FOODBORNE PATHOGENS AND DISEASE
Volume 0, Number 0, 2015

DOI: 10.1089/fpd.2014.1842

Review

A Possible Route for Foodborne Transmission of *Clostridium difficile?*

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Abstract

Spores of toxigenic *Clostridium difficile* and spores of food-poisoning strains of *Clostridium perfringens* show a similar prevalence in meats. Spores of both species are heat resistant and can survive cooking of foods. *C. perfringens* is a major cause of foodborne illness; studies are needed to determine whether *C. difficile* transmission by a similar route is a cause of infection.

Introduction

Clostridium difficile is a major cause of illness in hospitals and other healthcare settings and its incidence is recognized increasingly in the community (Hensgens *et al*., 2012). In a study in the United States, 32% of cases were classified as community associated (Lessa, 2013). Asymptomatic colonization of healthy individuals by *C. difficile* has been reported by several groups of workers. Colonization by toxigenic strains was found in 4.0% of 478 healthy individuals. Colonization by nontoxigenic strains was found in a further 3.1% of individuals (Ozaki *et al*., 2004). In many cases, colonization by a particular strain was transient, but in some cases it was persistent. Studies in healthcare settings and in the community have shown that a high proportion of cases of *C. difficile* infection (CDI) cannot be matched to previous CDI cases (Eyre *et al*., 2013; Wilcox, 2013). Thus, sources of *C. difficile* other than symptomatic patients may play an important role in transmission.

C. difficile and *C. perfringens* type A occur in foods, particularly meats, but also in vegetables and shellfish, and produce heat-resistant spores. The potential for *C. difficile* to be transmitted in food has been discussed by several workers including Weese (2009), Rupnik and Songer (2010), Hoover and Rodriguez-Palacios (2013), and Rodriguez-Palacios *et al*. (2013). *C. perfringens* is ranked as an important cause of foodborne illness in the United Kingdom (Tam *et al*., 2012, 2014) and in several countries (Grass *et al*., 2013) and was estimated as responsible for 10% of cases in the United States (Scallan *et al*., 2011). Cooked meat or poultry dishes are the predominant foods associated with *C. perfringens* food poisoning (Grass *et al*., 2013). Spores of *C. perfringens* survive cooking; if cooked food is allowed to remain at temperatures between ~12°C and 50°C, surviving spores can germinate and the vegetative bacteria can multiply. On consumption of the food containing high numbers of *C. perfringens*, some of the vegetative cells survive passage through the stomach and reach the intestine, where they can multiply and form toxin, causing gastroenteritis. The purpose of this article is to consider the common features of *C. difficile* and *C. perfringens* that may be relevant to possible foodborne transmission of *C. difficile*, and to identify further information that is needed.

Optimum Conditions for Detection and Growth of *C. difficile*

A major consideration in studying the transmission of *C. difficile,* and environmental sources, is the efficiency of methods for optimum germination of spores and growth of vegetative bacteria. Various culture media have been used to investigate the prevalence of the organism. Unheated spores of *C. difficile* or spores heated at 80°C for 10 min showed very low germination on a medium without lysozyme, but this was increased by a thousandfold in the presence of lysozyme, 5 μg/mL, and by a further 100-fold (giving 100% germination) following treatment with sodium thioglycolate and inoculation onto a medium containing lysozyme (Ionesco, 1978). In a similar manner, full recovery of spores of the majority of 108 strains of *C. difficile* (69 toxigenic, 39 nontoxigenic) heated at 80°C for 10 min required treatment with sodium thioglycolate and inoculation into a medium containing lysozyme (Nakamura *et al*., 1985). The addition of the bile salt sodium taurocholate to a selective medium increased recovery of spores of *C. difficile* isolated from clinical samples and heat shocked at 56°C for 10 min, to inactivate vegetative bacteria (Wilson *et al*., 1982). Taurocholate (and other cholates) and glycine were reported to act as co-germinants for *C. difficile* spores (Sorg and Sonensheim, 2008), but spores of different clinical isolates differ in response to bile salts (Heeg *et al*., 2012). In work by Paredes-Sabja *et al*.

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(2008), germination occurred in brain–heart infusion medium and was not increased by the inclusion of taurocholate.

Inclusion of lysozyme, 5 μg/mL, in a selective medium containing sodium cholate (a less expensive alternative to taurocholate), increased recovery of *C. difficile* from hospital ward environments, whereas pre-exposure of swabs to alkaline thioglycolate did not further increase recovery (Wilcox *et al*., 2000). Pre-enrichment in cooked meat broth before plating on a selective medium improved recovery; however, some samples were positive on direct culture but not after enrichment.

According to Limbago *et al*. (2012), the best recovery of a strain of *C. difficile* from spiked meat samples was obtained by enrichment in brain–heart infusion broth followed by plating on blood agar or on selective agar media with taurocholate. In an evaluation of culture methods for recovery of the toxigenic *C. difficile* type strain ATCC 9689 from stool and swab samples, the most sensitive method was heat shock (80°C for 10 min) followed by enrichment in a medium containing cycloserine–cefoxitin mannitol broth with taurocholate, lysozyme (5 μg/mL), and cysteine, followed by plating on a nutrient medium with 5% sheep blood (Hink *et al*., 2013). Tyrrell *et al*. (2013) reported that alcohol treatment followed by plating on a selective medium containing horse blood and taurocholate gave the highest recovery from toxin-positive fecal samples; a selective enrichment medium containing lysozyme and taurocholate, without alcohol pretreatment, followed by plating on selective medium containing horse blood and taurocholate enabled recovery of *C. difficile* from 9% of toxin-positive samples that were negative on direct plating.

A requirement for lysozyme for recovery of spores may result from damage to the spore coat and associated enzymes (Permpoonpattana *et al*., 2011, 2013). Removal of the exosporium from spores of *C. difficile* resulted in an increase of colony formation on medium containing taurocholate (Escobar-Cortés *et al*., 2013). The use of both lysozyme and taurocholate in a culture medium may give maximum recovery of *C. difficile* spores. The addition of blood to culture media may be a source of lysozyme (Flanagan and Lionetti, 1955).

Consideration is needed of the anaerobic conditions used for culture of *C. difficile*. Spores of many *Clostridium* spp. can survive and germinate in aerobic conditions, but the vegetative bacteria will be inactivated in such conditions. The initiation of growth of clostridia can be prevented by the presence of low levels of oxygen. *C. perfringens* is one of the most easily grown anaerobes, and is less sensitive to oxygen than some other species of anaerobic bacteria (Fredette *et al*., 1967). *C. difficile* is reported to require strictly anaerobic conditions (Songer, 2010). The effect of oxygen on initiation of growth of some clostridia is illustrated by its effect on nonproteolytic *C. botulinum* type E. In an atmosphere of N_2:H_2:CO_2, 84:10:5 by volume, the presence of 0.79% oxygen and a redox potential of +250 mV at pH 7, an inoculum of $>10^4$ spores was required to give growth in 5 days at 37°C; in the absence of detectable oxygen (<0.21%), at a redox potential of −400 mV, growth occurred from a single spore in 2 days (Lund *et al*., 1984). Thus, the most sensitive detection of low numbers of viable spores of some *Clostridium* spp. by culture media requires strictly anaerobic conditions. A recent article reported successful isolation of *C. difficile* in culture medium without incubation in an anaerobic chamber (Cadnum *et al*., 2014). The medium contained cysteine as a reducing agent and was boiled to eliminate oxygen before use; there was no demonstration that very low numbers of *C. difficile* could be isolated, and the sensitivity of the medium was not determined.

De-aerated liquid media containing reducing agents are used commonly for culture of *Clostridium* spp. using large inocula, but for epidemiological purposes there is a need to ensure that anaerobic conditions used are sufficient to ensure the most sensitive recovery of *C. difficile* from samples.

Reported Prevalence of *C. difficile* in Food Animals and in Food

C. difficile has been found in the intestinal tract of many types of food animals, including cattle, pigs, sheep, and poultry, as well as dogs and cats (Hensgens *et al*., 2012; Koene *et al*., 2012). Ribotypes in cattle, pigs, and poultry included those causing disease in humans. Reports of the prevalence of toxigenic *C. difficile* in retail samples of meat and, less frequently, in other foods have been summarized by Hensgens *et al*. (2012). In North America relatively high prevalence rates have been reported in uncooked meat products, in up to 42% of beef, 41% of pork and 44% of turkey samples, whereas lower prevalence rates, of up to 4.3% and 2.7% in ground beef/pork and chicken meat, respectively, have been reported in Europe. In contrast to many North American reports, a survey of 1755 retail meat samples, including ground beef, ground poultry, chicken breast, and pork chops, tested by U.S. state public health laboratories and 60 samples tested by Centers for Disease Control and Prevention, failed to detect *C. difficile* (Limbago *et al*., 2012). Differences in reported prevalence may be due in part to the use of different methodology. Toxigenic *C. difficile* was found in 23–50% of uncooked meats in the United States, in 14% of ready-to-eat summer sausage, and in 63% of ready-to-eat braunschweiger (Songer *et al*., 2009). Ribotypes 078 and 027, which are found commonly in human infection, were detected. Most strains isolated from foods have a genotype identical to those of human and animal isolates from the same geographic area or other parts of the world (Rupnik and Songer, 2010).

In a Canadian study, toxigenic *C. difficile* was isolated from 28 of 230 (12%) of samples of retail ground beef and ground pork (Weese *et al*., 2009). Of 28 positive samples, 20 were positive by enrichment but not by direct plating. The detection threshold of the enrichment method was stated as ≤10 spores/g. In four ground beef samples that were positive by direct culture, 20–240 spores/g were found and in four ground pork samples that were positive by direct culture 20–60 spores/g were detected. Toxigenic *C. difficile* was detected in 12.8% of chicken meat samples but could only be detected by enrichment, indicating that the numbers present were ≤10 spores/g (Weese *et al*., 2010).

These reports indicate that toxigenic *C. difficile* may be present in meat products, usually at a low concentration.

Reported Prevalence of Food-Poisoning Strains of *C. perfringens* Type A in Food

C. perfringens is often found in retail samples of raw meat and poultry. In order to determine the incidence of type A

strains carrying the *cpe* gene, which is essential for causing food poisoning, Wen and McClane (2004) surveyed 887 non-outbreak, retail samples of meats, poultry, and seafood. Of these samples, 31% were contaminated with *C. perfringens* with a most probable number (MPN) up to 32/g, 24% were contaminated with *C. perfringens* type A, and only 13 samples (~1.4%) were contaminated with *cpe*-positive strains of type A. In the majority of samples, *C. perfringens* was present as vegetative cells rather than as spores. In representative *cpe*-positive strains, the *cpe* gene was located on the chromosome, rather than on a plasmid; strains with a chromosomal *cpe* gene are reported to have a much higher heat resistance than strains with a plasmid *cpe* gene (Sarker *et al*., 2000). In 395 samples of cooked beef (kidney and flesh) sold in the streets in the Ivory Coast, the prevalence of *C. difficile* spores (12.4%) was reported greater than that of *C. perfringens* type A spores (5.1%); the numbers present were not determined (Kouassi *et al*., 2014).

Heat Resistance of Spores of *C. difficile* and *C. perfringens*

Studies in which heated spores of *C. difficile* were treated with alkaline thioglycolate and recovered on a medium with added lysozyme showed the highest heat resistance (Table 1). Kamiya *et al*. (1989) showed that when spores of four clinical strains were heated at 70°C for 10 min and plated on brain–heart infusion medium supplemented with glucose, soluble starch, and cysteine-HCl (GS-BHI), recovery rates were >10% for 2 strains and <0.01% for 2 strains. With all these strains, after heating at 80°C for 10 min, there was a marked decline in relative recovery rates of spores. After heating at 60°–75°C for 10 min, recovery of spores was increased to ~100% by inoculation onto GS-BSI plus taurocholate, but after heating at 80°C for 10 min the relative recovery rate was reduced to 2.9–0.05% of spores. When

Table 1. Heat Resistance of Spores of *Clostridium difficile* and *C. perfringens*

Heating medium	*Recovery of spores*	*Strains tested*	*D value at specified temperature*	*Reference*
C. difficile				
Phosphate buffer	Alkaline thioglycolate treatment. Medium plus lysozyme (10 μg/mL)	108 strains	$D_{100°C}$ = 2.5–33 min	Nakamura *et al*. (1985)
Distilled water	Alkaline thioglycolate treatment. Medium plus lysozyme (10 μg/mL)	4 strains	$D_{100°C}$ = ~4–6 min	Kamiya *et al*. (1989)
Phosphate-buffered saline	Medium, 5% sheep blood agar	20 strains	$D_{71°C}$ = ~30 min	Rodriguez-Palacios *et al*. (2010)
Phosphate –buffered saline	Blood agar	22 strains	$D_{85°C}$ = 6.0–8.5 min	Rodriguez-Palacios and Lejeune (2011)
Gravy, 0% fat; lean ground beef, 3% fat; ground beef 30% fat.	Blood agar	4 strains	$D_{96°C}$ = 0.59–1.19 min	Rodriguez-Palacios and Lejeune (2011)
Gravy, 0% fat; lean ground beef, 3% fat; ground beef 30% fat.	Blood agar	4 strains	$D_{85°C}$ = 2.5–3.3 min	Rodriguez-Palacios and Lejeune (2011)
Gravy, 0% fat; lean ground beef, 3% fat; ground beef 30% fat.	Blood agar	4 strains	$D_{71°C}$ = 47–71 min	Rodriguez-Palacios and Lejeune (2011)
C. perfringens				
Culture medium	Brain–heart infusion agar	5 strains; chromosomal *cpe* gene	$D_{100°C}$ = 30–124 min	Sarker *et al*. (2000)
Culture medium	Brain–heart infusion agar	7 strains; plasmid *cpe* gene	$D_{100°C}$ = 0.5–1.9 min	Sarker *et al*. (2000)
Culture medium	Brain–heart infusion agar	14 strains; chromosomal *cpe* gene	$D_{100°C}$ = 30–170 min	Wen and McClane (2004)
Phosphate buffer	Brain–heart infusion agar	10 strains; chromosomal (plus one plasmid) *cpe* gene	$D_{95°C}$ = >7.5 min	Grant *et al*. (2008)
Phosphate buffer	Brain–heart infusion agar	5 strains; plasmid *cpe* gene	$D_{95°C}$ = <7.5 min	Grant *et al*. (2008)

D value, time at specified temperature for 10-fold reduction in viable numbers.

spores heated at 85°C for 10 min were recovered by treatment with thioglycolate and inoculated onto GS-BHI plus lysozyme, recovery rates approaching 100% were obtained; after heating at 90°C for 10 min, relative recovery rates were 47.2–10.0% and heating at 100°C for 10 min reduced the relative recovery rates to 2.1–-0.20%. Studies by some workers involved recovery of survivors on media containing blood, which may be a source of lysozyme. Studies shown in Table 1, using multiple strains from animal and food sources and including genotypes of public health relevance, showed that spores were liable to survive heating at 71°C and higher temperatures.

Treatment of heated spores with alkaline thioglycolate probably ruptures disulphide bonds in the outer layers of the spore, increasing the spore permeability (Gould and Hitchins, 1963).

Enzymes with lysozyme activity have been reported in many types of raw food and in animal tissue (Lund and Peck, 1994). Hen egg-white lysozyme is stable to heat treatment, and some activity remained after heating 5–50 μg/mL at 90°C for 20 min in meat slurry pH 6.5–6.6 (Peck and Fernandez, 1995). The heat stability may be reduced by the presence of other proteins or increased in the presence of components such as some sugars and polysaccharides. The measured heat-resistance of spores of *C. difficile* was higher when heated spores were treated with alkaline thioglycolate and recovered on medium containing lysozyme (Table 1). This has also been reported for spores of nonproteolytic *C. botulinum* and *C. perfringens* (Peck *et al*., 1993; Barach *et al*., 1974).

Spores of *C. perfringens* can be sublethally injured by heat treatment, and spores heated at 105°C for up to 20 min or at 120°C for up to 25 s showed increased recovery on a medium containing lysozyme (Barach *et al*., 1974). Removal of exchangeable cations Ca^{++} and Na^{+} from *C. perfringens* spores by treatment with acid or alkali decreased survival of spores after heating at 95°C and plating on a medium without lysozyme; addition of lysozyme to the culture medium increased the number of survivors, and this was increased more dramatically by treatment of the heated spores with alkali before plating on medium containing lysozyme (Ando and Tsuzuki, 1983). Recovery on a medium without added lysozyme showed that spores had a very high heat-resistance, and spores of strains with a chromosomal *cpe* gene showed much higher heat-resistance than that of strains with a plasmid-borne *cpe* gene (Table 1). The majority of strains of *C. perfringens* associated with food poisoning carry a chromosomal *cpe* gene (Wen and McClane, 2004).

Results in Table 1 indicate that spores of *C. difficile* may be less heat resistant than those of *C. perfringens*, although variations in methodology may account for some of the reported differences. Importantly, however, spores of *C. difficile* as well as those of *C. perfringens* are likely to survive many processes for cooking foods, which involve heating to an internal temperature of 70° for up to 2 min or up to 74°C (WHO, 2006; ACMSF, 2007; FSA, 2010, 2013; FDA, 2013; Lund, 2014).

Spores of *C. perfringens* that survive cooking of foods can germinate and multiply if cooked foods are allowed to remain at temperatures between 12°C and 50°C. Guidelines in the United States and in the United Kingdom aim to ensure that exposure of cooked foods to this range of temperature is minimized, so that no more than a 10-fold increase in numbers of *C. perfringens* occurs during cooling of cooked food (Le Marc *et al*., 2008; FDA, 2013). A computer program is available that enables prediction of growth from surviving *C. perfringens* spores during any specified cooling curve (Le Marc *et al*., 2008).

Discussion

It is clear that spores of *C. difficile,* like those of enterotoxigenic *C. perfringens*, are liable to survive cooking of meat and other foods to a core temperature of 74°C or to 70°C for up to 2 min and therefore, like those of *C. perfringens*, they may germinate and allow growth of vegetative bacteria if cooked food is maintained at permissive temperatures, or they may remain as resistant spores. Although there have been no reported cases of food-associated CDI in humans, consumption of beef has been reported as a risk factor for *C. difficile* infection in patients in the community (Søes *et al*., 2014). For *C. perfringens*, there is information on the temperatures allowing germination and growth and on rates of growth, enabling determination of the rate of growth during cooling of cooked foods; this allows specification of temperature controls and a cooling regimen to prevent growth of this bacterium.

The optimum temperature for growth of *C. difficile* is stated as 30°–37°C, and the organism grows at 25°C and 45°C (Rainey *et al*., 2009), but there appears to be a lack of information on the minimum and maximum temperatures allowing growth, and on rates of growth. Thus, conditions required to prevent growth of the bacterium during cooling of cooked foods are not known.

In the case of *C. perfringens*, growth of bacteria to give $> 10^5$/g in the food consumed is considered, in general, to result in food poisoning (Heredia and Labbé, 2013). In the case of *C. difficile*, the infectious dose of spores or of vegetative bacteria that results in colonization, and possibly disease in vulnerable people, is unknown. In order to assess the risk of transmission of *C. difficile* in cooked foods and, if necessary, to devise conditions to prevent this transmission, the following information is needed, bearing in mind the wide genetic diversity of strains (Knetsch *et al*., 2012):

- the anaerobic conditions required for the most sensitive detection and isolation of the bacterium
- the optimum culture medium for maximum recovery of heated and unheated spores and vegetative bacteria
- further data on spore heat-resistance in foods
- the range of temperatures that allow growth
- the effect of temperature on rate of growth
- the effect of combinations of factors (e.g., temperature, pH, and NaCl) on growth
- an estimate of the number of vegetative bacteria or spores needed to result in colonization of healthy adults and of vulnerable members of the population

Conclusions

Research is needed to establish whether infection with *C. difficile* can be caused by transmission on food. Spores of *C. difficile*, like those of *C. perfringens*, can occur in meat and survive temperatures and times recommended for cooking. Germination of spores of *C. perfringens* and vegetative growth can occur in cooked meats if they are maintained at

temperatures of 12°–52°C, and following consumption gastroenteritis can result. Information is needed on conditions in which surviving spores of *C. difficile* would germinate and vegetative bacteria would multiply in cooked meat dishes, or whether the spores would persist, and whether the spores or vegetative bacteria would result in asymptomatic or symptomatic infection after consumption of the meat.

Acknowledgments

This work was supported by the BBSRC Institute Strategic Programme on Gut Health and Food Safety (grant number BB/J004529/1).

Disclosure Statement

No competing financial interests exist.

References

[ACMSF] Advisory Committee on the Microbiological Safety of Food. Report on the safe cooking of burgers. 2007. Food Standards Agency. Available at: http://www.food.gov.uk/multimedia/pdfs/acmsfburgers0807.pdf, accessed November 11, 2014.

Ando Y, Tsuzuki T. Mechanism of chemical manipulation of the heat resistance of *Clostridium perfringens* spores. J Appl Microbiol 1983;54:197–202.

Barach JT, Adams DM, Speck ML. Recovery of heated *Clostridium perfringens* type A spores on selective media. Appl Microbiol 1974;28:793–797.

Cadnum JL, Hurless KN, Deshpande A, Nerandzic MM, Kundrapu S, Curtis J. Sensitive and selective medium for detection of environmental *Clostridium difficile* isolates without requirement for anaerobic culture conditions. J Clin Microbiol 2014;52:3259–3263.

Escobar-Cortés K, Barra-Carrasco J, Paredes-Sabja D. (2013) Proteases and sonication specifically remove the exosporium layer of spores of *Clostridium difficile* strain 630. J Microbiol Methods 2013;93:25–31.

Eyre DW, Cule ML, Wilson DJ, Griffiths D, Vaughan A, O'Connor L, Ip CLC, Golubchik T, Batty EM, Finney JM, Wyllie DH, Didelot X, Piazza P, Bowden R, Dingle KE, Harding RM, Crook DW, Wilcox MH. Diverse sources of *C. difficile* infection identified on whole-genome sequencing. N Engl J Med 2013;369:1195–1205.

[FDA] Food and Drug Administration. FDA Food Code 2013. Available at: http://www.fda.gov, accessed November 11, 2014.

Flanagan P, Lionetti F. Lysozyme distribution in blood. Blood 1955;10:497–501.

Fredette V, Planté C, Roy A. Numerical data concerning the sensitivity of anaerobic bacteria to oxygen. J Bact 1967;93:2012–2017.

[FSA] Food Standards Agency. Caterers warned on chicken livers. 2010. Available at: http://www.food.gov.uk/news/newsarchive/2010/jul/livers, accessed November 11, 2014.

FSA. Safer food better business for caterers. 2013. Available at: http://www.food.gov.uk, accessed November 11, 2014.

Gould GW, Hitchins AD. Sensitization of bacterial spores to lysozyme and to hydrogens peroxide with agents which rupture disulphide bonds. J Gen Microbiol 1963;33:413–423.

Grant KA, Kenyon S, Nwafor I, Plowman J, Ohia C, Halford-Maw R, Peck MW, McLauchlin J. The identification and characterisation of *Clostridium perfringens* by real-time PCR, location of enterotoxin gene and heat resistance. Foodborne Pathog Dis 2008;5:629–639.

Grass JE, Gould LH, Mahon BE. Epidemiology of foodborne disease outbreaks caused by *Clostridium perfringens*, United States, 1998–2010. Foodborne Pathog Dis 2013;10:131–136.

Heeg D, Burns DA, Cartman ST, Minton NP. Spores of *Clostridium difficile* clinical isolates display diverse germination response to bile salts. PLoS ONE 2012;7:e2381.

Hensgens MPM, Keesen EC, Squire MM, Riley TV, Koene MGJ, de Boer E, Lipman LJA, Kuijper EJ on behalf of European Society for Clinical Microbiology and Infectious diseases Study Group for *Clostridium difficile* (ESGCD). *Clostridium difficile* infection in the community: A zoonotic disease? Clin Microbiol Infect 2012;18:634–645.

Heredia NL, Labbé RG. *Clostridium perfringens*. In: *Guide to Foodborne Pathogens*. 2nd ed. Labbé RG, Garcia S (eds.). Chichester, UK: John Wiley and Sons Ltd., 2013, pp. 82–90.

Hink T, Burnham C-AD, Dubberke ER. A systematic evaluation of methods to optimize culture-based recovery of *Clostridium difficile* from stool samples. Anaerobe 2013;19:39–43.

Hoover DG, Rodriguez-Palacios A. Transmission of *Clostridium difficile* in foods. Infect Dis Clin North Am 2013;27:675–685.

Ionesco H. Initiation de la germination des spores de *Clostridium difficile* par le lysozyme. C R Acad Sci Paris 1978;287:659–661. (In French.)

Kamiya S, Yamakawa K, Ogura H, Nakamura S. Recovery of spores of *Clostridium difficile* altered by heat or alkali. J Med Microbiol 1989;28:217–221.

Knetsch CW, Terveer EM, Lauber C, Gorbalenya AE, Harmanus C, Kuijper EJ, Corver J, van Leeuwen HC. Comparative analysis of an expanded *Clostridium difficile* reference strain collection reveals genetic diversity and evolution through six lineages. Infect Genet Evol 2012;12:1577–1586.

Koene MGJ, Mevius D, Wagenaar JA, Harmanus C, Hensgens MPM, Meetsma AM, Putirulan FF, van Bergen MAP, Kuijper EJ. *Clostridium difficile* in Dutch animals: Their presence, characteristics and similarities with human isolates. Clin Microbiol Infect 2012;18:778–784.

Kouassi KA, Dadie AT, N'Guessan KF, Dje KM, Loukou YG. *Clostridium perfringens* and *Clostridium difficile* in cooked beef sold in Côte d'Ivoire and their antimicrobial sensitivity. Anaerobe 2014;28:90–94.

Le Marc Y, Plowman J, Aldus CF, Munoz-Cuevasa M, Baranyi J, Peck MW. Modelling the growth of *Clostridium perfringens* during the cooling of bulked meat. Int J Food Microbiol 2008;128:41–50.

Lessa FC. Community-associated *Clostridium difficile* infection: How real is it? Anaerobe 2013;24:121–123.

Limbago B, Thompson AD, Greene SA, MacCannell D, MacGowan CE, Jolbitado B, Hardin HD, Estes SR, Weese JS, Songer JG, Gould LH. Development of a consensus method for culture of *Clostridium difficile* from meat and its use in a survey of U.S. retail meats. Food Microbiol 2012;32:448–451.

Lund BM. Microbiological food safety and a low microbial diet to protect vulnerable people. Foodborne Pathog Dis 2014;11:413–424.

Lund BM, Knox MR, Sims AP. The effect of oxygen and redox potential on growth of *Clostridium botulinum* type E from a spore inoculum. Food Microbiol 1984;1:277–287.

Lund BM, Peck MW. Heat resistance and recovery of spores of non-proteolytic *Clostridium botulinum* in relation to refrigerated, processed foods with an extended shelf-life. J Appl Bacteriol 1994;76:115S–128S.

Nakamura S, Yamakawa K, Izumi J, Nakashio S, Nishida S. Germinability and heat resistance of spores of *Clostridium difficile* strains. Microbiol Immunol 1985;29:113–118.

Ozaki E, Kato H, Kita H, Karasawa T, Maegawa T, Koino Y, Matsumoto K, Takada T, Nomoto K, Tanaka R, Nakamura S. *Clostridium difficile* colonization in healthy adults: Transient colonization and correlation with enterococcal colonization. J Med Microbiol 2004;53:167–172.

Paredes-Sabja D, Bond C, Carman RJ, Setlow P, Sarker MR. Germination of spores of *Clostridium difficile* strains, including isolates from a hospital outbreak of *Clostridium difficile*-associated disease (CDAD). Microbiology 2008;154: 2241–2250.

Peck MW, Fairbairn DA, Lund BM. Heat-resistance of spores of non-proteolytic *Clostridium botulinum* estimated on medium containing lysozyme. Lett Appl Microbiol 1993;16: 126–131.

Peck MW, Fernandez PS. Effect of lysozyme concentration, heating at 90°C, and then incubation at chilled temperatures on growth from spores of non-proteolytic *Clostridium botulinum*. Lett Appl Microbiol 1995;21:50–54.

Permpoonpattana P, Tolls EH, Nadem R, Brisson A, Cutting SM. Surface layers of *Clostridium difficile* endospores. J Bacteriol 2011;193:6461–6470.

Permpoonpattana P, Phetcharaburanin J, Mikelson A, Dembek M, Tan S, Brisson M-C, Ragione RL, Brisson A, Fairweather N, Hong HA, Cutting SM. Functional characterization of *Clostridium difficile* spore coat proteins. J Bacteriol 2013; 195:1492–1503.

Rainey FA, Hollen BJ, Small A. *Clostridium difficile.* In: *The Firmicutes*. *Bergey's Manual of Systematic Bacteriology*, 2nd ed., vol. 3. De Vos P, Garrity GM, Jones D, Kreig NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB (eds.). Dordrecht: Springer, 2009, pp. 771–772.

Rodriguez-Palacios A, Reid-Smith RJ, Staempfli HR, Weese JS. *Clostridium difficile* survives minimal temperature recommended for cooking ground meats. Anaerobe 2010;10: 540–542.

Rodriguez-Palacios A, LeJeune JT. Moist-heat resistance, spore aging, and superdormancy in *Clostridium difficile*. Appl Env Microbiol 2011;77:3085–3091 and Supplementary material.

Rodriguez-Palacios A, Borgman S, Kline TR, LeJeune JT. *Clostridium difficile* in food and animals. Animal Health Res Revs 2013;14:11–29.

Rupnik M, Songer JG. *Clostridium difficile*: Its potential as a source of foodborne disease. Adv Food Nutr Res 2010;60: 53–66.

Sarker MR, Shivers RP, Sparks SG, Juneja VK, McClane BA. Comparative experiments to examine the effects of heating on vegetative cells and spores of *Clostridium perfringens* isolates carrying plasmid enterotoxin genes versus chromosomal enterotoxin genes. Appl Env Microbiol 2000;66:3234–3240.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, Jones JL, Griffin PM. Foodborne illness acquired in the United States—Major pathogens. Emerg Infect Dis 2011;17:7–15.

Søes LM, Holt HM, Böttiger B, Nielsen HV, Andreasen V, Kemp M, Olsen KEP, Ethelberg S, Mølbak K. Risk factors for *Clostridium difficile* infection in the community: A case-control study in patients in general practice, Denmark, 2009–2011. Epidemiol Infect 2014;142:1437–1448.

Songer JG. Clostridia as agents of zoonotic disease. Vet Microbiol 2010;140:399–404.

Songer JG, Trinh HT, Killgore GE, Thompson AD, McDonald LC, Limbago BM. *Clostridium difficile* in retail meat products: USA, 2007. Emerg Infect Dis 2009;15:819–821.

Sorg JA, Sonensheim AL. Bile salts and glycine as cogerminants for *Clostridium difficile* spores. J Bact 2008;190:2505–2512.

Tam CC, Rodrigues LC, Viviani L, Dodds JP, Evans MR, Hunter PR, Gray JJ, Letley LH, Rait G, Tompkins DS, O'Brien SJ, on behalf of the IID2 Study Executive Committee. Longitudinal study of infectious intestinal disease in the UK (IID2 study): Incidence in the community and presenting to general practice. Gut 2012;61:69–77.

Tam CC, Larose T, O'Brien SJ on behalf of the Study Group. Costed extension to the Second Study of Infectious Intestinal Disease in the Community: Identifying the proportion of foodborne disease in the UK and attributing foodborne disease by food commodity. Project B18021 (FS231043). 2014. Available at: http://www.foodbase.org.uk, accessed November 11, 2014.

Tyrrell KL, Citron DM, Leoncio ES, Merriam CV, Goldstein EJC. Evaluation of cycloserine-cefotoxin fructose agar (CCFA), CCFA with horse blood and taurocholate, and cycloserine-cefotoxin mannitol broth with taurocholate and lysozyme for recovery of *Clostridium difficile* isolates from faecal samples. J Clin Microbiol 2013;51:3094–3096.

Weese JS. *Clostridium difficile* in food—Innocent bystander or serious threat? Clin Microbiol Infect 2009;16:3–10.

Weese JS, Avery BP, Rousseau J, Reid-Smith RJ. Detection and enumeration of *C. difficile* spores in retail beef and pork. Appl Env Microbiol 2009;75:5009–5011.

Weese JS, Reid-Smith RJ, Avery BP, Rousseau J. Detection and characterization of *Clostridium difficile* in retail chicken. Lett Appl Microbiol 2010;50:362–365.

Wen Q, McClane BA. Detection of enterotoxigenic *Clostridium perfringens* type A isolates in American retail foods. Appl Env Microbiol 2004;70:2685–2691.

Wilcox MH, Fawley WN, Parnell P. Value of lysozyme agar incorporation and alkaline thioglycollate exposure for the environmental recovery of *Clostridium difficile*. J Hosp Infect 2000;44:65–69.

Wilcox MH. *C. difficile* transmission in hospitals—Assumptions, theories and proof. Clostpath. 2013. 8th International Conference on the Molecular Biology and Pathogenesis of the Clostridia (abst).

Wilson KH, Kennedy MJ, Fekety FR. Use of sodium taurocholate to enhance spore recovery on a medium selective for *Clostridium difficile*. J Clin Microbiol 1982;15:443–446.

[WHO] World Health Organization. *Five Keys to Safer Food Manual*. 2006. Available at: http://www.who.int, accessed November 11, 2014.

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