Microbial update

E. coli

Dr Roy Betts, Head of Microbiology, Campden BRI, Chipping Campden, Gloucestershire, GL55 6LD, UK.
Produced as a service to the food industry by Oxoid, part of Thermo Fisher Scientific.

Perhaps of all foodborne bacteria, Escherichia coli is the most famous. It has been isolated and researched continuously for nearly 130 years, but it still poses great problems and can cause large unexpected outbreaks of food poisoning.

Theodor Escherich was a pioneering paediatrician, who studied bacteriology and believed that a study of gut micro-organisms could potentially solve many infant illnesses.

His work took him to laboratories around Europe, and he attended large outbreaks of illness such as the 1884 cholera epidemic in Naples. In 1885 Escherich published a paper describing 'Bacterium coli commune' – a bacterium that he isolated from the stools of infants suffering from enteritis.

Escherich died in 1911 and never saw his own name attached to this now famous organism, as this only occurred in 1920 when Castellani and Chalmers first described the Genus Escherichia, named in honour of Escherich.

The Genus contained the single species E. coli for 54 years, before it was joined by E. blattae in 1973, this being an odd organism isolated from the gut of a cockroach.

In the 1980s three more species were identified E. hermannii, E. vulneris and E. fergusonii. The final species E. albertii was added in 2003 being isolated from the stools of children in Bangladesh.

But of course, the best known of all species in the Genus remains E. coli, known by school children and students as probably the first bacterium they ever cultured, and by readers of the popular press as the cause of many outbreaks of food poisoning.

What is it?

E. coli is a Gram negative, non-sporeforming, rod shaped bacterium and a member of the Family Enterobacteriaceae. They are usually motile and have an optimum growth temperature of 37°C, but can grow over a wide temperature range from approximately 15-45°C.

The organism is a facultative anaerobe growing equally well under either aerobic or anaerobic conditions. The organism is widely dispersed in nature, its origin is usually the gut of animals, but from there it will spread to various soils and waters through animal faeces, and is often considered to be a 'marker of animal faecal contamination.

What does it cause?

E. coli is a very common micro-organism. The intestinal flora of man contains an abundant flora of Enterobacteriaceae, and of these E. coli is the predominant species. The organism can be found in the gut shortly after birth and soon colonises the large intestine. It is a saprophytic commensal organism and is believed to play an important role in various physiological functions of the gut.

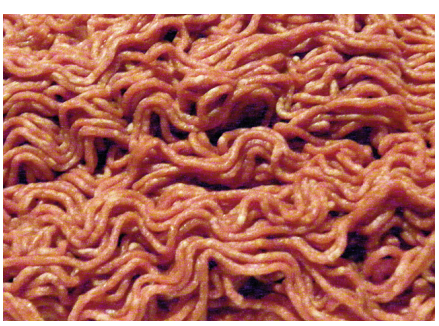
There are, however, many different strains of E. coli recognised and, whilst a vast majority are not just harmless but necessary for the good functioning of the gut, some are pathogens and will cause illness.

The types of illness caused by E. coli is very varied, all will usually result in some form of diarrhoea that begins approximately 24-48 hours after consuming the organism, but the severity of the resulting illness will vary from a mild and short lived problem, to a major life threatening disease.

The different pathogenic forms of E. coli are noted below:

- **Entero-pathogenic E. coli (EPEC).**
This group produces watery diarrhoea with vomiting and fever. It is usually self limiting. This group are usually associated with infants and young children and have in the past caused large outbreaks in hospitals. There have been few reported outbreaks in Europe or the USA since the 1970s but it can be an important illness for children in developing countries.

- **Entero-toxigenic E. coli (ETEC).**
This group produces watery diarrhoea with abdominal cramps, fever, malaise and vomiting. In very severe cases the disease can resemble the symptoms of cholera. It is a major cause of illness in children in developing countries and the major cause of travellers' diarrhoea.



- **Entero-invasive E. coli (EIEC).**
This group produce acute watery diarrhoea with a fever and abdominal cramps. Sometimes the stools can become bloody and mucoid.

- **Entero-haemorrhagic E. coli (EHEC) (also known as Verocytotoxin producing E. coli (VTEC) or Shiga Toxin producing E. coli (STEC).**
Also commonly known as VTEC or in the USA as STEC, this group were first described in 1977, but it was only in 1982 that they were first recognised as a cause of haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS).

The key strain of this group is E. coli O157:H7 but others (O111, O26, O103, O121, O45, and O145) can cause a similar illness. These strains cause the most severe form of food poisoning produced by E. coli, with symptoms of severe bloody diarrhoea and blood in the urine. Some of those affected suffer kidney failure and death from these infections can sometimes occur.

In recent years there has been considerable interest in non-O157 VTEC and in the USA there are now regulations in place that require all processed raw meats to be tested for serotypes O26, O45, O103, O111, O121, and O145 (known in the USA as the 'big 6') as well as O157.

In Europe the large outbreak of food poisoning associated with E. coli O104:H4 sprouted seeds in Germany and France in 2011 has resulted in new microbiological criteria being developed. These criteria will come into force mid way through 2013 and require seeds used for sprouting and sprouted seeds to be tested for VTEC serotypes O157, O26, O111, O103, O145 and O104:H4.

There are a limited number of methods for the detection of these serotypes, a number of kit manufacturers have produced methods that cover either VTEC in general, or the US big 6 serotypes, but at present there are no kits covering the serotypes in the European criteria. So in Europe we are left with an ISO Technical Specification (ISO/TS 13136:2012) covering O157, O26, O111, O103, O145, but not O104:H4.

In the future we will see increased testing for non-O157 VTEC serotypes as we increasingly recognise their public health significance.

● **Entero-aggregative E. coli (EAEC or EAggEC).**
These strains have been associated with persistent diarrhoea lasting for many days (up to 14 days) usually watery, with vomiting, dehydration and abdominal pain.

Where does it come from?

E. coli is an organism that will always originate in the animal gut, research has shown that most animals carry E. coli and in a majority of cases it causes no ill effects to the animal host. However, whilst individual strains cause no ill effects to their immediate hosts, if that strain were introduced to a different 'host' then illness could result.

In foods, contamination will always have a distant link to animals, this may be passed on through soil, plants (through close contact with soils or contaminated irrigation water), water itself (contamination occurring through field run off, or animals being present in water sources) or even human contact with food where poor hygienic practices are followed.

General E. coli measurements are often used as a hygiene indicator in food production systems, as they will tend to show up areas that have been improperly cleaned, or where the process has failed to eliminate or reduce contaminants to an acceptable level. E. coli is sometimes considered a 'faecal indicator' which is not strictly true. Whilst the organism will probably have always originated from a faecal source, it is naturally found in many soils and other 'natural' environments.

The Health Protection Agency (HPA) give guidance on levels of general E. coli in ready to eat foods, they note that less than 20/g is satisfactory, whilst between 20-100/g is considered 'borderline' and should result in review of processing and cleaning procedures and potentially more investigative sampling and testing. A level greater than 100/g is considered unsatisfactory according to HPA guidance.

Common sources for the most pathogenic forms of E. coli (VTEC) are raw meats, underprocessed or post process contaminated milk, uncooked salad items/vegetables, raw waters, unprocessed fruit juices, unprocessed flour. However, the importance of direct contamination should not be misjudged.

There have been cases of VTEC illness traced to person to person contamination, animal to person contamination (from zoos and animal parks) and even cases originating from children playing in a paddling pool that had become faecally contaminated.

Controls for E. coli

E. coli is not a difficult organism to control. It is not particularly heat resistant and can be prevented from growing by reducing pH or water activity of foods. But it is important that food producers consider the risks of E. coli in their own production environments, and introduce appropriate controls if required.

Adequate cooking will kill E. coli, and in the UK a process of 7°C for two minutes at the slowest heating point within the product, is considered appropriate to reduce the risk of the organism considerably from all products (note: milk pasteurisation treatments are covered by legislation and must be followed).

Adjusting product pH, water activity or decreasing the temperature will prevent the growth of the organism. However food producers using such techniques must remember that whilst growth may be prevented, if the organism is present in the food or ingredient, it will remain viable. Unlike salmonella, where all types are considered pathogenic and must be eliminated from ready to eat foods, relatively few strains of E. coli cause illness, and this has implications when considering microbial criteria for foods.

Pathogenic strains (E. coli O157) should not be present in ready to eat foods, and specific test methods for this type can be used to check for absence of the organism in 25g of product if required.

With other (non-pathogenic) E. coli, however, whilst the absence of the organism is always the target, the presence of very low levels can be accepted, as long as suitable checks are done to eliminate or reduce the problem from future production.

Key control factors that can be used to prevent the growth of VTEC and most other types of E. coli are pH <4.4, water activity <0.935, temperature <7°C. Combinations of these factors well above the values noted previously may be equally as inhibitory to growth, but these should be established using predictive microbiology or challenge testing techniques before they are used.

At one time E. coli O157 were thought to be more acid resistant that other types, but these concerns do appear to have been greatly reduced, and acid resistance in the organism is not now a major concern.

Detection and enumeration

Methods used to test foods for E. coli can broadly be split into three types:

● Direct plating methods for standard E. coli that use a specific diagnostic medium. These methods will tend to have a lower limit of detection of 20 cells/g and are simple to use.

● Broth based methods for standard E. coli using one or more broth media to detect or enumerate (via MPN) levels of E. coli less than 20/g.

● Specific enrichment based methods to detect pathogenic E. coli O157. These tend to be based on enrichment in a selective enrichment broth, followed by a separation phase using magnetic particles coated with antibodies specific to O157 (this is known as Immunomagnetic separation). The particles are finally spread onto selective and differential agar plates. These methods are designed to detect very low levels (a single organism in 25g of product) of E. coli O157.

There are a variety of new 'rapid methods' for E. coli. Some instrument based methods have been designed to automate testing for general E. coli, whilst there are a number of new tests for the O157 types that utilise immunoassays, or the polymerase chain reaction (PCR). These tests can reduce test times for the organism by one to two days.

Conclusions

E. coli is a very interesting organism. It is undoubtedly the best studied of all the bacteria we know. It is found in large numbers in the human intestine, and some consider it essential for good operation of a healthy gut. However, some strains can be very harmful, and give rise to life threatening illnesses. In foods, general 'non-pathogenic' E. coli are often used as indicators of hygiene.

The presence of very low levels is undesirable and requires investigation, but it is not considered a major risk to food safety.

However, the presence of even very low levels of E. coli O157 (and similar VTEC organisms) is a major risk to the consumer.■

✉ maureen.sanders@thermofisher.com

References

● US Food and Drug Administration, Center for Food Safety and Applied Nutrition. Bad Bug Book – Foodborne Pathogenic Micro-organisms and Natural Toxins Handbook – Escherichia coli O157:H7. www.cfsan.fda.gov/~mow/chap15.html

● Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection of E. coli O157, EN ISO 16654.

● Chapter 35 Pathogenic E. coli (2001). In Compendium of Methods for the Microbiological Examination of Foods, 4th Edition. Downes, F. P & Ito, K. APHA.

● Death of Professor Escherich. The Lancet, 1911, 1: 626.

● Pathogenic E. coli. C. Bell and A. Kyriakides. In 'Foodborne Pathogens' Eds. C. Blackburn and P. McClure. Woodhead Publishing.

● Escherichia coli (2000). G. Willshaw, T. Cheasty and H. Smith. In 'The Microbiological Safety and Quality of Food. Eds: B. M. Lund, A. C. Baird-Parker and G. Gould. Aspen Publications, Gaithersburg, MD, USA.

● ISO /TS 13136:2012. Horizontal method for the detection of STEC and the determination of O157, O26, O111, O103, O145 serogroups.