Microbial update

pathogenic E. coli

Produced as a service to the food industry by Oxoid Ltd.

Many of the most important enteric pathogens that cause disease in man are members of the enterobacteriaceae family – most notably salmonella, shigella, yersinia and Escherichia coli. Occurring naturally in the normal flora of the intestinal tract, most E. coli do not cause gastro-intestinal illnesses. There are, however, six main groups that can cause illness – five of which are associated with foodborne transmission.

Enterotoxigenic E. coli (ETEC) give rise to a clinical syndrome which includes watery diarrhoea, abdominal cramps, low grade fever, nausea and malaise – a condition frequently referred to as travellers' diarrhoea.

Enteroaggregative strains of E. coli (EAEC) cause vomiting and persistent diarrhoea, particularly in children, while infection with enteropathogenic (EPEC) strains can result in either watery or bloody diarrhoea, and is usually associated with disease in infants.

Watery diarrhoea also results from infection with enteroinvasive E. coli (EIEC), with an infective dose of just 10 EIEC organisms being sufficient to produce bacillary dysentry.

Of most concern, the enterohaemorrhagic group of E. coli (EHEC) is capable of causing potentially fatal illnesses such as haemorrhagic colitis and haemolytic uraemic syndrome (HUS), mainly in young children and the elderly.

Characteristic virulence factors of EHEC include the Shiga toxins, (Stx1 and Stx2) sometimes referred to as verotoxins, (VT1 and VT2). These Shiga toxins are closely associated with human illness. Diffusely adherent E. coli (DAEC) are also associated with diarrhoea in children, but these are not associated with foodborne outbreaks of disease.

All six of these groups have been categorised on the basis of virulence factors (virotyping), but classification on the basis of antigenic differences (serotyping) is also carried out. When used in combination, serotyping and virotyping provide a useful approach for grouping E. coli strains, although absolute categorisation cannot always be accomplished by this means.

The primary basis for serological classification are two E. coli surface components, namely the O antigen of the lipopolysaccharide cell wall and the H antigen of the flagella.

The O antigen is taken to identify the serogroup of a strain, while the combination of both O antigen and H antigen is used to identify its serotype (for example, E. coli O157:H7). Over 170 different serogroups of E. coli have been identified.

There is a degree of correlation between serogroup and virulence (virotype), but this correlation cannot be regarded as absolute. There is, however, a definite correlation between the O157:H7 serogroup and the EHEC virotype, with which food microbiologists are particularly concerned.

Undercooked minced beef has been implicated in outbreaks of illness associated with E. coli O157:H7 (Shutterstock).

E. coli in food

E. coli is present in large numbers amongst the normal flora of the gastrointestinal tract of man and other animals. For this reason, it is readily found in the environment where it can easily be transferred to raw materials and finished food products.

Its ubiquity makes it likely to be present in a very wide variety of foods, including some that are markedly acidic. It may frequently be found on crops irrigated with waste water, while fruit and vegetables may also be directly contaminated with animal faeces.

The risks are borne out by the broad spectrum of foods which have been implicated in many of the documented outbreaks of illness associated in particular with E. coli O157:H7. In addition to the more 'obvious' culprits of undercooked minced beef, raw milk and cheese curds, many other raw foods such as alfalfa sprouts, unpasteurised fruit juices and lettuce have also been found to be the cause of outbreaks in recent years.

Between August and October 2006, 199 people across the United States were reported to have fallen ill as a result of infection with E. coli O157:H7 following the consumption of fresh spinach – 16% of the victims developed HUS, and at least three people died.

On 11th December 2006, The Washington Post highlighted the suspected role of tomatoes and green onions in two other recent outbreaks of illness, while a Scripps Howard study of state health department reports to the US Centers for Disease Control and Prevention recently concluded that the risk of transmission from fresh vegetables is nearly as great as that from undercooked meat.

Isolation and detection

The phenotypic diversity of pathogenic E. coli is such that no single standard microbiological method is suitable to isolate all of them.

Traditionally, the formulae of the media used to culture E. coli were targeted towards detection of organisms as indicators of faecal contamination and general hygiene conditions, often using the Most Probable Number (MPN) technique.

It is only relatively recently, with the emer-

gence of EHEC strains as a cause of serious illness that media have begun to be formulated specifically for the purpose of selective enrichment and detection of these strains.

Several of the resulting formulations have been based on modification of either Tryptone Soya Broth or buffered peptone water through the addition of antibiotics.

EC Broth is a widely used selective medium for differentiating and enumerating faecal and non-faecal coliform organisms. This has been successfully supplemented by the addition of novobiocin and used in a method which uses immunomagnetic beads for concentration of E. coli O157.

Immunomagnetic separation of serotype O157 from selective enrichment cultures is now an established procedure and its use is detailed in the horizontal method described in the International Standard for the detection of E. coli O157 in food and animal feedstuffs – EN ISO 16654:2001.

The method details four successive stages for the detection of this pathogen. The test portion is initially homogenised in modified Tryptone Soya Broth containing novobiocin (mTSB + N) with incubation at 41.5°C (± 1°C) for six hours and subsequently for a further 12-18 hours. This is followed by immunomagnetic separation of the target organisms.

Isolation is then achieved by subculturing the immunomagnetic particles with adhering bacteria onto Cefixime Tellurite Sorbitol MacConkey Agar (CT-SMAC) and the user's choice of a second selective isolation, for example, Cefixime Rhamnose Sorbitol MacConkey Agar (CR-SMAC).

Confirmation of sorbitol-negative colonies from CT-SMAC and colonies typical of E. coli O157 on the second isolation agar is established on the basis of indole production and agglutination with E. coli O157 antiserum from a purified culture on nutrient agar.

The FDA Bacterial Analytical Manual (BAM) outlines an alternative method as follows. 25g of sample is homogenised in 225ml of EHEC Enrichment Broth and incubated at 3°C with agitation for six hours.

The following isolation procedure is then performed, before re-incubating the enrichment broth overnight at 37°C. 100µl of the six hour enrichment culture is spread onto a CT-SMAC plate, and one loopful onto a second CT-SMAC plate, with both plates then being incubated at 35°C overnight.

Pink sorbitol fermenting colonies are disregarded, while non-sorbitol fermenting neutral/grey colonies typical of O157:H7 are selected and transferred onto slants of TSA with 0.6% yeast extract and incubated overnight at 35°C.

If no colonies typical of O157:H7 grow on CT-SMAC plates, the re-incubated overnight enrichment culture is plated on CT-SMAC plates according to the enrichment procedure described above.

For confirmation of colonies picked from TSA, a test for indole production is performed using Kovac's reagent. Indole positive isolates can then be tested using commercial O157 antiserum.

Many manufacturers offer latex kits targeted at O157. Such latex kits and those for identifying other O serotype strains associated with virulence are available from Oxoid.

Such assays are both rapid and easy to use, and simply require a cell suspension to be made from a pure culture on an agar plate which is then mixed with a drop of antibody bound to coloured latex beads. If target bacteria are present, the antibody latex agglutinates with the cells to form visible clumps within a matter of minutes.

The use of automated DNA based PCR systems such as the BAX system enable detection of E. coli O157:H7.

New rapid methods

Methods of detecting E. coli in foods and environmental samples have traditionally relied on growth in culture media, followed by isolation, biochemical identification (to establish that the isolated organism definitely is E. coli), subsequently followed by serotyping – a process which is undeniably time consuming.

The introduction of products such as miniaturised biochemical kits (Microbact, Oxoid) and enzyme linked immunosorbent assay (ELISA) tests has gone some way to speeding up and simplifying the identification process.

More recently, advances in molecular techniques have helped make the overall detection and identification process very much quicker and more convenient.

The use of automated DNA based polymerase chain reaction (PCR) systems such as the BAX system (Dupont Qualicon) – distributed by Oxoid – enable detection of E. coli O157:H7 from raw ingredients, finished products and environmental samples to be obtained following enrichment in a matter of hours rather than days. Use of such systems allows commercially sensitive food safety decisions to be taken without delay.

However, the need to confirm positive results by appropriate methods should not be overlooked.

A positive reaction obtained with anti-O157 reagents is only a presumptive indication that the isolate may be of serotype O157:H7. Many E. coli of serotype O157, but not H7, have been isolated from samples.

These O157, non-H7, strains do not produce Shiga toxins and are generally regarded as non-pathogenic for humans. Because of the potential for false positive reactions it is important that further testing for the H7 antigen or the characteristic EHEC virulence markers should be carried out.

Oxoid offers a latex text for the confirmation of the presence of E. coli O157:H7 antigen and VTEC-RPLA (Oxoid) which enables detection of the two verotoxins (VT1 and VT2) which are among the characteristic virulence factors of EHEC organisms. ELISA kits are also available from some manufacturers for detection of the antigens associated with O157:H7 as well as just O157.

EHEC strains pose a very real threat to the reputations and profitability of food industry companies, and the importance of detecting them before products reach the consumer cannot be underestimated – particularly as the potentially fatal disease causing infective dose is extremely low.

It is fortunate that the microbiological technologies now evolving are proving themselves to be more than equal to the task. n

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