high priority. Alternative innovative and cost effective ways of delivering appropriate immunity may also prove effective in the future, such as the generation of passive immunity through the consumption of genetically engineered antibodies (Kühne et al., 2004; La Ragione et al., 2006).

Ultimately, although huge progress has been made, STEC still remains a significant zoonotic issue. Substantial reductions in the public health burden due to this infection will require a multi-pronged approach. The world-wide surveillance in humans, livestock and foods, using diagnostic and detection methods with high sensitivity and specificity, in conjunction with high-resolution subtyping of isolates, will be required to monitor future progress and detect STEC evolution. Increased awareness and education of the general public and food producers will be required to improve food hygiene and on-farm management and abattoir practices to reduce ruminant colonisation and meat contamination. Finally, there is an urgent need to develop appropriate and complementary interventions, including vaccines, to control STEC colonisation in livestock and prevent environmental contamination.

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Table 1: Current challenges for the investigation, control and prevention of STEC and the roles that novel and innovative detection, and diagnostic techniques could play in meeting these challenges.

Research challenge	**Importance**	**Research required, with particular relevance to detection & diagnostic techniques**	**Example references**
Prioritise the public health burden of STEC disease at national and regional levels.	Accurate and timely disease monitoring and data collection to prioritise public health and research funding.	Shift focus from O157 to all STEC. Sample collection and diagnostic tests that are suitable for monitoring all STEC disease in routine laboratories.	NA
Provide rapid response to STEC outbreaks.	Critical to detection of sources, limit outbreak and provide appropriate clinical care. Must be able to detect newly evolved strains (e.g. O104:H4).	Rapid tests of clinical disease with broad specificity to detect all STEC strains.	NA
Understand and monitor the population structure of all STEC.	Essential to understanding the pathogenicity, epidemiology and evolution of the pathogen.	Comparative genome sequencing of large collections of human, animal and environmental STEC strains. Requires large numbers of complete and closed genomes The observation of STEC population shifts over time require long-term monitoring, and accurate and relevant subtyping techniques.	Adams et al., 2016
Determine the role of non-O157 strains in human disease.	To inform risk assessment models.	Rapid, economic and easy to use laboratory methods for recovery, culture, detection and subtyping of non-O157, as well as O157, STEC strains in human and animal faeces.	Frank et al., 2011 Severi et al., 2016
Define the minimum infective dose and the variation in dose	To inform risk assessment models.	Volunteer studies not feasible, so epidemiological studies	Teunis et al., 2008

response for human STEC infections.		required. Rapid, portable, quantitative and comprehensive detection methods applicable for detailed epidemiological investigation of outbreaks. An alternative approach is to develop surrogate *in vivo/in vitro* models, with evidenced-based relevance to human STEC disease.	Newell et al., 2016
Define and characterise the virulence factors/genes essential for severe human disease.	To predict the clinical outcome of human disease and ensure appropriate and timely treatment.	Fundamental studies required to investigate the genetic-basis of virulence and then use to develop subtyping methods to distinguish levels of potential virulence in STEC strains.	Beutin et al., 2008 Shaaban et al., 2016 Franz et al., 2015 Delannoy et al., 2013
Prevent the severe consequences of human infection such as HUS.	Reduce mortality and chronic disease	Rapid, point of care tests indicating the level of STEC strain virulence, degree of colonisation and indicating appropriate treatments.	Vanaja et al 2013 Kruger and Lucchesi, 2015
Understand the genetic basis and evolution of antibiotic resistance in STEC.	Antimicrobial treatment is generally contraindicated for patients with EHEC infections, however when antimicrobials are required they must be appropriately targeted so that the unnecessary selection of resistance in residual flora is avoided.	Rapid point of care tests should include antimicrobial resistance targets.	NA
Determine and prioritise the sources, reservoirs and routes of transmission for all clinically important STEC.	To inform risk assessment models, develop regulatory frameworks and develop/prioritise intervention strategies.	Requires detailed epidemiological investigation of animals, environment, plants etc. using detection systems, which will quantify and subtype all STEC, be suitable in all environments and provide robust comparative data.	Mancusi & Trevsani, 2014
Understand the genetic basis of survival and persistence of STEC in	To develop control strategies for environmental contamination	Will enable the development of tests to determine which strains will survive on foods etc.	N/A

the environment including biofilms.		and the development of appropriate decontamination.	
Understand the mechanisms of colonisation of livestock by STEC and the factors influencing colonisation.	Reducing STEC in the food chain is the currently available public health measure.	Investigation of *in vivo* models expanded to non-O157 strains. Understanding genetic basis of STEC colonisation and "supershedding" will allow suitable tests for selection of resistant livestock.	NA
Develop interventions to effectively reduce/eliminate colonisation of livestock by STEC.	Controlling STEC dissemination in livestock will also reduce environmental and thus food contamination.	Rapid, cheap pen-side tests which are robust and quantitative required for determining factors including colonisation and monitoring effectiveness of interventions such as vaccines or feed supplements such as probiotics.	NA
Determine the most effective vaccine candidates for livestock and develop appropriate delivery systems.	To provide complementary control/prevention strategies in the food chain	Mining of WGS data required to identify appropriate antigens expressed only by virulent STEC.	Lupolova, Dallman, Matthews, Bono & Gally 2016
Investigate safe and effective decontamination methods to be used post-harvest and in the food chain environment.	To ensure delivery of safe food at retail	Improved recovery methods for all STEC strains to ensure test accuracy to assess bacterial survival. Rapid quantitative tests needed that can be inserted into food production lines for rapid on-line monitoring.	NA
Replace time and resource consuming routine STEC isolation techniques.	The surveillance of STEC in humans, foods and the environment requires diagnostic and detection tests appropriate for the sample, level of information required and laboratory conditions. Culture remains the "Gold Standard" and will remain important for many	For the foreseeable future STEC isolation will remain important. Low-tech and cost effective methods to improve STEC recovery, speed up culture and enable simple characterisation are still needed Increased availability of WGS data has enabled	NA

	routine public health laboratories.	rapid and specific genome- based tests for strain characterisation, such as LAMP to be developed. Such tests now need to be translated into low tech practical formats.	
In the near future WGS will be the preferred route for strain identification, subtyping and virulence assessment but what are the issues to be addressed with WGS to ensure best use of this data?	Modern genome-based techniques will provide new opportunities for improved diagnosis and approaches to treatment and intervention.	Development of international standards of interpretation and quality control measures The storage capacity needed, data ownership, security and ethics all need urgent consideration. Development and availability of bioinformatics pipelines and platforms using a standard language. Improved communication between expert bioinformaticists and non-expert microbiologists.	Kozyreva et al., 2017 ; Del Chierico et al., 2015; Bertelli & Greub, 2013
If public health laboratories shift to WGS for data storage, should historical and on-going strain collections still be retained?	Phenotypic and genotypic information are complementary.	Phenotypic studies are required to ensure rapid genetic tests developed are relevant and appropriate.	NA