Review.

A Review on Clostridium Perfringens Food Poisoning

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Foodborne diseases can be defined as those conditions that are commonly transmitted through ingested food. The two most common types of food borne diseases are intoxication and infection. Intoxication occurs when toxin produced by the pathogens cause food poisoning, while infection is caused by the ingestion of food containing pathogens. Some microorganisms can use our food as a source of nutrients for their growth. By growing on the food, metabolizing them and producing by-products, they not only render the food inedible but also pose health problems upon consumption. Clostridia produce the highest number of toxins of any type of bacteria. Among *Clostridium* species, *clostridium perfringens* is the largest toxin producer and also the most widespread, being found as part of the microbiota of animals and humans and in the soil. In most cases, clostridium perfringens food poisoning results by eating improperly cooked and stored foods. *Clostridium perfringens* food poisoning is quite common and it is an important cause of outbreaks worldwide. The prevention of growth of this organism is best accomplished by following the standard food service practices of rapidly chilling prepared foods in shallow containers and keeping cold food cold and hot food hot.

Keywords: Clostridium perfringens, Foodborne diseases, Intoxication, Poisoning

INTRODUCTION

Increases in the incidence of foodborne diseases, often associated with outbreaks, threaten global public health security and raise international concern. Foodborne diseases can be defined as those conditions that are commonly transmitted through ingested food [12]. Food Borne disease (FBD) outbreaks are common and often cause considerable morbidity and mortality. The two most common types of food borne diseases are intoxication and infection. Intoxication occurs when toxin produced by the pathogens cause food poisoning, while infection is caused by the ingestion of food containing pathogens [8].

Food poisoning results from ingestion of water and wide variety of food contaminated with pathogenic organisms (bacteria, viruses, parasites, and fungi), their toxin and chemicals. Food poisoning must be suspected when an acute illness with gastrointestinal or neurological manifestation affect two or more persons or animals who have shared a meal during the previous 72 hours [2]. Some microorganisms can use our food as a source of nutrients for their growth. By growing on the food, metabolizing them and producing by-products, they not only render the food inedible but also pose health problems upon consumption [10]. Intoxication involves food poisoning in which the organism grows in food and releases a toxin from the cells. When the toxin is ingested along with the food, it gives rise to the Food Poisoning Syndrome [9].

Clostridia produce the highest number of toxins of any type of bacteria. Among Clostridium species, clostridium perfringens is the largest toxin producer and also the most widespread, being found as part of the microbiota of animals and humans and in the soil. This bacterium is a gram-positive anaerobic bacillus that is classified into five types according to the production of four major toxins: alpha (a), beta (b), epsilon (ε) and iota (i). In addition to the major toxins, it can produce more than 15 other known toxins. Some of these additional virulence factors, including enterotoxin, necrotic enteritis B-like toxin (NetB) and beta-2 toxin, have received more attention than others due to their role in the pathogenesis of C. perfringens-associated disease (CPAD) in humans and animals [17, 6, 15]. *C. perfringens* food poisoning is caused by infection with the *C. perfringens* bacterium. *C. perfringens* is found frequently in the intestines of humans and many animals and is present in soil and areas contaminated by human or animal feces. In most cases, *C. perfringens* food poisoning results by eating improperly cooked and stored foods. Normally, bacteria are found on food after cooking, and these bacteria can multiply and cause *C. perfringens* food poisoning if the foods sit out and cool before refrigerating. Commonly infected foods include meats, meat products, and gravy [9, 10].

Symptoms of *C. perfringens* food poisoning include intense abdominal cramps and watery diarrhea. Symptoms usually appear 6 to 24 hours after eating foods containing large numbers of *C. perfringens*. The disease usually is over within 24 hours. Less severe symptoms may last for 1 or 2 weeks. C [4].

The prevention of growth of this organism is best accomplished by following the standard food service practices of rapidly chilling prepared foods in shallow containers and keeping cold food cold and hot food hot. Remember, always reduce the level of contamination by keeping all work areas clean and sanitary [18].

Hence, the objective of this paper is: To review the Clostridium Perfringens Food Poisoning.

Literature Review

Organism

Classification of clostridium

The Clostridiaceae are a family of the Clostridia, and contains the *Clostridium* genus. The genus *Clostridium* currently comprises 152 validly described species in Nomenclature. However, on the basis of 16S rRNA gene sequence analyses, only 73 of these validly described species fall within the radiation of the type species of the genus *Clostridium*. Most species of the genus *Clostridium* are saprophytic organisms found in many places in the environment, most notably the soil [7, 18].

Characteristics of clostridium perfringens

Clostridium perfringens, of the *Clostridium* genus, is non-motile, anaerobic, (few strains are aerotolerant), and spore forming bacteria (sub terminal spores) that are encapsulated in tissue smears. Vegetative cells are rod shaped, pleomorphic, and occur in pairs or short chains. It is catalase and superoxide dismutase negative, and has 5 toxigenic types (A-E), of which A and C strains are pathogenic for humans. It produces many different toxins. Colonies with double zone of hemolysis are produced when cultured at 37°C on blood agar overnight [7, 9].

Other pathogenic clostridium

Clostridium contains important pathogens. The main species responsible for disease in humans are: *Clostridium botulinum* can produce botulinum toxin in food or wounds and can cause botulism. *Clostridium difficile* can flourish when other gut flora bacteria are killed during antibiotic therapy, leading to superinfection and potentially fatal pseudomembranous colitis (a severe necrotizing disease of the large intestine). *Clostridium tetani* causes tetanus. *Clostridium sordellii* can cause a fatal infection in exceptionally rare cases after medical abortions [12].

Hosts and reservoirs

The Clostridium Perfringens are ubiquitous in nature, with humans and animals as the primary reservoirs. Probably it is one of the most widely occurring bacterial pathogens in the environment and is commonly found in a variety of foods. Humans (mainly type A; type C rarely); animals such as dogs, pigs and goats also become infected with type A and type C [16].

Food products commonly implicated with *Clostridium Perfringens* food poisonings

In most instances, the actual cause of poisoning by this organism is temperature abuse of cooked foods. Small numbers of the organism often are present after the food is cooked, due to germination of its spores, which can survive high heat and can multiply rapidly as a result of a fast doubling time (<10 minutes for vegetative cells), depending on temperature and food matrix. Therefore, during cool-down (109-113°F) and storage of prepared foods, this organism can reach levels that cause food poisoning much more quickly than can other bacteria [6].

Meats (especially beef and poultry), meatcontaining products (e.g., gravies and stews), and Mexican foods are important vehicles for *C. perfringens* foodborne illness, although it is also found on vegetable products, including spices and herbs, and in raw and processed foods. Spores of some *C. perfringens* strains can survive boiling water for an hour or longer in a relatively protective medium (e.g., a cooked-meat medium) [6, 17].

Enterotoxins and its characteristics

C. perfringens produces four typing toxins (alpha,

beta, epsilon, and iota) and at least eleven other toxins. *C. perfringens* is classified into five types, A through E, on the basis of its production of the four typing toxins. Clostridium Perfringens Enterotoxin (CPE) is one of the toxins produced by *C. perfringens*, evoking diarrhea and the enterotoxin gene is cloned and sequenced. Most CPE-producing *C. perfringens* belong to type A [15].

Clostridium intoxication

Clostridial Food Poisoning: Food poisoning can be caused by *C. perfringens* enterotoxin (CPE) produced by *C. perfringens* spores in the small intestine, which can germinate in foods such as meat and poultry. In the United States consumption of large amounts of *C. perfringens* is considered an important cause of watery diarrhea. Main symptoms of the disease are nausea, abdominal pain, and diarrhea. The disease is usually mild and self-limiting in healthy individuals, with symptoms resolving within 24 hours [15, 16].

Clostridial Myonecrosis (Gas Gangrene): *C. perfringens* is the most common cause of clostridial myonecrosis. The disease involves breakdown of muscle tissue due to the action of potent exotoxins, alpha and beta, produced by the bacteria. It is manifested by severe pain, edema, tenderness and pallor, followed by discoloration and hemorrhagic bullae, and production of gas at the site of wound. Systemic manifestations of the disease include shock, renal failure, hypotension, and bacteremia with intravascular hemolysis leading to coma and death [11].

Clostridial Cellulitis: *C. perfringens* is the most common cause of clostridial cellulitis, which is often associated with local trauma or recent surgery. Infection is less systemic than in clostridial myonecrosis, with localized infection and associated skin and soft tissue necrosis, but sparing of the fascia and deep muscles [1, 11].

Enteritis Necrotican (pigbel): Enteritis necroticans is a life threatening infection involving ischemic necrosis of the jejunum. The often fatal disease is caused by *C. perfringens* type C and is marked by hemorrhagic, inflammatory, or ischemic necrosis of the jejunum. Most cases occur sporadically, during outbreaks, or in underdeveloped countries [1].

Central Nervous System (CNS) manifestations of C. perfringens infection: CNS diseases due to C. *perfringens* infection are rare. The main manifestations of C. perfringens infection in CNS are meningitis and encephalitis. Clinical symptoms of the two diseases are similar and include tiredness, fever, headache, vomiting, hypersensitivity to light or noise, neck stiffness, or impaired consciousness and coma [11].

Vehicles of Transmission

C. perfringens can grow over a wide range of temperatures, but grows very slowly at low temperatures. Unlike most other food poisoning bacteria, these bacterial spores will germinate and grow best at temperatures between 100 degrees F and 117 degrees F. Unfortunately, this is a temperature range found guite frequently in warm food holding areas in food service facilities, such as steam tables and oven type warmers. The major reasons C. perfringens is associated with food service or mass feedings are that it requires an environment free from air, storage time, high temperatures and a strict nutrient supply. These conditions are usually met when meat stews, sauces, gravies and soups are improperly stored. The food is usually cooked in a large, deep container. After cooking, one assumes (incorrectly) that no bacteria are present, and the large container is placed in the cooler. Because of the container's size and depth, rapid cooling is impossible. The spores germinate and grow to large numbers. When the product is ready to be used, it is heated in the same container. Because of the container's size, reheating is difficult and the bacteria are not killed. When the food is eaten, a large number of people are usually affected [12, 18].

Distribution, public health and economic importance

The global burden of FBD caused by the 31 hazards in 2010 was 33 million Disability Adjusted Life Years (DALYs); children under five years old bore 40% of this burden. The 14 sub regions, defined on the basis of child and adult mortality, had considerably different burdens of FBD, with the greatest falling on the sub regions in Africa, followed by the sub regions in South-East Asia and the Eastern Mediterranean sub region [8]. *C. Perfringens* food poisoning is quite common and it is an important cause of outbreaks worldwide [13].

C. perfringens is probably one of the most widely occurring bacterial pathogens in the environment and, as a consequence of its ubiquity, is commonly found in a variety of foods. The food poisoning strains of *C. perfringens* exist in soils, water, food, dust, spices and intestinal tract of humans and other animals. Various investigators have reported that the incidence of the heat-resistant, non-hemolytic stains to range from 2% to 6% in the general population. Between 20% and 30% of healthy hospital personnel and their families have been found to carry these organisms in their feces, and the carrier rate of victims after 2 weeks may be 50% or as high as 88% [9, 10].

C. Perfringens poisoning is one of the most commonly reported foodborne illnesses in the U.S. The CDC estimates that 965,958 domestically acquired cases occur annually in the U.S., second only to *Salmonella* when considering bacterial causes of foodborne illness. Thirty-four outbreaks in 2006 (i.e., not including isolated cases) included 1,880 cases. At least 51 outbreaks were reported annually in the U.S. from 2001 to 2005. Typically, 50 to 100 people are affected in one outbreak. It is probable that many outbreaks go unreported, because the implicated foods and patients' feces are not tested routinely for *C. perfringens* or its toxin [16, 19].

It is estimated that each year there are 1.6 million (1.2-2.0 million) and 2.4 million (1.8-3.0 million) episodes of domestically acquired foodborne illness related to 30 known pathogens and unspecified agents, respectively, for a total estimate of 4.0 million (3.1-5.0 million) episodes of domestically acquired foodborne illness in Canada. Norovirus, Clostridium perfringens, Campylobacter spp., and nontyphoidal Salmonella spp. the leading pathogens and account are for approximately 90% of the pathogen-specific total [20].

C. perfringen type A food poisoning remains one of the most prevalent food born disease in western countries. In Japan and Europe, food-borne GI disease outbreaks caused by plasmid CPE were reported recently, while several food-borne isolates carry chromosomal CPE. 313 outbreaks of gastroenteritis reported due to *C. perfringens* in England and Wales from 1992 to 2012 [15]. A suspected outbreak of probably *C. perfringens* food poisoning was reported in the Eastern region of Ghana. [3].

Institutional settings (such as school cafeterias, hospitals, nursing homes, prisons, etc.), where large quantities of food are prepared several hours before serving, are the most common circumstance in which *C. perfringens* poisoning occurs. The young and elderly are the most frequent victims of *C. perfringens* poisoning. As with other infections, immunocompromised people are at higher risk of severe illness than are others; e.g., those with HIV/AIDS or undergoing cancer chemotherapy or immunosuppressive drugs for rheumatoid arthritis or other inflammatory conditions [18, 19].

Identification and isolation of clostridium enterotoxin

Despite the medical importance of enterotoxigenic *C. perfringens*, the ecology of these bacteria remains poorly understood. In part this is because, while *C. perfringens* has widespread distribution in the environment, only a small fraction (~1 to 5%) of the global *C. perfringens* population carries the enterotoxin (CPE) gene. A number of criteria have been proposed for establishing an outbreak of *C. perfringens* type A

food poisoning. These includes; a) more than 106 spores/gram feces from ill individuals; b) more than 105 cells/gram incriminated food; c) the presence of the same serotypes of *C. perfringens* in both contaminated food and feces; d) the presence of the same serotype in all ill individuals in an outbreak or detection of enterotoxin in feces of individuals [14, 17].

Standard bacteriological culturing procedures are used to detect the organism in implicated foods and in feces of patients. Homogenized food is diluted and plated on selective medium as well as Robertson cooked meat medium and incubated anaerobically. The isolated bacteria must be shown to produce enterotoxin. Serological assays are used for detecting enterotoxin in the feces of patients and for testing the ability of strains to produce toxin. With the introduction of Polymerase Chain Reaction (PCR)-based methods, toxin typing using antiserum neutralization tests in mice is no longer practical [11, 15].

Prevention and control measures

C. Perfringens are ubiquitous and are impossible to eliminate from the environment. Many foods such as meat and poultry may carry the organism, but the mere presence of *C. perfringens* in food is not enough to cause illness. Millions of growing cells are needed. The total destruction or significant reduction in the bacterial load in foods during growth, harvesting, processing, packaging, and storage prior to consumption has always been a general goal. However, the wide array of parameters for proliferation of foodborne pathogens is staggering. Some of the same methods for the control of organisms in the food supply are used separately or in combination in the preservation of foods [5, 6, 10, 11].

The prevention of growth of this organism is best accomplished by following the standard food service practices of rapidly chilling prepared foods in shallow containers and keeping cold food cold and hot food hot. Remember, always reduce the level of contamination by keeping all work areas clean and sanitary [11, 15].

Prevention of *C. perfringens* infections/intoxication requires strategies to interrupt various modes of transmission. Essentially these control programs include improvements in personal hygiene practices among healthcare workers and food handlers, decontamination of equipment, surfaces, and clothing, proper cooking and storage of foods, and screening programs [16, 18].

CONCLUSION AND RECOMMENDATIONS

Among *Clostridium* species, *C. perfringens* is the largest toxin producer and also the most widespread, being found as part of the microbiota of animals and humans

and in the soil. *C. perfringens* is an important causative agent for food poisoning outbreaks and major concern in public health programs worldwide. *C. Perfringens* poisoning is one of the most commonly reported foodborne illnesses. Anyone who consumes *C. perfringens* toxin can become ill. However, severity of the illness will depend on the age, sex, health status, amount of contaminated food and toxin consumed. In most cases, *C. perfringens* food poisoning results by eating improperly cooked and stored foods. Normally, bacteria are found on food after cooking, and these bacteria can multiply and cause *C. perfringens* food poisoning if the foods sit out and cool before refrigerating.

Based on above conclusion, the following recommendations are forwarded:

 \checkmark Do not allow foods sit out and cool before refrigerating, as well as eating improperly cooked and stored foods

 \checkmark Proper sanitation by Food handlers and should be encouraged to join the education and training to ensure good hygiene practices.

✓ Special care should be given for Institutional settings (such as school cafeterias, hospitals, nursing homes, prisons, etc.), and risk groups such as young and elderly.

✓ Considerable studying effort is still required for better understanding of the interactions between *C. perfringens* and food products, and of the mechanisms of CPE production in foodstuffs.

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