ISOLATION AND ANTIBIOTICS SUSCEPTIBILITY OF *CLOSTRIDIUM PERFRINGENS*

BY

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18/4775

A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES AND BIOTECHNOLOGY, COLLEGE OF PURE AND APPLIED SCIENCES, CALEB UNIVERSITY, IMOTA, LAGOS STATE, NIGERIA.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.)

IN MICROBIOLOGY AND INDUSTRIAL BIOTECHNOLOGY FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES AND BIOTECHNOLOGY, CALEB UNIVERSITY, IMOTA, LAGOS STATE.

JUNE, 2022

DECLARATION

I, Durotimi Flourish Oluseyi hereby declare that this project "Isolation and Antibiotic Susceptibility of *Clostridium perfringens"* is based on my own work under the supervision of Dr. E.A. Ademola, A references made from other sources have been given due credit and acknowledgement

Durotimi Flourish Oluseyi

_____ _____

Signature Date

CERTIFICATION

This is to certify that this research work "Isolation and Antibiotic Susceptibility of *Clostridium perfringens"* was carried out by DUROTIMI FLOURISH OLUSEYI with Matric number 18/4775 in the Department of Biological Sciences and Biotechnology, College of Pure and Applied Sciences, Caleb University, Imota, Lagos, Nigeria.

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DEDICATION

This research work is dedicated to Lord Almighty for everything and to my irreplaceable parent Baba and Mama Famijade.

ACKNOWLEDGEMENTS

I thank God for his faithfulness over my life and to my supervisor, Dr. E. A. Ademola for his time, efforts, advice and also moral support for this research to be a reality. God will bless you sir. I am also grateful to all my lecturers and my able Head of Department (DR. C.C. EZEANYA-BAKPA) for their great impact in my life. God bless you all.

My acknowledgement also goes to my parent (Baba and Mama Famijade) for everything.

TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

Clostridium perfringens is a Gram-positive bacillus that forms spores. *Clostridium perfringens* is a common bacteria that cause food poisoning, it is an emerging health problem in health setting. The ability of the spores to persist in the environment is a key factor in rates of infection caused by *Clostridium perfringens*. *Clostridium perfringens* has also been described as one of the leading cause of food poisioning. It causes a serious toxic-mediated enteric diseases in humans. This study aimed to investigate the isolation and antibiotic susceptibility of *Clostridium perfringens* from different samples. A total of forty five (45) samples comprising of sea food (22), wastewater (7), dumpsite soil (16) samples were collected for this study. Isolation was carried out using Reinforced clostridial agar in an anaerobic condition, while identification of this bacteria was done using morphological and biochemical tests. Antibiotic susceptibility was tested in this study using eight (8) antimicrobials disk via disc diffusion method. The isolate gotten from all the samples were eight (8) and all the isolates have the characteristics morphological features of *Clostridium perfringens* on Reinforced clostridial agar. The highest antibiotic susceptibility test of the isolates was recorded with Chloramphenicol (87%), tetracycline (75%) , Ciprofloxacin (75%), Ampicillin (62%), Vancomycin (62%), Gentamicin (62%). Erythromycin (25%) and Metronidazole (0). In this study, *Clostridium perfringens* was isolated more in the food samples than in the environmental samples.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Clostridium perfringens, a bacterium, have been identified as one of the major causes of food poisoning. It was discovered in 1891 by William H. Welch, a medical doctor, at the Johns Hopkins Hospital in an autopsy he conducted on a 38 years old man (Lucey *et al*., 2004). *Clostridium perfringens* food poisoning has frequently been identified as a problem in the food industry since most occurrences have been associated with mass feeding operation (Archaya, 2022). Previously, researchers were uncertain whether absorption with Staphylococcus and Salmonellosis causes food poisoning but recent changes in bacterial studies affirmed and outlined how they caused food poisoning (Zottola, 1971). This organism can be found in soil, poultry litter, dust, feaces, healthy birds and intestinal contents. According to Nishikido *et al*. (2017), *Clostridium perfringens* septicemia hardly occurs and, in most cases, proved very fatal, with a reported mortality rate of 60%. This efficiency lethality is due to a merging of the 7-min doubling time of the organism and the making of a mass of virulent toxins. *Clostridium perfringens* are ubiquitous in nature, with humans and animals as the primary reservoirs (Archaya, 2022). Almost certainly, it is one of the most widely occurrence bacterial pathogens in the environment and is commonly seen in different types of foods (Miyamoto 2008). The genus *Clostridium* consists of various groups of Gram-positive bacteria but do not grow in the presence of oxygen (anaerobic) and they have the ability to form heat-resistant endospores. Many of these anaerobes are pathogenic for both humans and animals and most of the resultant diseases, such as tetanus and botulism are mediated by the production of potent extracellular toxins (Brynestad, 2002). *C. perfrigens* is the cause of some human diseases such as gas gangrene (Clostridial myonecrosis), food

poisoning, necrotizing enteritis in infants and enteritis necroticans (pigbel) (Poka, 2013). It is also the cause of some animal diseases such as lamb dysentery, ovine enterotoxemia and pulpy kidney disease of sheep (Duke, 2013). *C. perfringens* produces multiple exotoxins with different pathogenic significance in different animal species and serve as the basis for classification from A – E. Type A is important in humans and can be located in the colon and soil (Sogo, 2012) . *C. perfrigens* produces a wide range of wounds and soft tissue infections (Kart, 2021).

C. perfringens causes an estimate of over 1 million illnesses per year and this makes it the second most common bacterial cause of food poisoning in the United States. It is also ranked the leading cause of food borne illness outbreaks in other countries like Japan, England, and Australia and Wales (Scallan, 2011). Outbreaks of *C. perfringens* illness are mostly associated with the consumption of contaminated meats and poultry products (Grass, 2013). *C. perfringens* spores grows in raw or cooked foods under anaerobic condition and when it gets ingested, these vegetative cells sporulate and allows the elaboration of the *C. perfringens* enterotoxin (CPE) that causes diseases in human (Wen and McClane, 2004; Brynestad and Granum, 2002; Juneja *et al.,* 2010).

1.2 STATEMENT OF PROBLEM

Clostridium perfringens has become one of the major causes of food borne illness leading to food poisoning according to the Center for Disease Control (CDC, 2021). It is responsible for an estimate of about one million cases per year in the United States. Although number of cases of food poisoning from *Clostridium perfringens* is the second largest but it is more deadly and have a very high rate of reproduction (100% within seven minutes). It proves quickly fatal which makes its mortality rate high. Because it rarely occurs, little or no attention has been given to it in the literature. Most of the available studies derived their

samples mostly from non-African states (such as Scallan *et al.,* 2011; Dalton *et al*., 2004; Komatsu *et al.,* 2012). At the time of this study, the researcher is unable to lay hands on any study that derived samples from Nigeria. Hence, this study is focused on the identification and determination of antibiotics susceptibility of *Clostridium perfringen.*

1.3 JUSTIFICATION FOR STUDY

Clostridium perfringens as a major cause of food borne illness which is responsible for food poisoning is very harmful to the humans and animals because the reproduction rate and the mortality rate is very high.

In view of the nature and current low level of research efforts accorded to *Clostridium perfringens*, findings of this study will add to available literature. It will be of immense benefits to other researchers in the field of microbiology especially food poisoning. Study findings will be valuable to food and beverages firms. It is expected to create awareness of the nature of *Clostridium perfringens* and its prevention. Findings of the study will be of value to medical personnel, hospitals and pharmaceutical firms in the treatment of food poisoning from *Clostridium perfringens* ingestion. Lastly, it will awaken the CDC in performing their duties more effectively.

1.4 AIM OF THE STUDY

To isolate, identify and determine antibiotics susceptibility of *Clostridium perfringens* in different samples.

OBJECTIVES

- To determine the isolates using their morphological and biochemical characteristics.
- To determine the antibiotics susceptibility of *Clostridium perfringens* isolates.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *CLOSTRIDIUM PERFRINGENS*

Clostridium perfringens is an anaerobic gram-positive spore-forming bacillus that is associated with acute gastrointestinal infection which ranges in severity from diarrhea to necrotizing enterocolitis and myonecrosis in humans. *Clostridium perfringens* is a major cause of food poisoning and food borne disease; and can be found on roasted beef, pork, turkey, chicken, animal intestines, sauces and the environment (CDC, 2021).

According to Centers for Disease Control and Prevention (CDC), *Clostridium perfringens* causes nearly one million illnesses in the United States yearly. *Clostridium perfringens* form spores and act like protective coatings that help the survival of the bacteria when foods are kept at unsafe temperature (between 40°F - 140°F), the bacteria grow and get multiplied and when the food gets ingested, it will produce a toxin that will cause diarrhea & abdominal cramps after 8-15 hours of ingestion of the contaminated food (CDC, 2021; Jeffery and Stanley, 2001).

C. perfringens is not communicable from one person to another and can be diagnosed with stool studies and *is* commonly found in the gastrointestinal tract of humans, several animals, soils, stools and sewages (Sogo, 2012). *C. perfrigens* is the cause of some human diseases such as gas gangrene (Clostridial myonecrosis), food poisoning, necrotizing enterocolitis in infants and enteritis necroticans (pigbel) (Poka, 2013). It is also the cause of some animal diseases such as lamb dysentery, ovine enterotoxemia and pulpy kidney disease of sheep. *C. perfringens* is one of the most prevalent problems in hospitals and nursing homes where patients frequently receive antibiotics and must be fully hydrated(Labbe, 1989). Antibiotics are very important because it helps to wipe out germs and protect the body against infections.

The antibiotics remain in the body for as long as several months. *C. perfringens* spreads when an individual with the disease has direct contact with food, surfaces or objects that are contaminated with feaces (Medline, 2016). *Clostridium perfringens* is a significant pathogen in healthcare today, impacting both hospitalized and community-based patients. The inability of the immune system to fight infection can be caused by a number of conditions including illness and disease (such as diabetes, HIV, malnutrition and drugs) (Rebecca, 2015). Immunocompromised patients, including those receiving immunosuppressant medications or patients with human immune deficiency virus (HIV) and transplants, seem to be at increased risk of hospitalization and reoccurrence of *Clostridium perfringens* Infection as the immune system is an important defense for both protection and recovery from infection (Oluwaseun *et al.,* 2018).

Immunocompromised patients experience a high incidence of *Clostridium perfringen*s infection, ranging from 6% to 33% in the hematology-oncology population, up to 23% among lung transplant recipients, and a rate of 7.1-8.3 cases per 1000 patients years in patients with human immune deficiency virus (Dalton, 2004). Re-occurrence of *Clostridium perfringens* infections among immune compromised patients is also high, with rates up to 40% in both the hematology oncology population and solid organ transplant recipients (Sogo,2012). This higher incidence of *Clostridium perfring*ens infection and re occurrence is believed to be secondary to frequent antimicrobial use, suppressed immune function, increased exposure to healthcare settings, and higher prevalence of *Clostridium perfringens* colonization (Sara and Silvia, 2019). Clostridial myonecrosis or gas gangrene is caused by contamination of injury with clostridial spores from several species that causes myonecrosis, most especially *C. perfringens. C. perfringens* can be isolated from soil and sediment samples from any geographic region. During severe sepsis with *C. perfringens*, rapid hemolytic anemia may occur due to hemolysis which is caused by the α-toxin. In the civilian population,

10% of wounds gotten from automobile accidents, surgeries majorly Gastrointestinal or biliary tract and septic abortions has shown to contain clostridial spores (Andrew &Wendy, 2020*)*.

Necrotizing enteritis which is caused by Type C toxins is an acute necrotizing condition of the jejunum. Symptoms likely to be discovered are abdominal pain, bloody diarrhea, shock and peritonitis. It is severe and mostly fatal and immunization with type C toxoid has been reported to protect against this condition known as Pigbel in Papua New Guinea, where it is very common in countries like Germany, East Africa, Thailand and Nepal. *C.perfringens* produces many enzymes and many biological active soluble substances and one of the enzymes is neuraminidase which is the most important enzymes that alters the cell surface receptors and promotes capillary permeability. Other soluble substance produced are histamine, fibrinolysin, a bursting factor that acts on muscle tissues and is involved in muscle lesions in gas gangrene and the circulating factor that helps to increase the sensitivity of adrenaline of the capillary membrane and inhibit phagocytosis (Kart, 2021).

Taxonomy of *Clostridium perfringens*

Kingdom - Bacteria

Phylum - Firmicutes

Class - Clostridia

Order - Clostridiales

Family - Clostridiaceae

Genus - Clostridium

Species - *Clostridium perfringens*

2.2 BIOLOGY OF CLOSTRIDIUM PERFRINGENS

2.2.1 Physiological Phases of *Clostridium perfringens*

C. perfringens causes over 80%- 90% of gas gangrene cases but other species can cause infection. In the order of prevalence, they are *C. novyi* (40%), *C. septicum* (20%), *C. histolyticum* (10%), *C. bifermentans* (10%), *C. fallax* (5%) and *C. sordellii*. These organisms are in the soil and organic waste especially when contaminated with fecal material. When a deep penetrating wound in the muscle tissue in an immunocompromised host, it is more likely to develop an infection when it is been compared to a host with healthy immune system and good status of nutrition. Wounds that are open and are properly dressed and cleaned are less infected when compared to wounds with crush injury and tissue ischemia (Takazawa*et al.,* 2015).

Clostridial organisms produce alpha and theta toxins that cause extensive tissue damage. The infection spreads quickly and within a matter of several hours, the patient may develop shock, sepsis and death. The infection can develop slowly over weeks or over hours which depend on oxygen tension of the tissue and the amount of organism inoculated (Janik *et al.,* 2019). The toxin gets activated by enteric proteases and increases intestinal permeability through the disruption of tight junctions and the lamina propria. It causes perivascular edema with rapid cellular swelling and is found to accumulate in the kidney ad brain but the mechanism remains exclusive (Popoff, 2011).

2.2.2 Epidemiology of Clostridium perfringens

Type A and Type C toxins are known to cause human disease. Type A is responsible for most of the *C. perfringens* associated food poisoning and non-food borne diarrheal disease. According to CDC epidemiology surveillance data for foodborne disease outbreaks, *C.*

perfringens accounts for 5% of outbreaks, 10% of people with the illness and 4% of people that are undergoing hospitalizations (Dewey et al. 2018). The most common mode of transmission of this infection is undercooked beef and poultry. Outbreaks are more severe majorly during November and December periods. The pathogen ranks within top five most common cause of food poisoning in the US and at higher rank worldwide (Grass *et al.* 2013; Scallan *et al,* 2011).

In 1980s, a vaccination campaign has helped to reduce the incidence but has not stopped to cause significant morbidity and mortality. In 1960s to 1970s, Pigbel was identified to be the most common cause of death in infants over 1 year old (Poka and Duke. 2003; Duke *et al,* 2013).

Type C toxins are associated with endemic enteritis necroticans in post-world war II Germany from 1944-1949 and in the Highlands of Papua New Guinea named Darmbrand and Pigbel respectively. It is hypothesized that severe malnutrition increases susceptibility to type C infection (Ma *et al.* 2012). Type A toxins are mostly common in human stools with several other types associated with food poisoning and enterotoxigenic disease.From 1975-1999, clostridium perfringens has caused about 238 foodborne illness outbreaks in Finland.

From 1998-2010, two hundred and eighty nine (289) confirmed outbreaks of Clostridium perfringens illness have been reported with over fifteen thousands (15,000) illnesses, eighty three (83) hospitalizations and over ten (10) deaths. The number of outbreaks reported every year ranges from sixteen (16)- thirty one (31) with no trend with time. The annual number of outbreaks associated illness ranges from 359 - 2,173 with median outbreak size of twenty four (24) illnesses. Restaurants (43%) were the most common setting of food preparation; others are catering facility (19%), private homes (16%), Jail/Prison (11%), and others (10%). Among the 144 (50%) outbreaks attributed to a food commodity (66 outbreaks, 46%), then

poultry (43 outbreaks, 30%), and lastly pork (23 outbreaks, 16%). Outbreaks caused by *C. perfringens* usually occur frequently and they are often large and cause serious morbidity rate but they can be prevented if the contamination of the raw meat and poultry is controlled at the farm or slaughterhouse or after contamination, they are properly handled and prepared particularly in restaurants and catering facilities (Grass *et al.* 2010).

Although the number of outbreaks fluctuates yearly at an estimated medical cost of $539 per case, confirmed *C. perfringens* Outbreaks alone cost an estimated $8,197,112. Over 90% of the bacteria is attributed to meat and poultry consistently and because *C. perfringens* grows readily under pH 6.0 – 7.0. *C. perfringens* type A and isolates that produces the CPE has been isolated from raw meat and poultry at retail and in food service establishments (Scharff *et al.,* 2009; Labbe and Juneja, 2006; Wen and McClane,2004; Bryan and McKinley, 1979).

Contamination of meats and other foods has been documented to occur through direct contact of carcasses with feces as well as cross contamination with other foods or contaminated surfaces during food processing or preparation (Juneja *et al.,* 2010). In less developed countries with patients having decreased access to healthcare and antibiotics, the incidence is higher but the number remains unknown. Even with the best care, early recognition of the bacteria, surgical care, antibiotic treatment, and hyperbaric oxygen therapy make the overall mortality rate 20% - 30% and in some cases 5% - 10% but if it's not treated, the disease has a 100% fatality. Host factors such as an immunocompromised state, diabetes mellitus and spontaneous infections there is tendency of higher mortality rates of 67% or more. If the infection involves the abdominal soft tissue or chest wall, the rate of mortality is as high as 60% when it is been compared to extreme infections with more mortality of 5% - 30% (Shindo *et al., 2015*; Lehnhardt *et al.,* 2008).

2.2.3 Sporulation of *clostridium perfringens*

The capacity of *Clostridium perfringens* to spore forming performs a key function in the course of transmission of this bacterium to cause disease. Of precise note, the spores produced with the aid of using food poisoning strains are frequently resistant to food environment stresses consisting of preservatives, cold, and heat which probably helps their survival in temperature-abused foods. The exceptional resistance properties of spores made by most type A food poisoning strains and some type C food borne disease strains involve their production of a variant small acid-soluble protein-4 that binds more tightly to spore DNA than to the small acid-soluble protein-4 made by most other *C. perfringens* strains. Finally, sporulation is important for production of *C. perfringens* enterotoxin, which is responsible for the signs of *C. perfringens* type A food poisoning, the second most common bacterial foodborne disease in the United States. During this foodborne disease, *C. perfringens* is ingested with food and then, by using sporulation-specific alternate sigma factors, this bacterium sporulates and produces the enterotoxin in the intestines (McClane, 2010).

2.3 Metabolic phases of Clostridium perfringens

C. perfringens is a common Gram-positive anaerobic spore-forming rod shaped bacterium. This bacterium can exist as a vegetative cell or in its dormant spore form. Like other Gram-positive bacteria, *C. perfringens* is composed of a cytoplasmic lipid membrane, a thick peptidoglycan layer containing teichoic acids, and capsule polysaccharides. The spores of *C. perfringens* persist in the environment produce a variety of toxins and enzymes. Spores survive cooking and then germinate and multiply during storage at room and high temperatures, slow cooling, or inadequate re-warming. *C. perfringens* is an obligate anaerobe;

therefore, it acquires energy by performing anaerobic respiration. It uses nitrate as its final electron acceptor during anaerobic respiration. There is an increase in growth

when *C. perfringens* is grown in the presence of nitrate, because nitrate allows more metabolite molecules to undergo substrate-level phosphorylation, which lead to an increase in energy production. *C. perfringens* also has all the enzymes necessary to carry out glycolysis and glycogen metabolism. Therefore, *C. perfringens* breaks down the sugars it produces as uses them as an energy (Fujisawa, 2012).

2.3.1 Mode of transmission

Food Poisoning: Food-borne illness acquired by ingestion of large number of *C. perfringens* vegetative cells present in the food. Food sources are usually cooked meat, vegetables, fish or poultry dishes which have been stored at ambient temperatures for a long time after cooking.

Enteritis Necroticans: Consumption of contaminated pork meat .

Gas Gangrene/ Anaerobic Cellulitis: Infection can occur through contamination of wounds (fractures, bullet wounds) with dirt or any foreign material contaminated with *C. perfringens* (Fabris, 1991).

2.3.2 Incubation period: Food Poisoning: 8-24 hours

Gas Gangrene: 1-4 days after the injury, but may also start within 10 hours (Fabris, 1991).

2.3.3 *Clostridium perfringens* Infection

Clostridium perfringens infection include tissue necrosis, bacteremia, emphysematous cholecystitis,gas gangrene.

* Tissue necrosis: Necrotic tissue is a clinical situation wherein there are useless cells to the body organ. The loss of life of the cells takes place because of loss of oxygen and interrupted blood supply. It reasons the cells to be acidic, liberating enzymes that damage the cells. The malfunctioning of cells could make different body elements inactive. It can later bring about puncture of the cellular membrane. Ultimately, the cellular partitions will explode because of the gathering of more fluid. Necrotic tissue is the end result of skin necrosis. Necrosis is a untimely loss of life of cells which happens because of autolysis (self-digestion of cells after launch of enzymes). These cells are part of the residing tissue withinside the skin. Necrosis happens because of outside harm or trauma in a specific organ. Necrotic tissue is skin necrosis, wherein many cells die withinside the equal organ. It is taken into consideration to be a dangerous fitness situation, as it may bring about extreme sicknesses like skin cancers. Yearly, there are greater new instances of skin cancers than the mixed prevalence of cancers of the breast, prostate, lung, and colon. Over 3 decades ago, greater humans have had pores and skin most cancers than all different cancers.

This infection can be diagnosed through blood test s, biopsy, MRI scan, physical exam, CT scan and it can be treated by the use of antibiotics (Wendy, 2020).

* Bacteremia: Bacteremia is the presence of bacteria withinside the bloodstream. It can arise spontaneously, at some point of positive tissue infections, with use of indwelling genitourinary or IV catheters, or after dental, gastrointestinal, genitourinary, wound-care, or different procedures. Bacteremia might also additionally motive metastatic infections, inclusive of endocarditis, specifically in sufferers with valvular coronary heart abnormalities.

Transient bacteremia is frequently asymptomatic however might also additionally motive fever. Development of different signs generally indicates extra extreme infection, which includes sepsis or septic shock.

Gram-negative bacteremia secondary to contamination typically originates withinside the genitourinary or gastrointestinal tract or withinside the pores and skin of sufferers with decubitus ulcers. Chronically unwell and immunocompromised sufferers have an expanded chance of gram-negative bacteremia. They may additionally expand bacteremia with gram-positive cocci and anaerobes, and are vulnerable to fungemia. Staphylococcal bacteremia is not common amongst injection drug users, sufferers with IV catheters, and sufferers with complex pores and skin and smooth tissue infections. Bacteroides bacteremia can also additionally expand in sufferers with infections of the stomach and the pelvis, specially the woman genital tract. If an contamination withinside the stomach reasons bacteremia, the organism is maximum in all likelihood a gram-negative bacillus. If an contamination above the diaphragm reasons bacteremia, the organism is maximum in all likelihood a gram-positive bacillus. Usually, bacteremia that outcomes from regular events, which includes dental procedures, is transient and reasons no symptoms. Bacteremia that outcomes from different situations can also additionally reason fever. If humans with bacteremia have fever, a speedy coronary heart rate, shaking chills, low blood pressure, gastrointestinal symptoms (which includes belly pain, nausea, vomiting, and diarrhea), speedy breathing, and/or come to be confused, they likely have sepsis or septic shock (Morgan, 2011).

* Gas gangrene: Gangrene is the dying of body tissue. Clostridial myonecrosis, also known as gas gangrene, is a fast-spreading and doubtlessly life-threatening shape of gangrene resulting from abacterial contamination from Clostridium bacteria. The contamination reasons

pollutants to shape withinside the tissues, cells, and blood vessels of the frame. These bacteria will launch pollutants that motive tissue dying and launch a gas. Most gangrene infections arise in conditions in which open wounds from an harm or surgical operation are uncovered to bacteria. Non-disturbing gas gangrene, a extra uncommon shape of gas gangrene, can increase while blood waft to frame tissues is compromised and micro organism receives inside. There is a more threat in human beings who've a peripheral vascular disease, atherosclerosis, or diabetes mellitus.

Gas gangrene can arise everywhere on the body, however it maximum generally influences the fingers or legs. Common signs and symptoms encompass improved coronary heart rate, fever, and air below the skin. Skin withinside the affected location additionally will become faded after which later modifications to darkish pink or purple. These signs and symptoms commonly increase six to forty eight hours after the preliminary contamination and development very fast. Treatment may also encompass antibiotics and surgical operation to do away with the lifeless tissue. Occasionally a hyperbaric oxygen chamber can be used. Surgery includes debridement (elimination of lifeless tissue) and once in a while amputation.

* Emphysematous cholecystitis, recognised much less typically as clostridial cholecystitis, is an acute contamination of the gallbladder wall resulting from gas-forming organisms (such as Clostridium or *Escherichia coli*) this is usually taken into consideration a surgical emergency. An infrequent, insidious, and unexpectedly modern shape of acute cholecystitis, emphysematous cholecystitis is characterised via way of means of early gangrene, perforation of the gallbladder and excessive mortality. Although this circumstance develops in about 1% of all instances of acute cholecystitis, as compared with traditional acute cholecystitis, emphysematous cholecystitis is related to a whole lot better rates of gangrene and perforation of the gallbladder and drastically accelerated rates of mortality (15-25%).

An anticipated 500,000 cholecystectomies are accomplished per year withinside the United States. Assuming all sufferers with emphysematous cholecystitis come to surgical procedure, this will suggest that 5000 cholecystectomies are accomplished per year for emphysematous cholecystitis. Although the wide variety of sufferers who're handled correctly with out surgical procedure is sincerely small, the wide variety of sufferers who die with out surgical procedure is unknown (Yoshida, 2015).

2.3.4 Method of detection

Clostridium perfringens is a vital pathogen of human gastrointestinal (GI) tract illnesses consisting of meals poisoning, antibiotic-related diarrhea, and sporadic diarrhea in addition to nosocomial diarrheal sickness outbreaks. The maximum vital toxin made via way of means of this bacterium while it reasons human GI tract illnesses is *Clostridium perfringens* enterotoxin (CPE) . Although *C. perfringens* is a ubiquitous bacterium withinside the surroundings, handiest a small subpopulation of this bacterium, commonly much less than 5%, harbors the CPE gene (cpe). Probably due to this rarity, preceding surveys identifying cpe-superb *C. perfringens* isolates in meals, human feces, and the surroundings have suggested diverse results. Moreover, the quantity of contaminated *C. perfringens* cells (cpe-superb and cpe-poor traces) found in maximum nonoutbreak meals samples has been suggested to be fewer than three the usage of the maximum-probable-quantity method. Therefore, to save you meals poisoning and nosocomial outbreaks, a way capable of detect cpe-superb traces with excessive sensitivity and applicability for the trying out of a big quantity of samples is essential to be used in epidemiological surveys. Microbial supply tracking (MST) techniques permit the identity of the forms of microbial contaminants, the quantity of contamination, and the viable supply of contamination. In MST, molecular procedures are beneficial gear for the detection of a low quantity of bacteria. Hence, the

molecular techniques used for MST have to correctly distinguish cpe-superb C. perfringens isolates from cpe-poor *C. perfringens* isolates in meals and the surroundings (Wen, 2004).

2.3.5 Classification of *Clostridium perfringens* Toxins

Clostridium perfringens is the most pathologic infection with 17 known toxins and the alpha toxin is the most toxic but it is classified based on the production of six important toxins which are: alpha-toxin (CPA), beta-toxin (CPB), iota-toxin (ITX), epsilon-toxin (ETX), enterotoxin (CPE), and necrotic enteritis B-like toxin (NetB).

Type A: CPA only

Type B: CPA, CPB and ETX

Type C: CPA, CPB and CPE

Type D: CPA, ETX and CPE

Type E: CPA, ITX and CPE. (Adams and Lacey, 2018).

Alpha toxin is a phospholipase (Lecithinase) that breaks down cell membranes which triggers platelet aggregation, thrombosis and release of histamine. Theta toxins cause direct vascular injury and leukocytes causing blunted host inflammatory response to the infection (Dempsey, 2012; Srivastava, 2017; Crum-Cianflone, 2006). The hallmark of *C. perfringens* infection is the histotoxic gas production through glucose fermentation (Chi, 1995). *C. perfringens* produces some toxins like CPA, CPB, CPE, ETX, ITX, NetB and PFO.

PFO (Perfringolysin O) is a pore-forming toxin with synergistic effects with CPA which has been implicated in gas gangrene pathogenesis. It operates by targeting red blood cells and causing coagulative necrosis and it shares homology with other pore-forming toxins found in Streptococcus, Bacillus and Listeria (Awad *et al.,* 2001; Kiu and Hall, 2018).

NetB (enterotoxin and necrotic enteritis B-like toxin) is a toxin that forms pores and has been identified in avian necrotic enteritis and has shown to have 38% sequence similarity with CPB. The discovery of NetB created the type G classification but has not been linked to pathogenicity in Humans (Keyburn *et al.,* 2008).

ITX (Iota Toxin) is a binary toxin which is produced as two proteins Ia and Ib. Ib binds to a cell surface receptor and associates with Ia. The complex is endocytosed. Ia will pass tpo the cytosol through membrane channel created by Ib and hence depolymerize the actin cytoskeleton via ADP-ribosylation (Takehara *et al.,* 2017).

ETX (Epsilon Toxin) is a toxin that is associated with hemorrhagic enteritis and enterotoxaemia in sheep. The toxin gets activated by enteric proteases and increases intestinal permeability through the disruption of tight junctions and the lamina propria. It causes perivascular edema with rapid cellular swelling and is found to accumulate in the kidney ad brain but the mechanism remains exclusive (Popoff, 2011).

CPE (Enterotoxin) is a toxin that forms pores and binds to claudin receptors on cell surface and forms a hexamer complex and allows influx of calcium. This toxin is the major cause of food poisoning and non-foodborne diarrhea. Calcium influx is dose-dependent and results to activation of calpain and later resulting to cell death. (Navarro *et al.,* 2018).

CPB (Beta Toxin) is a toxin that forms pores and helps to bind endothelial cells and have neurotoxic properties through the release of substance P. the toxin helps to play a crucial role in the pathogenesis of necrotizing enterocolitis (Nagahama *et al.,* 2015).

CPA (Alpha-Toxin) is an enzyme that breaks down phosphatidylcholine and sphingomyelin on the cellmembrane and helps to inhibit migration and maturation of neutrophils and activate arachidonic acid metabolism and helps to lead to vasoconstriction and platelet

aggregation. This toxin also creates micro-environment with poor tissue circulation and impairment of the innate immune response (Titball*et al.,* 1999; Takehara *et al.,* 2016).

2.4 DIAGNOSIS OF *CLOSTRIDIUM PERFRINGENS*

Clostridium perfringens food poisoning can be diagnosed in the laboratory by detecting the bacterial toxin in feces using quantitative anaerobic cultures or enzyme immunoassay. Serotyping of the isolates is conducted to determine if the same serotype of *C. perfringens* is present in the epidemiologically implicated outbreaks.

In the case of gas gangrene, specimens are necrotic tissues, muscle fragments and exudates from deeper part of the wound where the infection appears to be more active. Other specimens depend on the type of infection such as suspected food (to investigate food poisoning), feces etc. Blood culture may be positive for *C. perfringens* and *C. septicum.* Although *C. perfringens* bacteria may occur in the absence of gas gangrene. Swabs rubbed over the infected area or wound in exudates are not satisfactory. Specimens should be put into Robertson cooked meat broth and transported to the laboratory immediately (Acharya, 2022).

Gram-stained smears of aspirated material from myonecrosis reveal a necrotic background with a lack of inflammatory cells and presence of gram-positive bacilli with a morphology that resembles *C. perfringens* or other Clostridia. Gram-stained films provide clues about species of clostridia present. Thick, stubby, boxcar shaped, Gram-positive bacilli without spore are suggestive of *C. perfringens*. The cells are 0.8 – 1.5 µm long with blunt ends. Culture plates should be incubated anaerobically at 37^0C (Acharya, 2022).

In cases of Clostridial myonecrosis, X-ray or CT scan of the affected area should be obtained in addition to blood tests such as CBC, CMP, Blood culture, CK level, ABG and lactic acid (Yao and Annamaraju, 2022).

2.5 TREATMENT

As the infection rapidly progresses, it is important to treat patients aggressively with antibiotics, early surgical consultation with debridement, intravenous fluid resuscitation, Intensive Care Unit (ICU) monitoring and adjuvant hyperbaric oxygen therapy. It is important to get early surgical consultation without delay because this is an emergency case. Treatment should be carried out immensely with antibiotics. Fasciotomy is important to relieve compartment pressures. Surgical debridement should focus on removing all the necrotic tissue and foreign bodies such as soil, debris and shrapnel. It is important to irrigate the wounds with copious amounts of sterile normal saline. In a case of soft tissue infection, penicillin and Clindamycin should be added which will also treat group a streptococcal necrotizing fasciitis. Clindamycin should be put into consideration because it inhibits the clostridial exotoxins synthesis and will lessen the systematic effect of the toxin because clindamycin is bacteriostatic and not bactericidal so it can be used with another anti-microbial called Penicillin (Finsterer, 2007; Nichols and Smith, 1994; Shin *et al.* 2018; Yang *et al.* 2015; Devaney *et al.* 2015).

Surgery is the mainstay of prophylaxis and the effective treatment of gas gangrene. The cases are treated with adequate removal of tissues that are damaged and properly clearing the wounds to remove foreign materials, necrotic tissue and blood clots. Hyperbaric oxygen treatment is also very useful as antiserum against alpha toxin is no longer in use. The Antibiotics named Metronidazole is the most effective one and prophylactic use of it with surgery is highly effective. The drug is introduced intravenously three times a day at 8 hours intervals before surgery. Antibiotic prophylaxis using several antibiotics like gentamicin,

chloramphenicol, amoxicillin and metronidazole is very effective since occurrence of mixed infections with aerobic and anaerobic bacteria is frequent. But it is not recommended for *C. perfringens* food poisoning, for this proper hydration is very important (Kart, 2021).

Proper cooking, cooling and hot holding can help reduce the growth of *C. perfringens* and the formation of vegetative cells in food. To ensure that vegetative cells are eliminated, foods are to be cooked or reheated to a temperature of $\geq 75^0$C. There should be consumer education, inspection of restaurants and efforts should be increased in the environment where production of food takes place. Food should be handled with care and this will minimize food poisoning and contamination of foods.

2.6 PREVENTION

Most of the food poisoning from *C. perfringens* is due to undercooked meat and poultry. Therefore, people are to undergo sensitization concerning proper food handling, safety and protocols in order to limit the spread of the disease. Patients with underlying health issue such as diabetes or vascular disease must not delay seeking medical attention in the event of cellulitis or trauma due to an increased risk of severe infection and rapid progression (Yao and Annamaraju, 2022).

The most important way of preventing the growth of *C. perfringens* spores is by cooking the food, especially beef and poultry, thoroughly, to the recommended temperatures. Leftover food should be refrigerated to a temperature below 40 °F (4 °C) within two hours of preparation. Refrigeration is also an advised method of preserving food quality thereby preventing *C. perfringens* growth or any pathogenic or spoilage microbe. Leftovers should be reheated to at least 165 °F (74 °C) before serving. (Columbia Dispatch, 2018).

2.7 ANTIBIOTICS SUSCEPTIBILITY

The overall performance of antimicrobial susceptibility testing via way of means of the medical microbiology laboratory is essential to affirm susceptibility to selected empirical antimicrobial agents, or to discover resistance in individual bacterial isolates. Empirical remedy remains powerful for a few bacterial pathogens due to the fact resistance mechanisms have now no longer been found such as continued penicillin susceptibility of Streptococcus pyogenes . Susceptibility testing of isolates is essential with species that could own received resistance mechanisms (such as members of the Enterobacteriaceae, Enterococcus species, *Pseudomonas species, Streptococcus pneumoniae* and *Staphylococcus species*) (Melvin, 2009).

Commonly Used Susceptibility Testing Methods includes:

* Broth dilution tests: One of the earliest antimicrobial susceptibility test techniques changed into the macrobroth or tube-dilution approach. This manner concerned making ready two-fold dilutions of antibiotics (eg, 1, 2, 4, 8, and 16 μg/mL) in a liquid increase medium disbursed in test tubes. The antibiotic-containing tubes had been inoculated with a standardized bacterial suspension of 1–5×105CFU/mL. Following in overnight incubation at 35°C, the tubes had been tested for seen bacterial increase as evidenced with the aid of using turbidity. The lowest attention of antibiotic that averted increase represented the minimum inhibitory attention (MIC). The precision of this approach changed into taken into consideration to be plus or minus 1 two-fold attention, due in big component to the exercise of manually making ready serial dilutions of the antibiotics. The benefit of this method changed into the technology of a quantitative result (ie, the MIC). The primary negative aspects of the macrodilution approach had been the tedious, guide project of making ready the antibiotic answers for every test, the

opportunity of mistakes in coaching of the antibiotic answers, and the rather big quantity of reagents and area required for every test. The benefits of the microdilution manner consist of the era of MICs, the reproducibility and comfort of getting preprepared panels, and the financial system of reagents and area that takes place because of the miniaturization of the test. There is likewise help in producing automatic reviews if an automatic panel reader is used. The fundamental disadvantage of the microdilution technique is a few inflexibility of drug alternatives to be had in wellknown commercial panels (James, 2009).

* Antimicrobial gradient technique. The antimicrobial gradient diffusion technique makes use of the precept of status quo of an antimicrobial awareness gradient in an agar medium as a method of figuring out susceptibility. The Etest (bioMérieux AB BIODISK) is a commercial model withinside the United States. It employs skinny plastic check strips which might be impregnated on the bottom with a dried antibiotic awareness gradient and are marked at the top floor with a awareness scale. As many as five (5) or six (6) strips can be positioned in a radial style at the floor of the correct 150-mm agar plate that has been inoculated with a standardized organism suspension like that used for a disk diffusion test. After overnight incubation, the test are study through viewing the strips from the pinnacle of the plate. The MIC is decided through the intersection of the decrease a part of the ellipse shaped increase inhibition place with the test strip.

The gradient diffusion technique has intrinsic flexibility through having the ability to test the medication the laboratory chooses. Etest strips value about $2-$3 each and might constitute an pricey method if quite a number pills are examined. This technique is high-quality acceptable to conditions wherein an MIC for most effective 1 or 2 pills is wanted or whilst a fastidious organism requiring enriched medium or unique incubation surroundings is to be examined (eg, penicillin and ceftriaxone with pneumococci). Generally, Etest outcomes have

correlated nicely with MICs generated through broth or agar dilution methods. However, there are a few systematic biases closer to better or decrease MICs decided through the Etest whilst testing positive organism-antimicrobial agent combinations. This can constitute a capability shortcoming whilst trendy MIC interpretive standards derived from broth dilution checking out are implemented to Etest MICs that won't be identical (Barth, 2009).

* Disk diffusion check. The disk diffusion susceptibility technique is straightforward and realistic and has been nicely-standardized. The test is completed through making use of a bacterial inoculum of about 1–2×108CFU/mL to the floor of a large (150mm diameter) Mueller-Hinton agar plate. Plates are incubated for 16–24 h at 35°C previous to dedication of outcomes. The zones of increase inhibition round every of the antibiotic disks are measured to the closest millimeter. The diameter of the area is associated with the susceptibility of the isolate and to the diffusion price of the drug thru the agar medium. The area diameters of every drug are interpreted the use of the standards posted through the Clinical and Laboratory Standards Institute (CLSI, previously the National Committee for Clinical Laboratory Standards or NCCLS) or the ones protected withinside the US Food and Drug Administration (FDA)-accredited product inserts for the disks. The outcomes of the disk diffusion test are “qualitative,” in that a class of susceptibility (ie, susceptible, intermediate, or resistant) is derived from the check in preference to an MIC. However, a few commercially-to be had area reader structures declare to calculate an approximate MIC with a few organisms and antibiotics through evaluating area sizes with trendy curves of that species and drug saved in an algorithm.

The benefits of the disk technique are the test simplicity that doesn't require any unique equipment, the supply of specific outcomes effortlessly interpreted through all clinicians, and versatility in choice of disks for testing. It is the least pricey of all susceptibility methods

(about $2.50-$5 in step with test for materials). The hazards of the disk test are the dearth of mechanization or automation of the test. Although now no longer all fastidious or gradual developing micro organism may be as it should be examined through this technique, the disk test has been standardized for testing streptococci, Haemophilus influenzae, and N. meningitidis through use of specialised media, incubation conditions, and unique area length interpretive standards (Mary, 2009).

2.7.1 ANTIBIOTICS SUSCEPTIBILITY OF *CLOSTRIDIUM PERFRINGENS*

Antimicrobial susceptibilities and toxin types were determined for 275 *Clostridium perfringens* isolates collected in Ontario in the spring of 2005. Minimal inhibitory concentrations (MICs) of *C. perfringens* isolates for 12 antimicrobials used in therapy, prophylaxis, and growth promotion of cattle (n = 40), swine (n = 75), turkeys (n = 50), and chickens (n = 100) were determined using the microbroth dilution method. Statistical analyses and MIC distributions showed reduced susceptibility to bacitracin, clindamycin, erythromycin, florfenicol, and tetracycline for some isolates (Boerlin *et al*., 2011). Reduced susceptibility to bacitracin was identified in chicken (64%) and turkey (60%) isolates. Swine isolates had predominantly reduced susceptibility to clindamycin (28%) and erythromycin (31%), whereas bovine isolates had reduced susceptibility to clindamycin (10%) and florfenicol (10%) (Boerlin *et al*., 2011). Reduced susceptibility to tetracycline was spread across all species. Toxin typing revealed that *C. perfringens* type A was the dominant toxin type isolated in the study across all 4 host species (Boerlin *et al*., 2011). In general, *C. perfringes* show highest resistance to antibiotics such as tetracycline (56.2%), imipenem (24.9%), metronidazole (9.5%), penicillin G (9%), vancomycin (4.5%), chloramphenicol (3%) and ceftriaxone (1%) (Tansuphasiri *et al*., 2005).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 MATERIALS AND EQUIPMENT USED

MacCartney bottle, testubes, conical flask, spirit lamp, autoclave, syringe, ethanol, cotton wool, petridish, foil paper, paper tape, permanent marker, beaker, cryovial bottle, inoculating loop, glass slides, hand gloves, nose masks, testube rack, knife, water bath, microscope, egg, spatula, weighing balance, ziplock bag, measuring cylinder.

3.2 REAGENTS USED

The reagents used in this study are as follow:

Reinforced Clostridial Agar, nutrient agar, anaerogen, blood agar, urea solution, gram staining reagents, peptone water, kovacs reagents, hydrogen peroxide, normal saline, phenol red.

3.3 SAMPLE COLLECTION

Seafood, dumpsite soil and waste water samples were collected in March 2022. Seafood samples collected were twenty two (22) and were bought in Allison market, Ikorodu. The twenty two (22) samples consist of five (5) shrimps, eight (8) crabs, five (5) horse mackerel and four (4) catfish. The samples were kept in a sterile ziplock bag from the market to the laboratory.

Dumpsite soil and waste water were collected in Caleb University. These samples were twenty three (23) which consist four (4) dumpsite soil from girls hostel, six (6) dumpsite soil from water factory, six (6) dumpsite soil from Architecture building, two (2) waste water from girls hostel, a sample of waste water from cafeteria, a sample of waste water from water

factory, a sample of waste water from generator house, a sample of waste water from school gutter, a sample of waste water from boys hostel.

The dumpsite soil samples were collected into sterile petri dishes while the waste water samples were collected inside sterile universal bottle.

3.4 METHOD OF ISOLATION

The isolation of *Clostridium perfingens* was performed by preparing 25.5g of Reinforced Clostridial Agar into 500ml of distilled water in a conical flask. The agar was left for some time for the supernatant to separate from the deposit; the supernatant was poured into another conical flask. After separation, 20ml of the supernatal was dispensed into twenty three (23) Maccartney bottles. The Maccartney bottles that contain the broth were sterilized in an autoclave in $121^{O}C$ for 15mins. The broths were allowed to cool then the seafood gills and guts were picked with knife into the broth and were labeled. Water was heated in the water bath till the temperature reached $80^{O}C$ then the broths were put in the water bath for 15mins.

After heating the broths, they were incubated anaerobically in an anaerobic jar with the addition of anaerogen for 48hrs. After the incubation, I prepared 12.7g of Reinforced clostridial agar in 250ml of distilled water and it was sterilized for $121^{O}C$ for 15mins. After the sterilization, the agar was allowed to cool then after cooling; the agar was supplemented with 6ml of blood then dispensed into twenty three (23) petri dishes. After the supplemented agar solidified, the inoculating loop was flamed then dipped inside the incubated broth and picked a loopful of the seafood sample at the bottom of the bottle then streaked on the agar and incubated anaerobically with anaerogen for 48hrs.

Dumpsite soil and waste water sample isolation was prepared by weighing 15.3g of Reinforced clostridial agar in 300ml of distilled water and the broth was separated and 2ml of

the broth was dispensed into twenty three (23) testubes. The testubes were sterilized at 121^{O}C for 15mins. After the sterilization, small quantity of dumpsite soil and waste water sample were dispensed into the testube then incubated anaerobically for 48hrs with the addition of anaerogen. I prepared 12.7g of Reinforced clostridial agar in 250ml of distilled water and it was sterilized for 121^{O}C for 15mins. After the sterilization, the agar was allowed to cool then after cooling; the agar was supplemented with 6ml of blood then dispensed into twenty three (23) petri dishes. After the supplemented agar solidified, the inoculating loop was flamed and then dipped inside the incubated broth and picked a loopful of the dumpsite soil and waste water sample at the bottom of the testubes then streaked on the agar and incubated anaerobically with anaerogen for 48hrs.

3.5 BIOCHEMICAL TEST

The isolation was subjected to morphological and biochemical tests and their identity were confirmed using the characteristics. The test carried out includes:

Gram staining, sugar fermentation (salicin, lactose, sucrose, glucose e.t.c.), urea hydrolysis test, catalase, indole, motility, lecithinase and lipase.

GRAM STAINING TEST

The isolates were smeared on a clean, grease free microscopic slide and allowed to dry. The slide was flooded with crystal violet for a minute and then rinsed with distilled water, iodine for a minute and rinsed with distilled water, decolorizer (acetone) for few seconds and then rinsed with distilled water and then safranin for a minute and rinsed with distilled water and finally allowed to dry.

A drop of immersion oil was placed on the glass slide and microscopy examination was carried under x100 objective of light microscope.

SUGAR FERMENTATION

This was carried out using four (4) different fermentable sugar; salicin, lactose, sucrose, and glucose. Peptone water base was prepared, 1% of each sugar was added, phenol red indicator was added then dispensed into test tubes and sterilized. After sterilization, the sugars were inoculated with the isolates and incubated at 37^0C for 48hrs.

MOTILITY TEST

Half strength nutrient agar was prepared and dispensed into testubes and sterilized. After cooling, the agar was stabbed with the bacterial colonies and incubated at 37^0C for 48hrs.

INDOLE TEST

The isolates were inoculated into the peptone water and incubated at 37^0C for 24hrs. After incubation, a drop of kovac's reagent was added into the peptone water with the isolate.

HYDROGEN SULFIDE PRODUCTION

This test was carried out using triple sugar iron agar. The agar was inoculated with the isolate and incubated at 37^0C for 24hrs.

LECITHINASE TEST

The lecithinase test was carried out using nutrient agar supplemented with 10% egg yolk under aseptic condition and was dispensed inside petri dish. The cultures were streaked on the media plates and incubated.

UREA HYDROLYSIS TEST

The isolates were inoculated by gently streaking into an already prepared urease agar which was in a slant form following the manufacturer's procedure. This test helps in the

identification of microorganisms that has the ability to produce urease enzyme. This enzyme partakes in the hydrolysis of urea into NH_3 and CO_2.

CATALASE TEST

Colonies of the isolates were picked with a sterile inoculating loop and dropped on a clean grease free microscopic slides, a drop of Hydrogen Peroxide was added to the colony.

3.6 ANTIBIOTICS SUSCEPTIBILITY TEST

Antibiotics are drugs used to fight against bacteria and the susceptibility test is used to determine which specific antibiotic a particular bacterium is sensitive to. The following are the antibiotics used in this study: Ampicillin, Gentamicin, Ciprofloxacin, Vancomycin, Tetracycline, Chloramphenicol, Erythromycin and Metronidiazole. In this study, *Clostridium perfringens* was tested for antibiotics susceptibility using blood agar and the method used is disk diffusion method. The antibiotic disks were placed on the blood agar media and incubated anaerobically for 24hrs. After incubation, there were clear zone of inhibition on the plates and measurement was taken according to Clinical and Laboratory Standards Institute (CLSI, 2014).

CHAPTER FOUR

4.0 RESULTS

4.1 BIOCHEMICAL CHARACTERISATION

All the eight (8) isolates tested were Catalase negative, Alpha and beta haemolysis , Gram positive rods, indole negative, lecithinase positive, lipase negative, urease positive, non-motile and hydrogen sulfide positive. They all fermented the sugars used (Table 4.1).

Table 4.1: Biochemical tests results

SAMPLE	CATALASE	GRAM STAIN	SHAPE	MOTILITY	SALICIN	TSI	UREASE	LACTOSE	GLUCOSE	SUCROSE	LECITHINASE	LIPASE	INDOLE
Catfish 1	-	+	ROD	-	+	+	+	+	+	+	+	-	-
Mackerel 2	-	+	ROD	-	+	+	+	+	+	+	+	-	-
Mackerel 3	-	+	ROD	-	+	+	+	+	+	+	+	-	-
Mackerel 4	-	+	ROD	-	+	+	+	+	+	+	+	-	-
Crab 8	-	+	ROD	-	+	+	+	+	+	+	+	-	-
Crab 4	-	+	ROD	-	+	+	+	+	+	+	+	-	-
Dumpsite soil 2e	-	+	ROD	-	+	+	+	+	+	+	+	-	-
Dumpsite soil 3a	-	+	ROD	-	+	+	+	+	+	+	+	-	-

4.2 ANTIBIOTICS RESULTS

This table shows how the isolate were susceptible and resistant to the antibiotics listed below. The isolate with the most resistance capability is Mackerel 4 and all the isolates were resistant to antibiotic Metronidazole. Chloramphenicol has the highest susceptible percentage which is 87. 5% while metronidazole has no susceptible percentage.

Table 4.2: Antibiotics results of the isolates

SAMPLE	AMP	CN	CIP	VA	TE	C	E	MTZ
Catfish 1	R	S	S	S	R	S	R	R
Mackerel 2	S	S	S	S	S	S	S	R
Mackerel 3	S	R	R	S	S	S	R	R
Mackerel 4	S	R	R	R	R	R	R	R
Crab 8	R	S	S	R	S	S	R	R
Crab 4	R	S	S	R	S	S	R	R
Dumpsite soil 2e	S	R	S	S	S	S	S	R
Dumpsite soil 3a	S	S	S	S	S	S	R	R

KEY: AMP—AMPICILLIN, CN--- GENTAMICIN, CIP---CIPROFLOXACIN, VA---VANCOMYCIN, TE---TETRACYCLINE, C---CHLORAMPHENICOL, E--ERYTHROMYCIN, MTZ---- METRONIDAZOLE.

Table 4.3:

ANTIBIOTICS	SUSCEPTIBLE	RESISTANT
AMPICILLIN	5 (62.5%)	3 (37.5%)
GENTAMICIN	5 (62.5%)	3 (37.5%)
CIPROFLOXACIN	6 (75%)	2 (25%)
VANCOMYCIN	5 (62.5%)	3 (37.5%)
TETRACYCLINE	6 (75%)	2 (25%)
CHLORAMPHENICOL	7 (87.5%)	1 (12.5%)
ERYTHROMYCIN	2 (25%)	6 (75%)
METRONIDAZOLE	8 (0)	8 (100%)

CHAPTER FIVE

5.0 Discussion

Clostridium perfringens is an important microorganism in the clinical, food and veterinary areas. The diversity of toxins produced not only makes it a risk to human health but also to animal health. It causes subclinical diseases that generate great losses particularly in food industry because of its ability to produce various toxins some of which have already been identified and characterized.

However, the infection produced by this organism known as necrotic enteritis (NE) has become a problem in maintaining good health by being isolated from wide range of food which causes disease to occur and generating significant economic losses to human.

Seafood as one of the most consumed proteins and enough supply to consumers requires mass production strategies, making worse of the problem by infections caused by pathogen such as *Clostridium perfringens*. Due to this, there is a need to find economical, environmentally, friendly and efficient alternatives in the modulation of the intestinal microbiota which contribute to the efficient production of seafood to meet current and future demands.

Upon the microbiological investigation, a total of eight (8) isolates were gotten from 45 samples ehich comprises of twenty-two (22) seafood, seventeen (17) dumpsite soil and six (6) waste water samples which means the isolate gotten from these samples is small which is similar to a report from china (Cal, 2008) but lesser than the report In America (Wen and McClane, 2004). In this study, seafood has the higher isolate than dumpsite soil and waste water samples.

Moreover, attention should be focused on the antimicrobial susceptibility of this organism so as to be able to know and be certain of the antibiotics to be used for the treatment of this organism.

5.1 Conclusion

Colonies with double zone of hemolysis were produced when cultured at 37^0C on blood agar overnight. All the eight (8) isolates were Catalase negative, gram positive rod, sugar fermented, indole negative, lecithinase positive, lipase negative, urease positive, non- motile and hydrogen sulfide positive. All the isolates were resistant to antibiotic Metronidazole. Chloramphenicol has the highest susceptible percentage which is 87. 5% while metronidazole has no susceptible percentage.

In this study, *Clostridium perfringens* was found more in the food samples than in the environmental samples.

5.2 Recommendation

More research work should be carried out to determine the occurrence and antibiotic susceptibility on *Clostridium perfringens* isolates to avoid more health complications especially in food poisoning cases.

Further studies should be focused on this bacterial infection and is also needed to understand the drug resistance mechanism of *Clostridium perfringens*, particularly to Metronidazole, because it will provide new targets and new ideas for the development of antimicrobial agents and preventing *Clostridium perfringens* infections.

REFERENCES

Andrew B. and Wendy S. (2020). Diseases caused by Clostridium. *Mandell, Douglas and Bennett'sprinciples and practices of Infectious Diseases,246,2960-2968.e2.*

ArchayaTankeshwar (2022). *Clostridium perfringens*: Properties, Diseases, Diagnosis. *Retrieved from microbe online*

Awad M, Ellemor D, Boyd R, Emmins J and Rood J (2001). Synergistic Effects of Alpha-Toxin and Perfringolysin O in *Clostridium perfringens*-Mediated Gas Gangrene.*Infect Immun. Dec;69(12):7904-10.*

Bryan F. and McKinley T. (1979). Hazard Analysis and Control of Roast Beef Preparation in Foodservice Establishments*. J Food Prot; 42:4-18.*

Brynestad S. and Granum P. (2002). *Clostridium perfringens*and foodborne infections*. Int J Food Microbiol; 74:195-202.*

Chi C, Chen K, Huang J, Chuang Y, and Wu M (1995). Gas Composition in *Clostridium septicum* gas gangrene. *J Formos Med Assoc. Dec;94(12):757-9.*

Cristoferi G, Fabris G, Ronconi A, Bozza F, Gallassi G, Bucca D, Caria G and Duodeci S (1991). Gas Gangrene. Clinical considerations, Prognosis and Therapeutic Prospects in our Experience. *J Chir (Paris). May; 128 (5):243-6.*

Crum-Clanflone N. (2006). Infection and Musculoskeletal Conditions: Infectious Myositis. *Best Pract Res Clin Rheumatol. Dec;20(6):1083-97.*

Dalton C., Gregory J., Kirk M., Stafford R., Givney R., Kraa E. and Gould D. (2004). Foodborne disease Outbreaks in Australia, 1995-2000. *Commun Dis Intell; 28:211-224*.

Dempsey A. (2012). Serious Infection associated with Induced Abortion in the United States. *Clin Obstet Gynecol. Dec;55(4):888-92.*

Dewey-Mattia D, Manikonda K, Hall A.J., Wise M.E. and Crowe S.J (2018). Surveillance for Foodborne Disease Outbreaks- United States, 2009-2015. *MMWR Surveill Summ. Jul 27;67 (10):1-11.*

Duke T., Poka H, Myers S, Radcliffe J. and Pavlin B. (2013). Pigbel in the 21st century: still here, and still in need of an effective surveillance system. *P N G Med J. Sep-Dec;56(3-4):136-40.*

Edmund A. Zottola (1971). Department of Food Science and Industries and extension microbiologist. *Jun,30. 55101.*

Finsterer J and Hess B (2007). Neuromuscular and Central Nervous System Manifestations of Clostridium perfrigens Infections. *Dec;35(6):396-405.*

Garcia N, Cai J (2018). Aggressive Soft Tissue Infections. *Surg Clin North Am. Oct;98(5):1097-1108.*

Gormley F., Little C., Rawal N., Gillespie I., Lebaigue S., and Adak G. (2011). A 17 – Years Review of Foodborne Outbreaks: Describing the Continuing Decline in England and Wales (1992 – 2008) *Epidemiol Infect; 139:688-699.*

Grass J., Gould L. and Mahon B. (2013). Epidemiology of foodborne disease outbreaks caused by Clostridium perfrigens, United States, 1998-2010. *Foodborne Pathog Dis. Feb;10(2):131-6.*

Janik E, Ceremuga M, Saluk-Bijak J, Bijak M. (2019). Biological Toxins as the Potential Tools for Bio-terrorism. *Int J Mol Sci. Mar 08;20(5)*

Jeffery Rhodehamel and Stanley Harmon (2001). Bacteriological Analytical Manual. *Bacteriological Analytical Manual, 8th Edition, Revision A, Chapter 16.*

Juneja V., Novak J., and Labbe R. (2010). *Clostridium perfringens.* In Juneja V., Sofos J., editors. Pathogens and Toxins in Foods: Challenges and Interventions. Washington, DC: ASM *Press. Pp.53-70.*

Juneja V., Novak J., Eblen B., and McClane B. (2001). Heat Resistance of Clostridium perfringens vegetative cells affected by prior heat shock. *J Food Saf; 21:127-139.*

Kart B. (2021). Clostridium perfrigens – Bacteriology. *Brainkart.com/article/Clostridium perfringens*

Keyburn A, Boyce J, Vaz P, Bannam T, Ford M, Parker D, Di Rubbo A, Rood j and Moore R (2008). NetB, A New Toxin That is associated with Avian Necrotic Enteritis Caused by Clostridium perfringens. PLoS Pathog. Feb 08;4(2): e26.

Kiu R and Hall L (2018). An Update on the Human and Animal Enteric Pathogen *Clostridium perfringens. Emerg Microbes Infect. Aug 06;7(1):141.*

Komatsu H., Inui A., Sogo T., and Fujisawa T. (2012). Clostridium perfringens. Nihon Rinsho. 70:1357-1361

L, Barth Reller, Melvin Weistein, James H. Jorgensen, Mary Jane Ferraro (Decmber, 2009). Clinical Infectious Diseases, Volume 49, Issue 11.

Labbe R. (1989). *Clostridium perfringens*. In: Doyle MP, editor. Foodborne Bacterial Pathogens. New York: Marcel Dekker.*pp.192-234.*

Labbe R. and Juneja V. (2006). Clostridium perfringens gastroenteritis. In: Riemann HP, Cliver DO, editors. Foodborne infections and intoxications. San Diego: *Academic Press; pp.137-163*.

Lehnhardt M., Homann HH., Diageler A., Hauser J., Palka P., and Steinau H. (2008). Major and Lethal Complications of Liposuction: A Review of 72 cases in Germany between 1998-2002. *PlastReconstr Surg.jun;121(6):396e-403e.*

Limbago B., Thompson A., Greene S., Maccannell D., Macgowan C.,

Lucey B.P., Hutchins G.M., William H and Welch MD (2004). In the discovery of Bacillus welchii. *Arch Pathol Lab Med. Oct; 128 (10): 1193-5.*

Ma M., Li J. and McClane B. (2012). Genotypic and phenotypic characterization of Clostridium perfringens isolates from Darmbrand cases in post-world war II Germany. *Infect Immun.Dec;80(12):4354-63.*

McClane B. (2007). *Clostridium perfringens*. *In: Doyle MP, Beuchat LR, editors. Food Microbiology: Fundamentals and Frontiers, Washington, DC: ASM Press;2007. pp.423-444.*

Morgan S. Barham (2011). Clostridium perfringens. *Microbewiki.kenyon.edu*

Nagahama M, Ochi S, Oda M, Miyamoto K, Takehara M and Kobayashi K (2015). Recent Insights into Clostridium perfringens beta-toxin. Toxins (Basel). *Feb 03;7(2):396-406.*

Navarro M, McClane B and Uzal F. Mechanisms of Action and Cell Death Associated with *Clostridium perfringens* Toxins. Toxins (Basel). *May 22;10(5).*

Navarro M.A., McClane B.A and Uzal F.A. (2018). Mechanisms of Action and Cell Death Associated with Clostridium perfringens Toxins. *Toxins (Basel) May 22;10(5)*

Nichols R and Smith J (1994). Anaerobes from a surgical perspective. *Clin Infect Dis. May;18 Suppl 4: S280-6.*

OluwaseunShogbesan,DilliRamPoudel,SamjerisVictor,AsadJehangir,OpeyemiFadahunsi,GbengaShogbesan,andAnthonyDonato (2018). Asystematic reviewoftheefficacyandsafetyoffaecalmicrobiotatransplantforClostridium perfringens infectioninimmunocompromisedpatients,*CanadianJournalof GastroenterologyandHepatology2018;1-10.*

Poka H., and Duke T. (2003). In search of Pigbel: gone or just forgotten in the highlands of Papua New Guinea? *P N G Med J. Sep-Dec; 46(3-4):135-42*

Popoff M (2011). Epsilon-Toxin: A Fascinating Pore-Forming Toxin. *FEBS J. Dec;278(23):4602-15*

RebeccaSchreler(2015).Infectionsintheimmunocompromisedhost,DrugsandDiseases>Paediatrics.RetrievedonJanuary20,2020 from https://emedicine.medscape.com/article/973120-overview.

Roberts E, Martucci J., and Wu D (2018). The Unusual Presence of Gas from a Puncture Wound: A Case Report. *J Foot Ankle Surg. Jul-Aug;57(4):785-789.*

SaraR.andSilviaM.(2019).Clostridium perfringens inimmunocompromised hosts*:A reviewofepidemiology,riskfactors,treatmentandprevention,Clinical Infectious Diseases,68:2144*-2153

Sarker M., Shivers R., Sparks S., Juneja V., and McClane B. (2000). Comparative Experiments to examine the Effects of Heating on Vegetative Cells and Spores of Clostridium perfringens isolates carrying plasmid genes versus chromosomal enterotoxin genes. *Appl Environ Microbiol; 66:3234-3240.*

Scallan E., Hoekstra R. Angulo F., Tauxe R., Widdowson M., Roy S., Jones J. and Griffin P. (2011). Foodborne illness acquired in the United States-major pathogens. *Emerg Infect Dis. Jan;17(1):7-15.*

Scharff R, McDowell J, and Medeiros L. (2009). Economic Cost of Foodborne illness in Ohio. *J Food Prot; 72:128-136*

Shin S, Park I, Kang J, Lee Y, and Chung Y. (2018) Vacuum-Assisted Closure Using Multiple Foam Pieces for Hidden Space Drainage through Less Exposure in Musculoskeletal Infections. *J Hand Surg Asian Pac Vol. sep;23(3):369-376.*

Shindo Y., Dobashi Y, Sakai T, Monma C., Miyatani H., and Yoshida Y. (2015). Epidemiological and Pathobiological Profiles of Clostridium perfringens infections: Review of Consecutive Series of 33 Cases Over a 13-year Period. *Int J Clin Exp Pathol;8(1):569-77.*

Srivastava I, Aldape M, Bryant A and Stevens D. (2017). Spontaneous C. septicum gas gangrene: A literature review. Anaerobe. *Dec; 48:165-171.*

Takazawa K, Otsuka H, Nakagawa Y, Inokuchi S. (2015). Clinical Features of Non Clostridial Gas Gangrene and Risk Factors for In-hospital Mortality. *Tokai J Exp Clin Med Sep 20;40(3):124-9.*

Takehara M, Takagishi T, Seike S, Oda M, Sakaguchi Y, Hisatsune J, Ochi S, Kobayashi K and Nagahama M (2017). Cellular Entry of *Clostridium perfringens* Iota-Toxin and Clostridium botulinum C2 Toxin. Toxins (Basel). *Aug 11;9(8).*

Takehara M, Takagishi T, Seike S, Ohtani K, Kobayashi K, Miyamoto K, Shimizu T and Nagahama M (2016*).Clostridium perfringens* Alpha-Toxin Impairs Innate Immunity Via Inhibition of Neutrophil Differentiation. *Sci Rep. Jun16;6:28192.*

Titball R, Naylor C and Basak A (1999). The *Clostridium perfringens* Alpha-Toxin. Anaerobe. *Apr;5(2):51-64.*

Wen Q. and McClane B. (2004). Detection of enterotoxigenic *Clostridium perfringens* type A isolates in American retail foods. *Appl Environ Microbiol; 70:2685-2691.*

Yao P and Annamaraju P (2022). Closstridium perfringens. *In statpearls. Treasureisland*