

Clostridium

Clostridium is a genus of Gram-positive bacteria. They are obligate anaerobes capable of producing endospores.^{[1][2]} Individual cells are rod-shaped, which gives them their name, from the Greek *kloster* or spindle. These characteristics traditionally defined the genus, however many species originally classified as *Clostridium* have been reclassified in other genera.

Overview

Clostridium consists of around 100 species^[3] that include common free-living bacteria as well as important pathogens.^[4] There are five main species responsible for disease in humans:

- *C. botulinum*, an organism that produces botulinum toxin in food/wound and can cause botulism.^[5] Honey sometimes contains spores of *Clostridium botulinum*, which may cause infant botulism in humans one year old and younger. The toxin eventually paralyzes the infant's breathing muscles.^[6] Adults and older children can eat honey safely, because *Clostridium* do not compete well with the other rapidly growing bacteria present in the gastrointestinal tract. This same toxin is known as "Botox" and is used cosmetically to paralyze facial muscles to reduce the signs of aging; it also has numerous therapeutic uses.
- *C. difficile*, which can flourish when other bacteria in the gut are killed during antibiotic therapy, leading to pseudomembranous colitis (a cause of antibiotic-associated diarrhea).^[7]
- *C. perfringens*, formerly called *C. welchii*, causes a wide range of symptoms, from food poisoning to gas gangrene. Also responsible for enterotoxemia in sheep and goats.^[8] *C. perfringens* also takes the place

of yeast in the making of salt rising bread. The name *perfringens* means 'breaking through' or 'breaking in pieces'.

- *C. tetani*, the causative organism of tetanus.^[9] The name derives from "of a tension", referring to the tension (caused by tetanus) in the muscles.
- *C. sordellii* has been linked to the deaths of more than a dozen women after childbirth. Fatal *Clostridium sordellii* infections after medical abortions.

Scientific classification

Domain: Bacteria

Phylum : Firmicutes

Class : Clostridia

Order : Clostridiales

Family : Clostridiaceae

Genus : ***Clostridium***

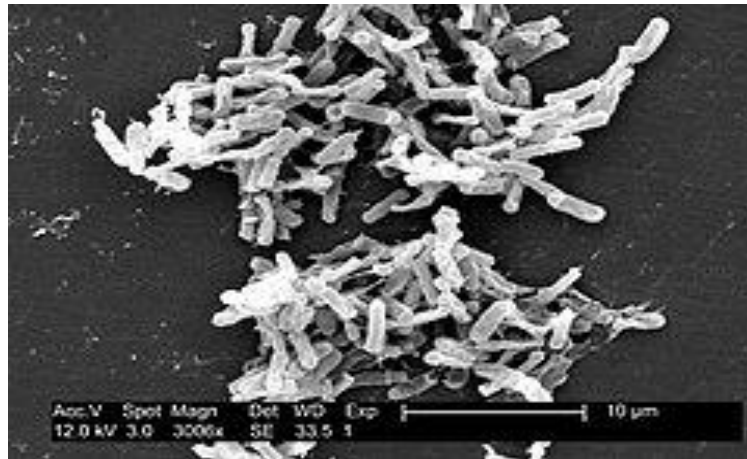
Selected species

C. difficile

C. perfringens

C. tetani

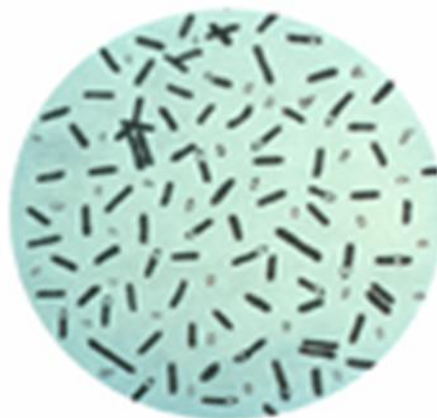
C. botulinum



SEM micrograph of *Clostridium difficile* colonies from a stool sample.

Clostridium botulinum

Clostridium botulinum is a Gram-positive, rod-shaped bacterium that produces several toxins. The best known are its neurotoxins, subdivided in types A-G, that cause the flaccid muscular paralysis. It is also the main paralytic agent in botox. *C. botulinum* is an anaerobic spore-former, which produces oval, subterminal endospores and is commonly found in soil. In addition it produces two ADP-ribosyltransferases: C2 and Clostridium botulinum C3 toxin. C2 acts on monomeric G-actin, while C3 causes the ADP- ribosylation of rho G-proteins with dramatic consequences for cellular integrity.



Clostridium botulinum stained with gentian violet.

Biology

Clostridium botulinum is a rod-shaped microorganism. It is an obligate anaerobe, meaning that oxygen is poisonous to the cells. However, *C. botulinum* tolerates traces of oxygen due to the enzyme superoxide dismutase (SOD) which is an important antioxidant defense in nearly all cells exposed to oxygen.^[10] *C. botulinum* is only able to produce the neurotoxin during sporulation, which can only happen in an anaerobic environment.

In the laboratory *Clostridium botulinum* is usually isolated in tryptose sulfite cycloserine (TSC) growth media in an anaerobic environment with less than 2% of oxygen. This can be achieved by several commercial kits that use a chemical reaction to replace O₂ with CO₂ (E.J. GasPak System). *C. botulinum* is a lipase negative microorganism that grows between pH of 4.8 and 7 and it can't use lactose as a primary carbon source, characteristics important during a biochemical identification.^[11]

Pathology

Botulism poisoning can occur due to improperly preserved or home-canned, low-acid food that was not processed using correct preservation times and/or pressure.

Neurotoxin types

Neurotoxin production is the unifying feature of the species *C. botulinum*. Seven types of toxins have been identified and allocated a letter (A-G). Most strains produce one type of neurotoxin but strains producing multiple toxins have been described. *Clostridium botulinum* producing B and F toxin types have been isolated from human botulism cases in New Mexico and California.^[12] The toxin type has been designated Bf as the

type B toxin was found in excess to the type F. Similarly, strains producing Ab and Af toxins have been reported.

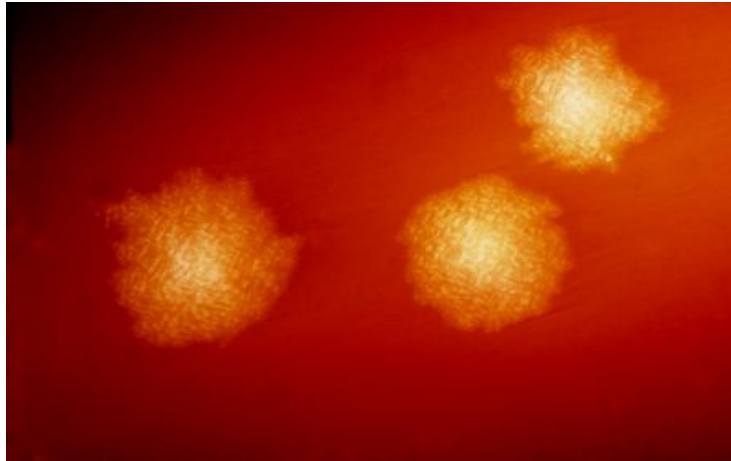
Only types A, B, E, and F cause disease in humans while types C and D cause disease in cows, birds, and other animals but not in humans. The "gold standard" for determining toxin type is a mouse bioassay, but the genes for types A, B, E, and F can now be readily differentiated using Real-time polymerase chain reaction (PCR).^[13]

A few strains from organisms genetically identified as other *Clostridium* species have caused human botulism: *Clostridium butyricum* has produced type E toxin^[14] and *Clostridium baratii* had produced type F toxin.^{[15][16]}

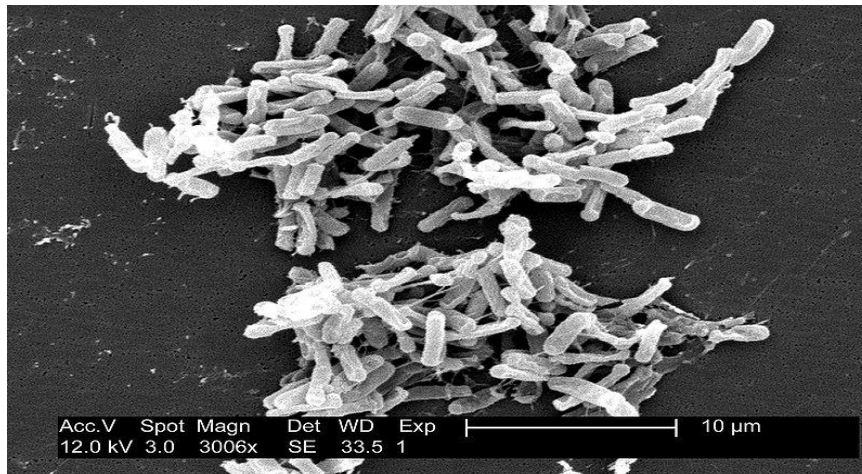
Clostridium difficile

Clostridium difficile (pronunciation below) (from the Greek *kloster* , spindle, and Latin *difficile*,^[17] difficult), is a species of Gram-positive bacteria of the genus *Clostridium* that causes severe diarrhea and other intestinal disease when competing bacteria in the gut flora have been wiped out by antibiotics.

Clostridia are anaerobic, spore-forming rods (bacilli).^[18] *C. difficile* is the most serious cause of antibiotic-associated diarrhea (AAD) and can lead to pseudomembranous colitis, a severe inflammation of the colon, often resulting from eradication of the normal gut flora by antibiotics.^[19]



C. difficile colonies on a blood agar plate



Micrograph of *Clostridium difficile*

Latent symptoms of *C. difficile* infection often mimic some flu-like symptoms and can mimic disease flare in patients with inflammatory bowel disease-associated colitis.^[20] Mild cases of *C. difficile* infection can often be cured by discontinuing the antibiotics responsible.^[18] In more serious cases, oral administration of, first, metronidazole and - if that fails - then, second, vancomycin are currently the treatments of choice. Relapses of *C. difficile* AAD have been reported in up to 20% of cases.^[18]

Bacteriology

Individual, drumstick-shaped *C. difficile* bacilli seen through scanning electron microscopy. Clostridia are motile bacteria that are ubiquitous in nature and are especially prevalent in soil. Under the microscope, clostridia appear as long, irregularly (often "drumstick" or "spindle") shaped cells with a bulge at their terminal ends. Under Gram staining, *Clostridium difficile* cells are Gram-positive and show optimum growth on blood agar at human body temperatures in the absence of oxygen. When stressed, the bacteria produce spores that can tolerate extreme conditions that the active bacteria cannot tolerate.^[18]

Virulence factor

Pathogenic *C. difficile* strains produce several known toxins. The most well-characterized are enterotoxin (Clostridium difficile toxin A) and cytotoxin (Clostridium difficile toxin B), both of which are responsible for the diarrhea and inflammation seen in infected patients, although their relative contributions have been debated.^[18] Toxins A and B are glucosyltransferases that target and inactivate the Rho family of GTPases. Clostridium difficile toxin B (cytotoxin) induces actin depolymerization by a mechanism correlated with a decrease in the ADP-ribosylation of the low molecular mass GTP-binding Rho proteins.^[21] Another toxin, binary toxin, has also been described, but its role in disease is not yet fully understood.^[22]

Prevention

The most effective method for preventing CDAD is proper antimicrobial prescribing. Infection control measures, such as wearing gloves when caring for patients with CDAD, have been proven to be effective at prevention. This works by limiting the spread of *C. difficile* in the

hospital setting. In addition, washing with soap and water will eliminate the spores from contaminated hands, but alcohol-based hand rubs are ineffective.^[23] sodium hypochlorite have been shown to kill the spores and prevent transmission between patients.^[24]

Treatment with various oral supplements containing live bacteria has been studied in efforts to prevent *Clostridium difficile*-associated infection/disease. A randomized controlled trial using a probiotic drink containing *Lactobacillus casei*, *L. bulgaricus*, and *Streptococcus salivarius subsp. thermophilus* was reported to have some efficacy. This study was sponsored by the company that produces the drink studied.^[25]

Hydrogen peroxide vapor (HPV) systems used to sterilize a patient room post discharge has been shown to reduce infection rates and to reduce risk of infection to subsequent patients.

In a limited clinical trial, a *C. difficile* anti-toxoid vaccine was reported to improve patient outcomes. Further testing will be required to validate this trial.^[26]

Clostridium perfringens

Clostridium perfringens (formerly known as *C. welchii*) is a Gram-positive, rod-shaped, anaerobic, spore-forming bacterium of the genus *Clostridium*.^[27] *C. perfringens* is ever present in nature and can be found as a normal component of decaying vegetation, marine sediment, the intestinal tract of humans and other vertebrates, insects, and soil.

C. perfringens is the third most common cause of food poisoning in the United Kingdom and the United States^[28] though it can sometimes be ingested and cause no harm.^[29]

Infections due to *C. perfringens* show evidence of tissue necrosis, bacteremia, emphysematous cholecystitis, and gas gangrene, which is also known as clostridial myonecrosis. The toxin involved in gas gangrene is known as α -toxin, which inserts into the plasma membrane of cells, producing gaps in the membrane that disrupt normal cellular function.^[28] *C. perfringens* can participate in polymicrobial anaerobic infections.^[30] *Clostridium perfringens* is commonly encountered in infections as a component of the normal flora.^[31] In this case, its role in disease is minor.

The action of *C. perfringens* on dead bodies is known to mortuary workers as tissue gas and can be halted only by embalming.



Photomicrograph of gram-positive *Clostridium perfringens* bacilli

Food poisoning

In the United Kingdom and United States, *C. perfringens* bacteria are the third-most-common cause of food-borne illness, with poorly prepared meat and poultry the main culprits in harboring the bacterium.^[28] The clostridium perfringens enterotoxin (CPE) mediating the disease is heat-labile (inactivated at 74 °C) and can be detected in contaminated food, if not heated properly, and feces.^[32] Incubation time is between six and 24 (commonly 10-12) hours after ingestion of contaminated food. Symptoms

typically include abdominal cramping and diarrhea; vomiting and fever are unusual. Very rare, fatal cases of clostridial necrotizing enteritis (also known as pigbel) have been known to involve "Type C" strains of the organism, which produce a potentially ulcerative β -toxin.

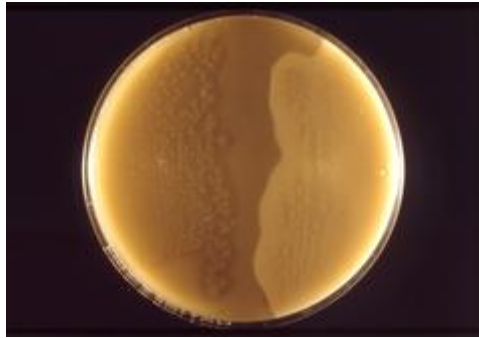
Despite its potential dangers, *C. perfringens* is used as the leavening agent in salt rising bread. The baking process is thought to reduce the bacterial contamination, precluding negative effects.^[29]

Infection

Clostridium perfringens is the most common bacterial agent for gas gangrene, which is necrosis, putrefaction of tissues, and gas production. It is caused primarily by *Clostridium perfringens* alpha toxin. The gases form bubbles in muscle (crepitus) and the characteristic smell in decomposing tissue. After rapid and destructive local spread (which can take hours), systemic spread of bacteria and bacterial toxins may result in death. This is a problem in major trauma and in military contexts. *C. perfringens* grows readily on blood agar plate in anaerobic conditions and often produces a zone of hemolysis.

Colony characteristics

On blood agar plates, *C. perfringens* grown anaerobically produces β -haemolytic, flat, spreading, rough, translucent colonies with irregular margins. A distinguishing characteristic of *C. perfringens* is a zone of double beta haemolysis. Nagler agar plates, containing 5-10% egg yolk, are used to identify strains that produce α -toxin, a diffusible lecithinase that interacts with the lipids in egg yolk to produce a characteristic precipitate around the colonies. One-half of the plate is inoculated with antitoxin to act as a control in the identification.



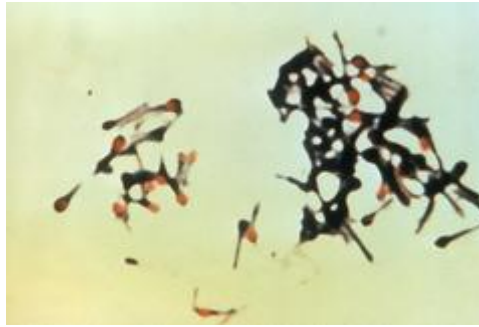
C. perfringens colonies on an egg yolk agar plate showing a white precipitate

Treatment

If detected on clinical grounds, treatment should begin without waiting for lab results. Traumatic wounds should be cleaned. Wounds that cannot be cleaned should not be stitched shut. Penicillin prophylaxis kills clostridia, and is thus useful for dirty wounds and lower leg amputations.

Clostridium tetani

Clostridium tetani is a rod-shaped, anaerobic bacterium of the genus *Clostridium*. Like other *Clostridium* species, it is Gram-positive, and its appearance on a gram stain resembles tennis rackets or drumsticks.^[33] *C. tetani* is found as spores in soil or in the gastrointestinal tract of animals. *C. tetani* produces a potent biological toxin, tetanospasmin, and is the causative agent of tetanus, a disease characterized by painful muscular spasms that can lead to respiratory failure and, in up to 40% of cases, death.



Clostridium tetani with characteristic 'tennis racket' appearance.

Characteristics

C. tetani is a rod-shaped, obligate anaerobe which stains Gram positive in fresh cultures; established cultures may stain Gram negative.^[33] During vegetative growth, the organism cannot survive in the presence of oxygen, is heat-sensitive and exhibits flagellarmotility. As the bacterium matures, it develops a terminal spore, which gives the organism its characteristic appearance. *C. tetani* spores are extremely hardy as they are resistant to heat and most antiseptics.^[35] The spores are distributed widely in manure-treated soils and can also be found on human skin and in contaminated heroin.^[34]

Toxicity

C. tetani usually enters a host through a wound to the skin, then it replicates. Once an infection is established, *C. tetani* produces two exotoxins, tetanolysin and tetanospasmin. Eleven strains of *C. tetani* have been identified, which differ primarily in flagellar antigens and in their ability to produce tetanospasmin. The genes for toxin production are encoded on a plasmid which is present in all toxigenic strains, and all strains that are capable of producing toxin produce identical toxins.^[36]

Tetanospasmin is a neurotoxin that causes the clinical manifestations of tetanus. Tetanus toxin is generated in living bacteria, and is released

when the bacteria lyse, such as during spore germination or vegetative growth. A minimal amount of spore germination and vegetative cell growth are required for toxin production.^[36]

Toxin action

Tetanospasmin released in the wound is absorbed into the circulation and reaches the ends of motor neurons all over the body. The toxin acts at several sites within the central nervous system, including nerve terminals, the spinal cord, and brain, and within the sympathetic nervous system. By binding to peripheral motor neuron terminals, the toxin enters the nerve axons, and is transported across synaptic junctions to the nerve-cell body in the brain stem and spinal cord by retrograde intraneuronal transport, until it reaches the central nervous system, where it rapidly binds to gangliosides at the presynaptic membrane of inhibitory motor nerve endings.^[34]

The clinical manifestations of tetanus are caused when tetanus toxin blocks inhibitory impulses, by interfering with the release of neurotransmitters, including glycine and gamma-aminobutyric acid. These inhibitory neurotransmitters inhibit the alpha motor neurons. With diminished inhibition, the resting firing rate of the alpha motor neuron increases, producing rigidity, unopposed muscle contraction and spasm.

Prevention

The most effective method for preventing CDAD is proper antimicrobial prescribing.

wearing gloves when caring for patients with CDAD

washing with soap and water will eliminate the spores from contaminated hands, but alcohol-based hand rubs are ineffective

sodium hypochlorite have been shown to kill the spores and prevent transmission between patients .

Vaccination

Tetanus can be prevented through the highly effective tetanus vaccine, which is a tetanus toxin inactivated with formaldehyde to be immunogenic but not pathogenic. The vaccine can be formulated as simple or adsorbed tetanus vaccine, combined tetanus and killed polio vaccine, or the older (diphtheria, tetanus, pertussis) (DPT) vaccine. Side effects are rare, but if they do occur, include fever, pain at the injection site, unexplained crying in infants, and irritability in older children or adults.

References

1. Ryan KJ, Ray CG (editors) (2004). *Sherrie Medical Microbiology* (4th ed.). McGraw Hill. ISBN 0-8385-8529-9.
2. Bruggemann H, Gottschalk G (editors). (2009). *Clostridia: Molecular Biology in the Post-genomic Era*. Caister Academic Press. ISBN 978-1-904455-38-7.
3. Evaluations and Standards Laboratory (July 14, 2008). "Identification of *Clostridium* Species". pp. 14. <http://www.hpa-standardmethods.org.uk/documents/bsopid/pdf/bsopid8.pdf>. Retrieved June 22, 2009.
4. Wells CL, Wilkins TD (1996). *Clostridia: Sporeforming Anaerobic Bacilli* in: *Baron's Medical Microbiology* (Baron S *et al.*, eds.) (4th ed.). Univ of Texas Medical Branch. ISBN 0-9631172-1-1. <http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mmed.section.1050>.

5. Wells CL, Wilkins TD (1996). *Botulism and Clostridium botulinum* in: *Baron's Medical Microbiology* (Baron S *et al.*, eds.) (4th ed.). Univ of Texas Medical Branch. ISBN 0-9631172-1-1. <http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mmed.section.1108>.
6. Tanzi MG, Gabay MP (2002). "Association between honey consumption and infant botulism". *Pharmacotherapy* **22** (11): 1479–83. doi:10.1592/phco.22.16.1479.33696. PMID 12432974.
7. Wells CL, Wilkins TD (1996). *Antibiotic-Associated Diarrhea, Pseudomembranous Colitis, and Clostridium difficile* in: *Baron's Medical Microbiology* (Baron S *et al.*, eds.) (4th ed.). Univ of Texas Medical Branch. ISBN 0-9631172-1-1. <http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mmed.section.1122>.
8. Wells CL, Wilkins TD (1996). *Other Pathogenic Clostridia Food Poisoning and Clostridium perfringens* in: *Baron's Medical Microbiology* (Baron S *et al.*, eds.) (4th ed.). Univ of Texas Medical Branch. ISBN 0963117211. <http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mmed.section.1131>.
9. Wells CL, Wilkins TD (1996). *Tetanus and Clostridium tetani* in: *Baron's Medical Microbiology* (Baron S *et al.*, eds.) (4th ed.). Univ of Texas Medical Branch. ISBN 0-9631172-1-1. <http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mmed.section.1099>.
10. Doyle, Michael P. (2007). *Food Microbiology: Fundamentals and Frontiers*. ASM Press. ISBN 1555812082.
11. (2005). *Brock Biology of Microorganisms* (11th ed.). Prentice Hall. ISBN 0131443291.

12. Hatheway C. L., McCroskey L. M. (1987). "Examination of faeces for diagnosis of infant botulism in 336 patients". *J. Clin. Microbiol* 25 (12): 2334–2338. PMC 269483. PMID 3323228. <http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pmcentrez&artid=269483>.
13. Satterfield, B. A.; Stewart, A. F.; Lew, C. S.; Pickett, D. O.; Cohen, M. N.; Moore, E. A.; Luedtke, P. F.; O'Neill, K. L. et al (2010). "A quadruplex real-time PCR assay for rapid detection and differentiation of the *Clostridium botulinum* toxin genes A, B, E and F". *J Med Microbiol* 59 (Pt 1): 55–64. doi:10.1099/jmm.0.012567-0. PMID 19779029.
14. Aureli, P.; Fenicia, L.; Pasolini, B.; Gianfrancesche, M.; McCroskey, J. M.; Hatheway, C. L. (1986). "Two cases of type E infant botulism caused by neurotoxigenic *Clostridium botulinum* in Italy". *J. Infect. Dis.* 154 (2): 207–211. PMID 3722863.
15. Hall, J. D.; McCroskey, L. M.; Pincomb, B. J.; Hatheway, C. L. (1985). "Isolation of an organism resembling *Clostridium baratii* which produces a type F botulinum toxin from an infant with botulism". *J. Clin. Microbiol* 21 (4): 654–655. PMC 271744. PMID 3988908. <http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pmcentrez&artid=271744>.
16. Notermans S., Havellar A. H. (1980). "Removal and inactivation of botulinum toxin during production of drinking water from surface water". *Antonie van Leeuwenhoek* 46: 511–514. doi:10.1007/BF00395840.

17. *American Heritage Dictionary* (4th ed.). 2000.
<http://www.bartleby.com/61/67/D0216700.html>. Retrieved 2009-02-15.
18. Ryan KJ, Ray CG (editors) (2004). *Sherris Medical Microbiology* (4th ed.). McGraw Hill. pp. 322–4. ISBN 0-8385-8529-9.
19. Curry J (2007-07-20). "Pseudomembranous Colitis". *eMedicine*. WebMD.
<http://www.emedicine.com/med/topic1942.htm#section~introduction>. Retrieved 2008-11-17.
20. Binion, David G (2010). "*Clostridium difficile* and IBD". *Inflammatory Bowel Disease Monitor* 11 (1): 7–14.
<http://www.remicaajournals.com/Inflammatory-Bowel-Disease-Monitor/BrowseIssues/Volume-11-Issue-1/Article-Clostridium-difficile-and-IBD>.
21. Just I, Selzer J, von Eichel-Streiber C, Aktories K (March 1995). "The low molecular mass GTP-binding protein Rho is affected by toxin A from *Clostridium difficile*". *J. Clin. Invest.* 95 (3): 1026–31. doi:10.1172/JCI117747. PMC 441436. PMID 7883950.
<http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pmcentrez&artid=441436>.
22. Barth H, Aktories K, Popoff M, Stiles B (2004). "Binary bacterial toxins: biochemistry, biology, and applications of common *Clostridium* and *Bacillus* proteins". *Microbiol Mol Biol Rev* 68 (3): 373–402, table of contents. doi:10.1128/MMBR.68.3.373-402.2004. PMC 515256. PMID 15353562.
<http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pmcentrez&artid=515256>.

23. <http://www.medscape.com/viewarticle/563232>
24. Savidge, Tor C; Urvil, Petri; Oezguen, Numan; Ali, Kausar; Choudhury, Aprateem; Acharya, Vinay; Pinchuk, Irina; Torres, Alfredo G et al (2011). "Host S-nitrosylation inhibits clostridial small molecule-activated glucosylating toxins". *Nature Medicine* 17 (9): 1136–41. doi:10.1038/nm.2405. PMID 21857653. Lay summary – *ScienceDaily* (August 21, 2011).
25. Hickson, M.; d'Souza, A. L; Muthu, N.; Rogers, T. R; Want, S.; Rajkumar, C.; Bulpitt, C. J (2007). "Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: Randomised double blind placebo controlled trial". *BMJ* 335 (7610): 80. doi:10.1136/bmj.39231.599815.55. PMC 1914504. PMID 17604300.
<http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pmcentrez&artid=1914504>.
26. Mattila, Eero; Anttila, Veli-Jukka; Broas, Markku; Marttila, Harri; Poukka, Paula; Kuusisto, Kaisa; Pusa, Liana; Sammalkorpi, Kari et al (2008). "A randomized, double-blind study comparing *Clostridium difficile* immune whey and metronidazole for recurrent *Clostridium difficile*-associated diarrhoea: Efficacy and safety data of a prematurely interrupted trial". *Scandinavian Journal of Infectious Diseases* 40 (9): 1–7. doi:10.1080/00365540801964960.
27. Ryan KJ; Ray CG (editors) (2004). *Sherris Medical Microbiology* (4th ed.). McGraw Hill. ISBN 0-8385-8529-9.
28. Warrell et al. (2003). *Oxford Textbook of Medicine* (4th ed.). Oxford University Press. ISBN 0-19-262922-0.

29. Juckett, G; Bardwell, G; McClane, B; Brown, S (2008). "Microbiology of salt rising bread". *The West Virginia medical journal* 104 (4): 26–7. PMID 18646681.
30. Brook, I (2007). "The role of anaerobic bacteria in cutaneous and soft tissue abscesses and infected cysts". *Anaerobe* 13 (5–6): 171–7. doi:10.1016/j.anaerobe.2007.08.004. PMID 17923425.
31. Wells CL, Wilkins TD (1996). *Clostridia: Sporeforming Anaerobic Bacilli*. In: *Barron's Medical Microbiology* (Barron S *et al.*, eds.) (4th ed.). Univ of Texas Medical Branch. ISBN 0-9631172-1-1. (via NCBI Bookshelf).
32. Murray *et al.* (2009). *Medical Microbiology* (6th ed.). Mosby Elsevier. ISBN 978-0-323-05470-6.
33. Ryan KJ; Ray CG (editors) (2004). *Sherris Medical Microbiology* (4th ed. ed.). McGraw Hill. ISBN 0838585299.
34. Atkinson W, Hamborsky J, McIntyre L, Wolfe S (eds). (2006). *Epidemiology and Prevention of Vaccine-Preventable Diseases (The Pink Book)* (10th ed. ed.). Public Health Foundation. <http://www.cdc.gov/vaccines/pubs/pinkbook/downloads/tetanus.pdf>.
35. Madigan M; Martinko J (editors). (2005). *Brock Biology of Microorganisms* (11th ed. ed.). Prentice Hall. ISBN 0131443291.
36. Todar, Ken (2005) Pathogenic Clostridia, Ken Todar's Microbial World, University of Wisconsin - Madison.