Microbial update

Clostridia

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Clostridia are anaerobic, spore forming bacteria occurring naturally in soil, dust and aquatic environments. For this reason the organism can also be found in a wide range of foods, including meat, dairy and canned products.

Clostridium was first described in detail in 1892 by Welch and Nuttall. It was recognised as a cause of foodborne illness as early as 18952 and the link was firmly established though epidemiological studies by McClung in 1945.

Species of the genus Clostridium, most commonly involved in foodborne illness, are C. perfringens and C. botulinum however infective dose, symptoms and severity are very different.

This article will concentrate on C. perfringens, the aetiology of the disease, sources of infection and the isolation of the organism from contaminated food.

C. perfringens is a Gram positive, anaerobic sporulating bacillus, unusual amongst the clostridia as it is non-motile.

It appears to naturally inhabit the human gut but because it possesses a number of necrotising and lethal enzymes and toxins, it has considerable pathogenic potential in both animals and man.

Toxin production

C. perfringens strains are subdivided into five types, A, B, C, D and E, dependant upon the presence or absence of four major exotoxins (soluble antigens): alpha, beta, epsilon and iota.

Types A, C and D are pathogenic to humans, whereas all types have been recognised as affecting animals. The enterotoxin responsible for food poisoning in humans is produced by type A and C strains.

Both types cause the acute diarrhoea predominantly associated with C. perfringens however less common disease can occur in the form of gas gangrene, necrotising colitis, peripheral pyrexia and septicaemia from type A and enteritis necroticans jejunitis ('pig-bel') from type C.

Illness can occur after the ingestion of large numbers of enterotoxin producing vegetative cells. Some of these cells will survive through the low pH of the stomach and can subsequently lodge in the large intestine, sporulating in the intestinal lumen.

During lysis and sporulation the enterotoxin is released and converted to a more active toxin by the action of trypsin and chymotripsin. This more active toxin will bind to receptors in the brush border membrane of the intestinal epithelial cells and cause pores to form.

This, in turn, causes the cells to become permeable to ions and small molecules and thus reverses the transportation of water across the intestinal wall.

Food poisoning

Food poisoning arises as a result of ingesting large numbers of viable organisms (around 10^8) which, typically, have multiplied in meat dishes prepared in large quantities and which have received insufficient heating. Their bulk means that cooling would have been slow thus allowing maximum opportunity for rapid multiplication.

C. perfringens is one of the top three organisms to cause food poisoning, sharing the podium with salmonella and campylobacter. Reported outbreaks in the UK show a downward trend in line with the Food Standard Agency's target to reduce cases of food poisoning by 20% between 1991 and 2006 (Fig. 1).

Having said this, it is thought that only one in 343 cases of C. perfringens infection is ever reported to the Health Protection Agency (HPA) due to the short lived nature of the infection and the difficulty to detect the organism in laboratory samples.

C. perfringens enteritis is not a classical intoxication because toxin is not preformed in the food although this has been observed on rare occasions. Symptoms of intoxication are abdominal pain and diarrhoea 8-24 hours after eating the contaminated food.

Vomiting and fever does not occur. Duration of the illness is short, usually lasting 12-24 hours. On rare occasions fatalities may occur, particularly amongst the elderly, as a result of dehydration.

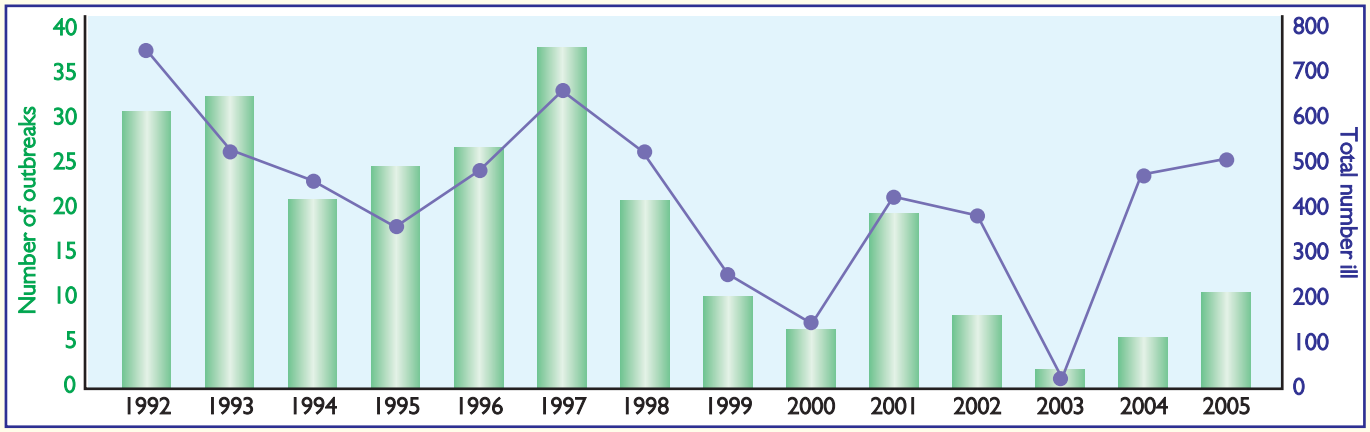
C. perfringens has the ability to multiply over a temperature range of 15-50°C and at the optimum temperature of approximately 45°C, cell numbers will double in about 12 minutes. Many organisms that accompany C. perfringens in foods are inhibited at temperatures above 40°C.

This means that the competition for nutrients is much reduced and C. perfringens is able to multiply freely.

Prevention of food poisoning is achieved by the proper use of refrigeration during storage and rapid cooling of cooked foods containing meat, fish or poultry.

C. perfringens is generally present in faeces in much lower numbers than E. coli and

Fig. 1. General outbreaks of Clostridium perfringens gastroenteritis reported to the Health Protection Agency Centre for Infections England and Wales, 1992-2006..



enterococci, and so is a less sensitive indicator of faecal contamination.

Despite this C. perfringens is often used in addition to other indicators as it produces resistant spores that survive in water and the environment for much longer periods than the vegetative cells of E. coli and coliforms. If C. perfringens is detected in a sample when other faecal indicator organisms are no longer detectable, it is an indication of remote or intermittent pollution.

Detection of C. perfringens

Culture media to detect C. perfringens in food almost all use the ability of the organism to reduce sulphite and produce lecithinase (phospholipase c).

These diagnostic features, in conjunction with a selective agent, mean that a presumptive identification can be made on organisms growing as black colonies with a zone of opacity around the colony.

BS ISO EN 7937:2004 Microbiology of food and animal feeding stuffs and Government of Canada MFHPB-23, November 2001 both recommend the use of Tryptose sulphite cycloserine (TSC) agar.

D-cycloserine is used as the selective agent and Sodium metabisulphite and ferric ammonium citrate are used as an indicator of sulphite reduction by C. perfringens which produce black colonies.

FDA/USDA methods recommend the same medium with a 50% egg yolk emulsion while NMKL recommend the use of a second plate in conjunction with TSC agar above.

The second plate recommended by NMKL is Shahidi-Ferguson-Perfringens (SFP) agar which uses the same base as TSC agar but with a different selective agent. Kanamycin sulphate and polymixin B sulphate used in SFP agar offer a greater recovery of both vegetative cells and spores of C. perfringens.

Identification

Morphological and cultural characteristics of C. perfringens greatly assist in the initial recognition of the organism on selective differential media. Production of black colonies surrounded by haloes of lecithinase activity in a medium containing egg yolk indicates organisms that should be examined further.

Microscopically C. perfringens appears as Gram positive, straight, square or blunt-ended rods that occur singly or in pairs. Generally spores are absent.

Initial confirmation that the organism is an anaerobe can be shown by demonstrating susceptibility to metranidazole in an anaerobic environment.

Alternatively, growth of a suspected anaerobe can be sub-cultured on two plates of medium, incubating one anaerobically and the other in air; anaerobic organisms will not grow in aerobic culture.

A number of sulphite reducing Clostridium species share with C. perfringens the ability to produce lecithinase and show a positive egg yolk reaction. The Nagler test, which uses C. perfringens type A antitoxin to neutralise lecithinase activity is useful, but positive results are not confined entirely to C. perfringens.

The possibility that a strain of C. perfringens does not produce lecithinase must always be considered.

The reversed CAMP test is an alternative to the Nagler test and is generally more satisfactory because it is specific for C. perfringens. Although this test is very reliable it is not applicable to non-haemolytic strains.

In a comparative study of standard methods the reversed CAMP test correctly identified 94.2% of all investigated colonies of C. perfringens. The combination of tests for motility, lactose fermentation, gelatine liquefaction and nitrate reduction correctly identified 89.2% of colonies and LS medium only 43.1%.

C. perfringens may also be identified immunologically using immunofluorescence microscopy or biochemically using for example RapID (Oxoid) or API 20A (bioMerieux).

Regulations

C. perfringens is present to some level in almost all foods however the presence of the organism does not necessarily indicate a threat to public health.

Commission Regulation (EC) No. 2073/2005 does not mandate testing for this organism however routine testing can be used to verify that a HACCP based system is in place and functioning effectively. n

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