PREVALENCE OF *Staphylococcus aureus* AND *Bacillus cereus* IN READY-TO-EAT GAME MEAT SOLID IN DIFFERENT CITIES IN SOUTHWESTERN NIGERIA

BY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, MOUNTAIN TOP UNIVERSITY, MAKOGI, IBAFO, OGUN STATE, NIGERIA

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

SEPTEMBER 2022

DECLARATION

I hereby declare that this project report written under the supervision of Dr. O.E. Fayemi is a product of my research work, scientific information derived from various sources has been duly acknowledged in the text and a list of references provided. This research project report has not been previously presented anywhere for the award of any degree or certificate.

___________________________ ________________________

OHUNYON RICHARD K. Date

CERTIFICATION

This is to certify that this research project titled "**PREVALENCE OF *Staphylococcus aureus* AND *Bacillus cereus* IN READY-TO-EAT GAME MEAT SOLID IN DIFFERENT CITIES IN SOUTHWESTERN NIGERIA"** was carried out by **OHUNYON, Richard Kelvin**, with matriculation number **18010101039**. This project meets the requirement governing the award of **Bachelor of Science (B.Sc.)** degree in **Microbiology** from the Department of Biological sciences of Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literacy presentation.

__________________________ __________________________

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Ag. HEAD OF DEPARTMENT

DEDICATION

This project is dedicated to God Almighty for providing me with good health, and the grace to complete this project; to my beloved parents, Mr. and Mrs. Ohunyon, for their prayers, counsel, and sacrifice.

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ABSTRACT

Safety of meat and meat products is a key issue of public health concern. Game meat is readily available to people in various open marketplaces of Nigeria, but the safety and consistency of such game-meat is always unknown and can be affected either by physical, biological and chemicals hazards. However, ready-to-eat (RTE) game meat can be a potential source of transmission of foodborne diseases. The aim of this study was to determine the prevalence of *Bacillus* spp. and *Staphylococcus aureus* in game meat sold in various cities within southwestern Nigeria. Isolation and Identification of these two pathogens associated with game meat were performed using culture method and molecular technique respectively. A total of 55 samples were analyzed for the presence of *Bacillus* spp. and *Staphylococcus aureus.* It was observed that the microbial counts was very high in all the game meat sampled from various states. The isolates were confirmed by the presence of the 16SrRNA gene using Simplex PCR. The presence of *Bacillus* spp. and *Staphylococcus aureus*, in game meat from various states in Nigeria is a public health concern which could lead to foodborne illnesses such as bacteremia, endocarditis, diarrhea, septicemia and food poisoning.

Keywords: Game-meat, public-health, *Bacillus* spp., *Staphylococcus aureus*, Food safety

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Bacillus cereus is found in soil, dust, and water and has a wide distribution in nature (Arnesen *et al.,* 2008). It can be isolated from a variety of processed and raw foods, especially plant that comes into direct touch with the soil, because of its widespread and widespread prevalence in the environment (Tewari and Abdullahi, 2015). Its presence in food, on the other hand, does not pose a serious threat hazard unless the organism can thrive and create toxins that cause foodborne illness (Østensvik *et al.,* 2004; Yu *et al.,* 2020; Jovanovic *et al.,* 2021). The presence of significant amounts of *B. cereus* in food, on the other hand, suggests that the organisms are involved in food poisoning (Tewari and Abdullahi, 2015). The consumption of food containing more than 10^5 viable toxigenic *B. cereus* organisms per gram of food has resulted in outbreaks of food poisoning (Granum and Lindbäck, 2012). Although *B. cereus* was long thought to be a harmless saprophyte, its significance as a possible foodborne pathogen associated with food poisoning was recognized as early as 1906 in Europe (Griffiths and Schraft, 2017 Jessberger *et al.,* 2020). Vanilla puddings, cooked meats and vegetable dishes, boiled and fried rice, dairy goods, and vegetable sprouts have all been linked to previous food poisoning outbreaks (Glasset *et al.,* 2016; Granum and Lindbäck, 2012).

Staphylococcus aureus is a leading cause of potentially fatal bacterial infections (Boscolo-Rizzo, P., and Da Mosto, 2009; Leech *et al.,* 2017). Each year, it is responsible for 241,188 illnesses, 1,064 hospitalizations, and 6 deaths. *Staphylococcus aureus* infects roughly 400,000 hospital patients (Rojas-Moreno and Salzer, 2016). Around 100,000 of these individuals die as a result of infection-related complications (Hal *et al.,* 2012; Bryant and Sebire, 2018). *Staphylococcus aureus* is a Gram-positive, oxidase-negative, non-motile, facultatively anaerobic bacteria (Thushani and Edirisinghe, 2016; Khan *et al.,* 2019). *Staphylococcus aureus* is one of roughly 32 bacterium species in the *Staphylococcus* genus (Foster, 2022). The majority of the other species are exclusively found in mammals and do not infect humans (Foster, 2022). *Staphylococcus aureus* developed from prehistoric soil germs, according to current beliefs. In 1884, German physician

Anton Rosenbach published the definitive description of *Staphylococcus aureus* (DePaolo, 2016). When permitted to proliferate in foods, the enterotoxins, among the many metabolites produced by *Staphylococcus aureus*, pose the greatest risk to consumer health. *S. aureus* can produce enough enterotoxins to make people sick if they eat infected food (Ei-Jakee, 2013). Meat and meat products, salads, cream-filled bread products, and dairy products are some of the foods linked to staphylococcal food poisoning (Mohammed *et al*., 2018). Many of these foods are infected during preparation in homes or restaurants, and then mistreated before being consumed (Bennett *et al.,* 2013; Bintsis, 2017).

In many African countries, game meat is regarded as a delicacy, which has led to its commercialization (Hoffman and Cawthorn, 2012). Despite the fact that hunting and trafficking wild animals for meat is a global phenomena, the difficulties related with its consumption have received little attention. Zoonoses, or animal-borne diseases linked to wildlife hunting and consumption, have emerged as a major source of concern around the world (Cantas and Suer, 2014). Game meat contamination can occur through the hunting process, slaughtering and processing stages including washing with water that has been contaminated as well as cross-contamination of ready-to-eat game meat (Sofos, 2014). Across the globe, outbreaks of illness linked to game meat have been connected to a wide range of bacteria, viruses and protozoa (Rahman *et al.,* 2020). *Staphylococcus aureus* and *Bacillus cereus* have been found from previous studies to be a part of the aetiological agents of infections related to game meat (Izah, and Seiyaboh, 2018; Katani *et al.,* 2019; Ratsimba *et al.,* 2019).

1.2 STATEMENT OF THE PROBLEM

Game meat brings humans into close contact with wildlife, facilitating the spread of foodborne and viral diseases such as Ebola and diarrhea as well as new developing infectious diseases. The spread of these disease could be wider from the consumption of unsafe game meats that are potential reservoirs of the causative pathogens.

1.3 SIGNIFICANCE OF THE STUDY

It is important to investigate the microbiological safety of game meat in order to ascertain the level of pathogens present in it and identify the most prevalent organisms. Samples of various game meat from different locations in Nigeria would be examined for the presence of *Staphylococcus*

aureus and *Bacillus cereus* in them which if present poses the risk of morbidity and mortality associated with the consumption of contaminated game meat.

1.4 AIMS AND OBJECTIVES OF THE STUDY

- To isolate *Staphylococcus aureus* and *Bacillus cereus* in ready-to-eat game meat sold in the various market areas of Nigeria using cultural methods.
- To identify isolated *Staphylococcus aureus* and *Bacillus cereus* using molecular techniques

CHAPTER TWO

LITERATURE REVIEW

2.1 GAME MEAT

The term "game meat" refers to any non-domesticated terrestrial mammal, bird, reptile, or amphibian harvested for food, and it can refer to any step in the supply chain, such as the acquisition, trade, and consumption of wild meat (Velden *et al.,* 2018). For many people, game meat is both a source of protein and a source of income (Velden *et al.,* 2018).

Approximately 75% of human Emerging Infectious Diseases (EIDs) are of zoonotic origin, which means the pathogen originated in animals and was transmitted to people (Gebreyes, 2014; Galindo-González, 2022). Despite the fact that many zoonotic pathogen spillovers occur in domestic animals, such as livestock, wildlife species account for the bulk of zoonotic EIDs (71.8%) (Kurpiers *et al.,* 2016).

Zoonotic diseases can spread between animal hosts and humans in a variety of ways (Karesh *et al*., 2012; Estrada-Peña, 2014), including;

- shared vectors, such as mosquitoes for malaria, (Vazirianzadeh and Rahdar, 2013)
- indirect contact, such as peridomestic exposure to rodent feces (Himsworth *et al.,* 2013; Li *et al.,* 2021), or
- direct contact with an animal through consumption, animal bites, scratches, body fluids, tissues, and excrement (Delahoy *et al.,* 2018).

Most infections that afflict animals do not spread to humans, however 33 percent of zoonotic pathogens that have spread (286 out of 868 zoonotic pathogen species investigated) are known to be transmissible between humans (Kurpiers *et al.,* 2016). Zoonotic spillovers from wildlife have been highlighted as the most serious and expanding hazard to global health among all EIDs (Gebreyes *et al.,* 2014; Brierly *et al.,* 2016; Grange *et al.,* 2021).

Although research has primarily focused on mammals and, to a lesser degree, birds, any wildlife species collected for bushmeat could conceivably be a source of zoonotic illness that can spread

during the hunting, slaughtering, and preparation process (Friant *et al.,* 2015). Hunters are at risk of being injured by live animals, which could enable animal blood into the hunter's bloodstream through open wounds (Kurpiers *et al.,* 2016). Small animals can be carried in bags, but large animals are typically carried on the hunter's shoulder or back, bringing the hunter into intimate contact with the animal and allowing the transmission of body fluids (Cantlay, 2015). The greatest danger of disease transmission occurs during animal butchering, such as skinning, opening the body cavity, removing organs, and cutting meat (Lysholm *et al.,* 2022). More individuals butcher than hunt animals (LeBreton *et al.* 2006), and slaughtering requires the use of sharp equipment, which may result in cuts during the procedure. Subramanian (2012) discovered that 38% of respondents cut themselves on a regular basis during butchering. Women are especially vulnerable to illness transmission because they slaughter and prepare food more frequently than men (Kamins *et al.,* 2015).

Despite the fact that pathogens are common and can be found in large quantities in almost all animals, only a small percentage of these pathogens will infect humans (Cleaveland *et al.,* 2007). However, when spillover incidents do occur, they can be fatal as well as costly (Kurpiers *et al.,* 2016; Sokolow *et al.,* 2019). For instance, the United Nations Development Program (2015) estimates that the 2014–2015 Ebola outbreak cost West Africa $3.6 billion per year between 2014 and 2017 (Novelli *et al.,* 2018).

2.2 MICROORGANISMS ASSOCIATED WITH GAME MEAT

2.2.1 Viral pathogens associated with game meat

Viruses are obligate intracellular parasites distinguished mostly by the composition of their nucleic acids (DNA or RNA; single or double stranded, etc.) (Louten, 2016). They are the most abundant type of life on the planet; several viruses have been identified as key disease-causing agents, and they are subject to regular mutation and consequently evolution (Acter *et al.,* 2020). In terms of game-meat, it is now evident that several viral variants are prevalent in hunted nonhuman primate species, which have garnered the majority of scientific focus, and that these variants have crossed between nonhuman primates and humans multiple times (Cawthorn *et al.,* 2015). Bats and rodents are also significant zoonotic virus vectors (Zhou *et al.,* 2022).

Human immunodeficiency virus (HIV) is the most well-known virus to have emerged from the game-meat interface (Cunningham and Wood, 2017). While the origins of HIV have long been

unknown, human HIV-1 and HIV-2 are thought to have originated from strains of simian immunodeficiency virus (SIV) (Schmitt *et al.,* 2017). Evidence implies that SIV spread to humans through blood contact when hunters had an exposed open wound or were harmed while killing nonhuman primates (Devaux *et al.,* 2019). SIVcpz and SIVgor, from common chimps and western gorillas in west central Africa, are the closest to HIV-1 discovered among nonhuman primates, and at least four different spillovers have occurred (Locatelli and Peeters, 2012). HIV-2 is derivedfrom SIVsmm from sooty mangabeys (Cercocebus atys) in West Africa, where viral genetic diversity is abundant and transmission is thought to have occurred at least eight times (Kurpiers *etal.,* 2016).

2.2.2 Human t-cell Lymphotropic Virus (HTLV)

Human T-lymphotropic viruses (HTLV), like HIV, are connected to simian viral lineages with great variation (Peeters and Delaporte 2014). STLV/HTLV variations have been found in all three Sub-Saharan great apes and 30 more nonhuman primates, while a range of HTLV viruses have been found in animals and central African hunters (Kurpiers *et al.,* 2016, Schüpbach, 2016). Dual infections with more than one form have been recorded in nonhuman primates and in humans, similar to HIV/SIV (Peeters and Delaporte 2014; Lu *et al.,* 2020).

2.2.3 Simian Foamy Retroviruses (SFV)

Simian Foamy Retroviruses (SFV) are prevalent in most African primates (Peeters and Delaporte 2012) and have been shown to transmit to humans (Gessain *et al.,* 2013). SFV is genetically varied and host species-specific (Muniz *et al.,* 2015). Wolfe *et al.,* (2004b) discovered three geographically distinct SFV infections in Cameroon, which were linked to De Brazza's monkey (*Cercopithecus neglectus*), mandrill (*Mandrillus sphinx*), and western gorilla. Similarly, Mouinga-Ondémé *et al.,* (2012) identified human spillover episodes involving different strains of SFV in Gabon, with sick humans having been bitten by infected common chimps, western gorillas, or mandrills.

2.2.4 Ebola and Marburg Viruses

There are now seven species of filoviruses, five of which are found in Sub-Saharan Africa: Tai forest ebolavirus (TAFV), Sudan ebolavirus (SUDV), Zaire ebolavirus (EBOV), Bundibugyo virus (BDBV); Marburg virus (MARV) (Rougeron *et al.,* 2015). These pathogens are hemorrhagicfever-causing viruses that appear on a regular basis, usually as a result of a single spillover event

(Nakayama and Saijo, 2013). While the zoonotic source of this outbreak is unknown, three previous Ebola virus outbreaks in the Democratic Republic of the Congo between 1976 and 1979 involved victims who were reported to have handled western gorilla or common chimpanzee carcasses or had physical contact with people who touched the animals. Similarly, laboratory workers who dissected imported grivet were the first to be infected with Marburgvirus (Chlorocebus aethiops) (Weyer *et al.,* 2015).

Following a wave of common chimpanzee deaths in the Tai National Park in Côte d'Ivoire, a single case of TAFV was discovered in an ethnologist who was presumably infected while doing a necropsy on a dead common chimpanzee (Cannas, 2015). Other incidental hosts in the wild, such as duikers (Cephalophus spp.) have been shown to be possible (Leendertz *et al.,* 2015). Both dogs (naturally) and pigs (at least experimentally) can be infected (Pickering *et al.,* 2016). It was discovered that over 30% seroprevalence in dogs residing in villages with EBOV human and animal cases during the EBOV outbreak in Gabon in 2001–2002 (EFSA, 2015; Kumar *et al.,* 2017). Those canines appeared to be unaffected, and it was assumed that they had been exposed by scavenging wild animals (EFSA, 2015; Kumar *et al.,* 2017).

Despite the fact that accidental hosts play an essential role in the ecology of these viruses, particularly when moribund or dead animals are consumed, strong evidence suggests that bats are the natural reservoir hosts for at least Marburgvirus and EBOV (Kurpiers *et al.,* 2016; Malmlov, 2018). The Egyptian rousette fruit bat (Rousettus aegyptiacus), which lives in caves and is densely packed, is now well-documented as a Marburgvirus reservoir host (Ruiz-Aravena *et al.* 2022).

2.2.5 Henipaviruses and other Paramyxoviruses

Hendra virus and Nipah virus (HNVs) are paramyxoviruses in the genus Henipavirus that arose in Australia and southeast Asia, respectively, with fruitbats in the genus Pteropus (family Pteropodidae) serving as reservoir hosts (Kurpiers *et al.,* 2016). However, Henipavirus and Henipa-like viruses have been detected in Sub-Saharan African fruit bats, which are a phylogenetically separate group of pteropodid bats that do not overlap in distribution with any Pteropus species (Kurpiers *et al.,* 2016). The presence of Henipavirus and associated RNA and anti-Henipavirus antibodies in the African straw-colored fruit bat (Eidolon helvum) is apparent (Baker *et al.,* 2013; Olufemi *et al.,* 2015). This bat species is a popular hunting target and a substantial protein source in areas where it is found (Weiss *et al.,* 2012; Ameh *et al.,* 2021)

discovered these group of viruses in live bats found in bushmeat markets. Antibodies against HNVs in human samples from Cameroon, providing strong evidence of spillover was also discovered (Watkinson and Lee, 2016). These seropositive human samples were virtually entirely detected in people who reported butchering these bats (Watkinson and Lee, 2016). This bat is also a long-distance migrant with extensive panmixia across the continent, which may aid in virus transmission between bats (Peel *et al.,* 2017).

2.2.6 Rabies and other Lyssaviruses

Rabies is the oldest zoonotic EID identified. Each year, an estimated 25,000 humans die in Africa from rabies (Adesina *et al.,* 2020), some of whom may have been exposed to bushmeat-related activities, though the majority of human cases can be ascribed to domestic dogs. The Lyssavirus genus includes the Rabies virus (RABV) (Brunker and Mollentze, 2018). In Africa, it is joined by at least five other species: the Lagos bat virus (LBV), the Mokola virus (MOKV), the Duvenhage virus (DUVV), the Shimoni bat virus (SHIBV), and the newly suggested Ikoma lyssavirus (IKOV) (Nokireki *et al.,* 2018). With two exceptions, these viruses have bats as reservoir hosts (Markotter *et al.,* 2020). Shrews (*Crocidura* spp.), rusty-bellied brush-furred rats (*Lophuromys sikapusi*), and companion animals have been discovered to carry the Mokola virus (Kurpiers *et al.,* 2016; Banyard *et al.,* 2020).

The Ikoma virus has only been found in African civets so far *(Civettictis civetta)* (Wicker *et al*., 2017). Rabies has been observed in a number of non-human primate species, particularly those encountered in the bushmeat trade, and a variety of animal species can be secondary hosts of the disease (Kurpiers *et al.,* 2016; Devaux *et al.,* 2019). Lyssaviruses are distributed all across the world, although Africa has the most genetic variety, and the Lagos bat virus may be more than one species (Coertse *et al.,* 2020). While the majority of human cases are caused by the rabies virus, human fatalities have been linked to the Duvenhage virus, which was likely spread by bat scratches (Gossner *et al.,* 2020). The Mokolo virus has been found in two human cases, neither of which has resulted in death (Kurpiers *et al.,* 2016).

2.2.7 Lassa and other Arenaviruses

Arenaviruses are a group of zoonotic viruses that are spread from rodents to humans (Ogola *et al.*, 2021). The Lassa virus is the most well-known of the viral hemorrhagic arenaviruses in Africa, with outbreaks in Guinea, Sierra Leone, Nigeria, and Liberia (Olayiwola and Bakarey, 2017). The

primary risk arises from peridomestic exposure to the rodent host, the natal mastomys (*Mastomys natalensis*), via urine or fecal contents (Kurpiers *et al.,* 2016).

2.2.8 Human Monkeypox Virus

Despite its name, rodents, not monkeys or humans, are the reservoir hosts of the human monkeypox virus (MPX) (Alakunle *et al.,* 2020). Human monkeypox initially appeared in the Democratic Republic of the Congo in 1970, with epidemics in Liberia, Sierra Leone, Côte d'Ivoire, Nigeria, and the Democratic Republic of the Congo (Durski *et al.,* 2018). The discontinuance of the human smallpox vaccine, which imparted some immunity to other pox viruses, has been blamed for recent MPX rises in the Democratic Republic of the Congo and countries (Hoff, 2014; WHO, 2022).

Infected species include squirrels (e.g., Thomas's rope squirrel, *Funisciurus anerythrus*; African ground squirrels; *Xerus sp.*), dormice (*Graphiurus sp.*), and gigantic pouched rats (*Cricetomys sp.*) (Kurpiers *et al.,* 2016). Human monkeypox was connected to rope squirrels, dormice, and pouched rats in the 2007 outbreak in the United States, which was caused by exposure to rodents in the illicit pet trade (Kurpiers *et al.,* 2016). While dormice are small and unlikely to be targeted, diurnal and highly conspicuous squirrels and gigantic pouched rats are regularly hunted, making a human spillover quite possible (Milton *et al.,* 2020).

2.2.9 Prions

Only 5% of prion disorders are acquired (as opposed to hereditary), but they include the well-publicized scrapie, bovine spongiform encephalopathy (BSE, or "mad cow disease"), and chronic wasting disease (CWD) outbreaks in ungulates from Europe and North America (Kurpiers *et al.,* 2016). Only BSE has been found in humans and captive primates (Comoy *et al.,* 2015), most likely as a result of eating tainted meat. Although the scientists identified no reports of infectious prion disorders in Africa, this understudied pathogen type could very well be prevalent on the world's second biggest continent (Blackburn *et al.,* 2019). Prions may be present in nearly all tissues and are resistant to breakdown, even by boiling, making them a possible disease to be concerned about when it comes to bushmeat-related behaviors (Kurpiers *et al.,* 2016).

2.2.10 Helminths

Many parasites with zoonotic potential are found in helminths or "worm-like" animals, though it was found that helminths are less likely to cause EIDs (Feirrera *et al.,* 2021). Humans who engage in bushmeat-related activities are likely to be exposed to these infections by fecal material in which eggs are shed, transcutaneous exposure to infected larvae, or undercooked meat eating (Kurpiers *et al.,* 2016). Several studies have looked at the frequency of helminths in bushmeat market animals and discovered high rates of various species (Messenger *et al.,* 2014; Vliet *et al.,* 2017; White *et al.,* 2019; Philavong *et al.,* 2020). Adejinmi and Emikpe (2011), for example, collected feces samples from greater cane rats (Thryonomys swinderianus) and bush duikers (Sylvicapra grimmia) in Nigerian bushmeat markets and found high prevalence rates of helminth ova in feces as well as larvae from fecal cultures (83.3 percent and 53.8 percent, respectively) (Kurpiers *et al.,* 2016).

Similarly, Magwedere *et al.,* 2012 and Mukaratirwa *et al.,* 2013 examined the evidence for Trichinella infection in humans, livestock, and wildlife in Sub-Saharan Africa, noting that bush-pigs (*Potamochoerus spp.)* and desert warthogs (*Phacochoerus aethiopicus*) are sources of human infection. Humans and nonhuman primates are both susceptible to various parasitic helminth species, as is the case with many other infections (Medkour *et al.,* 2020). Pourrut *et al.,* 2011 collected gastrointestinal parasites from 78 wild monkeys of nine species from Cameroon's bushmeat markets and found high helminth burdens, including those that are known to infect people. Gillespie *et al.,* 2010 discovered comparable results in ordinary chimp feces.

2.2.11 Protozoan

Protozoans, which include amoebas and giardia, are a paraphyletic group of eukaryotic organisms that are neither animals, plants, nor fungi (Burki *et al.,* 2020). The risk of protozoan spillover from bushmeat-related activities is similar to that of helminths and bacteria in that disease can be transmitted to a willing human host through feces, body fluids, and even meat (Kurpiers *et al.,* 2016). A variety of protozoans are significant pathogens with the potential to spread zoonotic disease (Rahman *et al.,* 2020). The amoebozoa, which induce diarrhea and have been found in a number of mammals, including bushmeat species like nonhuman monkeys, are perhaps the finest example (Cameira *et al.,* 2018).

In common chimpanzees, Gillespie *et al.,* 2010 found the amoeba *Entamoeba histolytica* and the ciliated protozoan *Balantidium coli,* both of which are human diseases (although the direction of

spillover is uncertain, as common chimpanzees and other primates may have obtained this parasite from humans). Both protozoans were found in common chimps, western gorillas, agile mangabeys, and humans living in the same region of the Central African Republic (Pafčo *et al.,* 2018). A number of other nonhuman primates have also been infected with *E. histolytica. Toxoplasma gondii,* which causes toxoplasmosis but was not found during a recent, albeit small-scale, investigation of bushmeat and water/foodborne parasites like *Giardia,* is another protozoan example (Kurpiers *et al.,* 2016). Recent research has shown Giardia in a range of bushmeat species, including the western gorilla and the African buffalo (*Syncerus caffer*) (Vliet *et al.,* 2017; Odeniran *et al.,* 2018; Recht *et al.,* 2020; Dibakou *et al.,* 2021).

2.2.12 Fungi

Fungus are rapidly being recognized as major infections that may emerge, even in humans (Chowdhary *et al.,* 2017; Fisher *et al.,* 2018), and a variety of fungi are regarded therapeutically relevant (Gräser *et al.,* 2008). Fungal infections are especially problematic for people who are immunocompromised (e.g., due to HIV infection), as their immune systems are unable to combat the infection effectively (Perfect, 2012; Limper *et al.,* 2017). Nonetheless, there has been no examples of EIDs in Africa caused by fungal pathogens unrelated to human immunosuppression, as even the 1950s outbreak of cryptococcal meningitis in the Democratic Republic of the Congo was likely linked to HIV co-infection (Rycker *et al.,* 2018).

2.2.13 Bacteria

According to Tabish, 2009, bacteria cause 54.3 percent of EID incidents, and there is excellent evidence to show that bacterial pathogens may be as important as viruses when it comes to those that may spread owing to bushmeat-related activities (Dell, 2020). Bacterial pathogens can be transmitted directly through body fluids or excrement, but they can also be transmitted indirectly through contact with disease vectors such as fleas and ticks when handling animals (Overgaauw *et al.,* 2020). Bachand *et al.,* 2012 tested muscle from 128 bushmeat carcasses from diverse species at markets in Gabon for the presence of *Campylobacter, Salmonella,* and *Shigella* in a rare investigation of bacterial infections that might spread via bushmeat-related activities.

While they only found *Salmonella*, the risk of contamination and consequently enteric pathogen overflow from carcass handling remains significant, particularly in the days after purchase when infections are still replicating (Kurpiers *et al.,* 2016). Bacteria in the genus *Leptospira* are

indigenous to Sub-Saharan Africa and are lost in urine, posing a high risk of spillover during bushmeat-related activities (Dell *et al.,* 2020). Jobbins and Alexander, 2015 found *Leptospira* in a wide range of wild mammals, birds, and reptiles, indicating that wildlife (bats, mongoose, velvet monkey, barn owl) which may be consumed as bushmeat may play a role in leptospirosis transmission. The bushmeat interface may also play a role in human anthrax cases caused by *Bacillus anthracis,* a disease mostly affecting grazing herbivorous mammals but also affecting common chimps (Alexander *et al.,* 2018). The risk of human epidemics increases if bushmeat includes not only killing apparently healthy animals but also sick animals or salvaging contaminated carcasses (Omoleke *et al.,* 2016).

A variety of bacterial infections are vector-borne, making them unlikely to spread through bushmeat-related activities on the surface (Pauly, 2015). However, animal handling could represent a risk, especially for bacteria that are spread by fleas or ticks, rather than mosquitoes (El-Sayed *et al.,* 2020). The plague, caused by the bacteria *Yersinia pestis* and spread by infected rodent fleas, is the most terrifying of the vector-borne bacterial infections (Bramanti *et al.,* 2016). Rickettsial pathogens, such as Rickettsia africae, which causes African tick-bite fever (ATBF), are transmitted by fleas and ticks (Mazhetese *et al*., 2021). Ticks from duikers and a pangolin living in close proximity to people were collected by Mediannikov *et al.,* 2012 in Guinea, and *R. africae* was discovered in 10% of ticks obtained from the tree pangolin (*Manis tricuspis*), indicating the possibility of spillover with the close handling of these animals.

2.3 Staphylococcus aureus

2.3.1 Background history

Staphylococci have existed from the beginning of time, although they were only discovered as a bacterial disease in the nineteenth century (Zaffiri *et al.,* 2012). Alexander Ogston identified grape-like clusters of bacteria in pus from a surgical abscess in a knee joint in 1880 and named them *Staphylococcus* (Greek staphyle, "a bunch of grapes; kokkos, "grain or berry") (Khan, 2017). Friedrich Julius Rosenbach, a German physician, was able to cultivate the organisms in pure culture in 1884 and classify them based on the color produced (Mottola, 2017). *Staphylococcus aureus* is an opportunistic bacterium that can colonize up to 30% of the human population and causes a wide range of diseases in people and animals (Haag *et al.,* 2019).

Staphylococcus aureus has been linked to foodborne disease since 1884, when spherical organisms in cheese caused a significant food poisoning outbreak in the United States (Hennekinne, 2018). Other prior outbreaks linked to staphylococcal contamination of foods occurred in France in 1894, Michigan in 1907, and the Philippines in 1914 (Argaw and Addis, 2015). Dr. Gail Dack and his colleagues at the University of Chicago demonstrated in 1930 that the cause of food poisoning caused by eating contaminated sponge cake with cream filling was a toxin produced by isolated *Staphylococci* (Bennett and Monday, 2003).

2.3.2 Characteristics of *staphylococcus aureus*

Staphylococcus aureus is a Gram-positive (purple by Gram stain) bacteria that is cocci-shaped and organized in clusters that are described as "grape-like" (Taylor and Unakal, 2021). These organisms may grow in medium containing up to 10% salt, and colonies are frequently golden or yellow (aureus means golden or yellow) (Habib *et al.,* 2015). These organisms can develop aerobically or anaerobically (facultatively), and at temperatures ranging from 18 to 40 degrees Celsius (Hussien *et al.,* 2020). *S. aureus* are catalase positive (all pathogenic *Staphylococcus* species), coagulase positive (to distinguish *Staphylococcus aureus* from other Staphylococcus species), novobiocin sensitive (to distinguish from *Staphylococcus saprophyticus*), and mannitol fermentation positive are typical biochemical identification tests (Adetutu *et al*., 2017). Scanning electron microscopy reveals cells that are about spherical in shape and have a smooth surface, the cells' diameters range from 0.5 to 1.0 M (Li *et al.,* 2016). Cells with robust cell walls, distinctive cytoplasmic membranes, and amorphous cytoplasm are visible under transmission electron microscopy (Rohde, 2019).

Staphylococcus aureus requires thiamine, nicotic acid, inorganic salts, and amino acids as nitrogen sources (Omotani *et al.,* 2017). It requires B vitamins (nicotic acid, thiamine), amino acids, and inorganic salts for growth, while cysteine, valine, glutamic acid, agrinine, and tyrosine do not aid in growth but are a key source of enterotoxin synthesis (Chen, 2018; Kehiller, 2019). *Staphylococcus aureus* has an extraordinarily long lifespan, it develops in a wide temperature range because it is Mesophilic; 7- 47.8°C (optional 35°C); pH: 4.5- 9.3 (opt. 7.0-7.5). Low levels of water activity (aw) of 0.83 (optional>0.99) (Landgraf and Destro, 2013; Nunes and Caldas, 2017; Lu *et al.,* 2020). *Staphylococcus aureus* can exist in a latent state for years if growth

conditions (such as temperature or nutrition availability) are not favorable (essentially, being inactive and lying in wait for a good time to begin growing (Michailova *et al.,* 2007).

Exoproteins produced by *S. aureus* contribute to the bacteria's capacity to colonize and cause illness in mammalian hosts (Kong *et al.,* 2016; Rudra and Boyd, 2020). A set of enzymes and cytotoxins secreted by nearly all strains comprises four hemolysins (alpha, beta, gamma, and delta), nucleases, proteases, lipases, hyaluronidase, and collagenase (Hassan *et al.,* 2012; Tam and Torres, 2019). These proteins' primary role could be to transform local host tissues into nutrients needed for bacterial growth Ciborowski and Jeljaszewicz, 2018). Toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxins (SEA, (SEB, SECn, SED, SEE, SEG, SEH, and SEI), exfoliative toxins (ETA and ETB), and leukocidin are among the extra exoproteins produced by some strains (Bernando *et al.,* 2002).

S. aureus is found in the environment as well as in normal human flora, and is found on the skin and mucous membranes (most commonly the nasal area) of most healthy people (Taylor and Unakal, 2021). *S. aureus* does not generally cause illness on healthy skin; but, if these bacteria reach the circulation or internal tissues, they can cause a number of potentially dangerous infections (Hussain *et al.,* 2018). Direct contact is generally used for transmission of staphylococcal infections (Tenover and Gorwitz, 2006). However, certain illnesses are spread through different means (Taylor and Unakal, 2021).

S. aureus food colonization has long been linked to a kind of gastroenteritis characterized clinically by emesis with or without diarrhea (Castro *et al.,* 2016). This illness is known as staphylococcal food poisoning (SFP) and is caused by the consumption of one or more preformed staphylococcal enterotoxins (SEs) on food contaminated with *S. aureus* (Fetsch and Johler, 2018). Systemic toxicity symptoms such as fever and hypotension are uncommon in SFP instances (Pereira *et al.,* 2021). Furthermore, SFP is a self-limiting illness that usually cures within 24 to 48 hours of start (Argaw and Addis, 2015). The prevalence of SFP is unknown, however it is most likely the most common cause of food poisoning in the United States (Sergelidis and Angelidis, 2017).

It is unknown whether humans develop long-term immunity to SFP (Argaw and Addis, 2015). Antibodies to a single SE, however, would not necessarily provide immunity to SFP because several SEs are capable of causing illness (Reddy *et al.,* 2017). Antibodies generated against one

SE may give cross-protection against another SE in some cases, because these two SEs share antibody binding epitopes, heterologous antibodies to SEB may provide cross-protection against staphylococcal enterotoxin C (SEC) (Augustyniak *et al.,* 2017).

2.3.3 Epidemiology of *Staphylococcus aureus*

Staphylococcus aureus including drug-resistant strains such as Methicillin-resistant *Staphylococcus aureus* (MRSA) is present on the skin and mucous membranes, and humans are the primary reservoir for these organisms (Taylor and Unakal, 2021). It is estimated that between 20 and 80% of people have *Staphylococcus aureus* in their anterior nares (Brown *et al.,* 2014). Some populations, including as health care professionals, those who use needles on a frequent basis (such as diabetics and IV drug users), hospitalized patients, and immunocompromised people, have higher rates *of S. aureus* colonization (Tenover and Gorwitz, 2006). *S. aureus* can be passed from person to person via direct touch or via fomites (Xiao *et al.,* 2019).

2.3.4 Pathogenesis of *Staphylococcus aureus*

S. aureus is the causative agent of many human infections, including bacteremia, infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema) and gastroenteritis (David and Daum, 2017). These bacteria can induce invasive infections and/or toxin-mediated illnesses depending on the strains involved and the site of infection (Taylor and Unakal, 2021). The pathophysiology of *S. aureus* infection differs widely (Taponen and Pyörälä, 2009).

Antiphagocytic capsule synthesis, sequestering of host antibodies or antigen masking by Protein A, biofilm formation, intracellular survival, and preventing leukocyte chemotaxis are all mechanisms for evading the host immune response (Taylor and Unakal, 2021). In infectious endocarditis, bacterial cell wall-associated proteins such as fibrinogen-binding proteins, clumping factors, and teichoic acids mediate bacterial attachment to extracellular matrix proteins and fibronectin (Heilmannm, 2011). Infectious endocarditis, sepsis, and toxic shock syndrome all have Staphylococcal superantigens (TSST-1 or toxic shock syndrome toxin 1) as key virulence factors (Kulhankova *et al.,* 2014).

Pneumonia infections are linked to the development of PVL (Panton-Valentine leukocidin), Protein A, and alpha-hemolysin by bacteria, and they're more likely after an influenza virus infection or a Cystic Fibrosis diagnosis (Radke, 2021). Infections of prosthetic devices are frequently mediated by *S. aureus* strains' ability to build biofilms and communicate utilizing quorum sensing in a bacterial cell density-dependent way (Taylor and Unakal, 2021).

2.3.5 Treatment of *Staphylococcus aureus* infections

S. aureus infections are treated differently depending on the type of infection and whether or not drug-resistant strains are present (Jones, 2008). When antimicrobial therapy is required, the length and style of treatment are largely determined by the type of infection as well as other factors (Spauldiing *et al.,* 2018). If the isolates are sensitive (MSSA, or methicillin sensitive *S. aureus* strains), penicillin is the drug of choice, and vancomycin is the drug of choice for MRSA strains (Dibah *et al.,* 2014). Alternative therapy may be required in addition to antimicrobial therapy in some circumstances. For toxin-mediated sickness, for example, fluid replacement is frequently required, as is the removal of foreign devices for prosthetic value endocarditis or catheter-associated infections (Taylor and Unakal, 2021). MRSA infections are becoming a significant disease in both hospital and community settings since many MRSA strains are resistant to various antibiotics (Lee *et al.,* 2018).

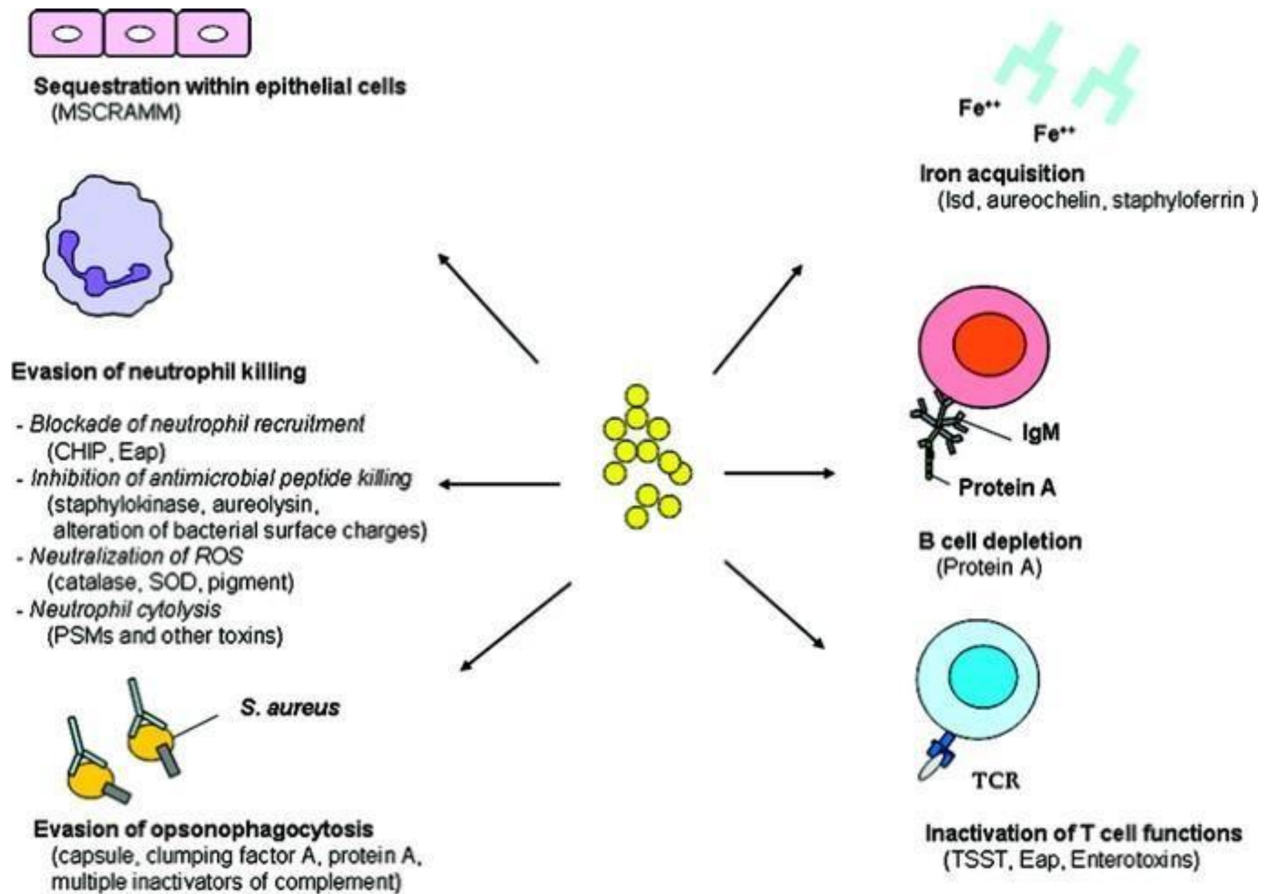

Figure 2.1: Survival strategies of *S.aureus* during infection (Liu, 2009)

2.3.6 ANTIMICROBIAL RESISTANCE OF *Staphylococcus aureus*

Beta-lactamase, a serine protease that hydrolyzes the beta-lactam ring, deactivates penicillin. All penicillinase-resistant penicillins and cephalosporins are resistant to methicillin resistance (Altshuler *et al.,* 2019). The presence of the mec gene, which encodes penicillin-binding protein 2a, is required for resistance (Jousselin *et al.,* 2015). Although many methicillin-resistant strains appear to be descended from a small number of clones, others appear to be multiclonal in origin, implying horizontal mec DNA transfer (Raphael *et al.,* 2017). Other staphylococcal genes, such as bla (for -lactamase) and fem (for factors required for methicillin resistance), influence resistance expression (Lee *et al.,* 2018). Resistance to methicillin is frequently diverse, with the fraction of a bacterial population expressing the resistance phenotype varying according to environmental factors (Brauner *et al.,* 2016).

There is growing concern regarding the advent of vancomycin-resistant *S. aureus* strains (Yousefi *et al.,* 2017). Vancomycin resistance has been identified in clinical isolates of *S. haemolyticus*, a coagulase-negative species (Akinkunmi and Lamikanra, 2010; Al-Tamimi *et al.,* 2020). The vancomycin resistance gene from an enterococcal plasmid has been transmitted to *S. aureus* in vitro by conjugation (Haaber *et al.,* 2017). Four case reports (one from Japan and three from the United States) describe the isolation of clinical strains with intermediate vancomycin sensitivity (minimum inhibitory concentration, 8 g per milliliter) (Sieradzki *et al.,* 2003).

2.4 Bacillus cereus

2.4.1 Background history of *Bacillus cereus*

B. cereus was first isolated from air in a cow shed and was described as a highly motile bacterium that appeared as single cells most of the time but occasionally formed longer threads and caused rapid gelatin liquefaction (Ehling-Schulz *et al.,* 2019). *B. cereus* was identified as a prevalent food contamination responsible for two forms of poisonings in the second part of the twentieth century (Tewari and Abdullah, 2015). *B. cereus* has recently been identified as a causative agent of both localized wound and ocular infections as well as systemic illnesses (Jovanovic *et al.,* 2021).

Hauge demonstrated in the 1950s that consuming *B. cereus*-contaminated vanilla sauce might cause diarrhea (Hariram, 2015). Since Hauges' groundbreaking discovery, two protein toxin complexes (Nhe and Hbl) as well as a single-protein toxin (CytK) have been identified, all of which are thought to play a role in diarrheal food poisoning (Messelhäußer and Ehling-Schulz, 2018). Exoproteins having enzymatic activity, such as proteases or phospholipases, have lately been proposed as potential virulence factors in the diarrheal illness (Tuipulotu *et al.,* 2021).

2.4.2 Characteristics of *Bacillus cereus*

Bacillus cereus is a gram-positive rod that is known for producing spores (McDowell *et al.,* 2021). An endospore and a vegetative cell are the two morphological forms of the organism (Wang *et al.,* 2015). The organism has a minimum growth temperature range of 10-12ºC, a maximum range of 48-50ºC, and an ideal growth temperature range of 28-35ºC (Marc *et al.,* 2021). The organism's generation time is between 18 and 27 minutes (Soleimani *et al.,* 2018). Growth has been observed throughout a pH range of 4.9 to 9.3, and the organism can withstand salt concentrations of up to 75% (Silva, 2016). With a germination frequency of up to 100%, the organism has a heat resistance

similar to that of other mesophiles (Luo *et al.,* 2021). The sporangium does not inflate, which distinguishes the spore.

The endospore is resistant to a variety of environmental and unfavorable circumstances, and it does not sporulate when exposed to air (Egan *et al.,* 2021). *Bacillus cereus* vegetative cells are facultative aerobic rods with a width of 1.0-1.2 micrometers and a length of 3.0-5.0 micrometers (Baek *et al.,* 2019). Long chains of rods tend to form. Although non-motile strains have been reported, motile *B.cereus* has peritrichous flagellae (Gordons, 2019).

2.4.3 Epidemiology of *Bacillus cereus*

Thousands of food poisoning incidents go unreported to health agencies in charge of collecting epidemiological data in order to track cases and outbreaks of foodborne illnesses (Mohammad *et al*., 2018). In order to create epidemiological profiles, thorough documentation of specific epidemics is required. Despite adequate documentation, *B. cereus* outbreaks continue to occur (Griffiths and Schraft, 2017).

In the United States from 1993 to 1997, bacterial pathogens were responsible for the highest percentage of outbreaks (75%) and cases (86%) among outbreaks with an etiology (Dewey-Mattia *et al.,* 2018). Food contamination by *B. cereus* was blamed for 14 (0.5%) of the outbreaks that occurred during this time period, resulting in 691 (0.8%) cases.

2.4.4 *Bacillus cereus* Toxins

Exotoxins from *B. cereus* have been linked to its pathogenicity, although the true contribution and significance of these toxins in GI and non-GI illnesses remains unknown (Ehling-Schulz *et al.,* 2019). Four *B. cereus* exotoxins have been connected to food poisoning thus far (Jessberger *et al.,* 2019). After toxicoinfections with *B. cereus* spore-contaminated food, three pore-forming enterotoxins (Nhe, a three-component nonhemolytic enterotoxin, Hbl, a three-component hemolytic enterotoxin, and the single protein cytotoxin CytK) are thought to cause diarrhea (Bhunia, 2018; Ehling-Schulz *et al.,* 2019). In vivo proof of Hbl enterotoxic activity on rabbit intestine has been proven, but there is no direct in vivo proof of Nhe or CytK activity (Dietrich *et al.,* 2021).

Nhe was discovered in a *B. cereus* strain responsible for a serious foodborne outbreak in Norway (1988), while CytK was discovered in a *B. cereus* strain responsible for a severe foodborne

outbreak in which several patients suffered bloody diarrhea and three elderly people died (Dietrich *et al.,* 2021). Enterotoxins can work in concert with one another and other virulence variables, making it difficult to estimate a strain's overall enterotoxic potential (Osman *et al*., 2018). On Vero and Caco-2 cells, as well as other human cells, Nhe, Hbl, and CytK have cytotoxic action (Jeßberger *et al.,* 2015). Different cell lines are susceptible to diverse toxins in different ways, implying different modalities of toxin action (Jovanovic *et al.,* 2021).

Symptoms usually appear after 6 to 12 hours and are self-limiting (Ehling-Schulz *et al.,* 2019). However, in rare situations, the severity of symptoms necessitates hospitalization, and fatalities have been observed in neonates and immunocompromised people (Barrett and Fhogartaigh, 2017).

The fourth toxin, known as cereulide, has been related to emetic *B. cereus* food poisoning (Rouzeau-Szynalski *et al.,* 2020). Cereulide is a depsipeptide toxin that is structurally linked to the potassium ionophore valinomycin and is made up of alternating -amino and -hydroxy acids (D-O-Leu–D-Ala–L-O-Val– L-Val) (Defourny, 2011). The cereulide peptide synthetase Ces, a novel type of non-ribosomal peptide synthetase, is responsible for its production (Lücking *et al.,* 2015). Cereulide is usually generated in food, and symptoms usually appear 15 minutes to 6 hours after consuming contaminated food (Messelhäusser *et al.,* 2014)

2.4.5 *Bacillus cereus* and Gastrointestinal Infections

B. cereus pathogenicity is based on a slew of poorly understood virulence factors that contribute to a variety of human diseases (Waterfield and Wren, 2004). *B. cereus* can cause two forms of food poisoning, with diarrhea and emesis as the most common symptoms (Griffiths and Schraft, 2017). Different enterotoxins considered to be created following spore outgrowth, taken up with contaminated meals, and active in the small intestine have been associated to diarrheal illness (Jovanovic *et al.,* 2021). The emetic sickness is induced by intoxication from the single depsipeptide toxin cereulide, which is found in food (Vidic *et al.,* 2020). The genes generating the cereulide synthetase toxin are primarily found in a subset of *B. cereus*, but the genes encoding the enterotoxin are widely dispersed across *B. cereus* members (Dietrich *et al.,* 2021).

Cereulide intoxication has been linked to serious clinical manifestations such abrupt liver failure and acute encephalopathy (Schreiber *et al.,* 2021). Because of the cereulide toxin's strong biological activity, liver transplantation is sometimes required as a life-saving intervention

(Acheson, 2009). In general, *B. cereus* strains' pathogenic potential is highly variable, ranging from strains that demonstrate minimal cytotoxic activity in cell culture experiments to strains thatare extremely cytotoxic (Jeßberger *et al.,* 2015). Virulence is influenced by environmental factorsin addition to innate strain-related changes in pathogenic potential.

Aside from its potential for food poisoning, *B. cereus* is becoming more widely recognized as an opportunistic pathogen that can cause both local and systemic illnesses (Messelhäußer and Ehling-Schulz, 2018). Hospital-acquired *B. cereus* infections, including as infections of the central nervous system, endocarditis, respiratory and urinary tract infections, wound infections, and septicemia, are especially dangerous for immunocompromised persons and neonates (Sikora and Zahra, 2021). *B. cereus* has been related to eye infections such as endophthalmitis and keratitis outside of hospital-acquired illnesses (Sadaka *et al*., 2012). Furthermore, in ruminants, *B. cereus* can cause severe mammary gland infections (Savini, 2016).

2.4.6 Pathogenesis of *Bacillus cereus*

During the exponential development phase, most *Bacillus cereus* strains are capable of producing a wide spectrum of extracellular metabolites (Ehling-Schulz *et al.,* 2019). These metabolites comprise a variety of poisons, including 'virulence factors,' which have been established in animal models, tissue culture, and cell lines (Dietrich *et al.,* 2019). Toxins produced by *B. cereus* are thought to be major elements in its pathogenicity. Enterotoxin-mediated diarrheal food poisoning infections are thought to be caused by a variety of proteins with varying properties and molecular mass values (Berthold-Pluta *et al.,* 2015). Enterotoxins found in the culture filtrates of the enterotoxigenic strains of *B. cereus* have been linked to hemolytic, cytotoxic, and dermonecrotic activity, vascular permeability, and fluid buildup in ligated rabbit ileal loops (McDowell *et al.,* 2021).

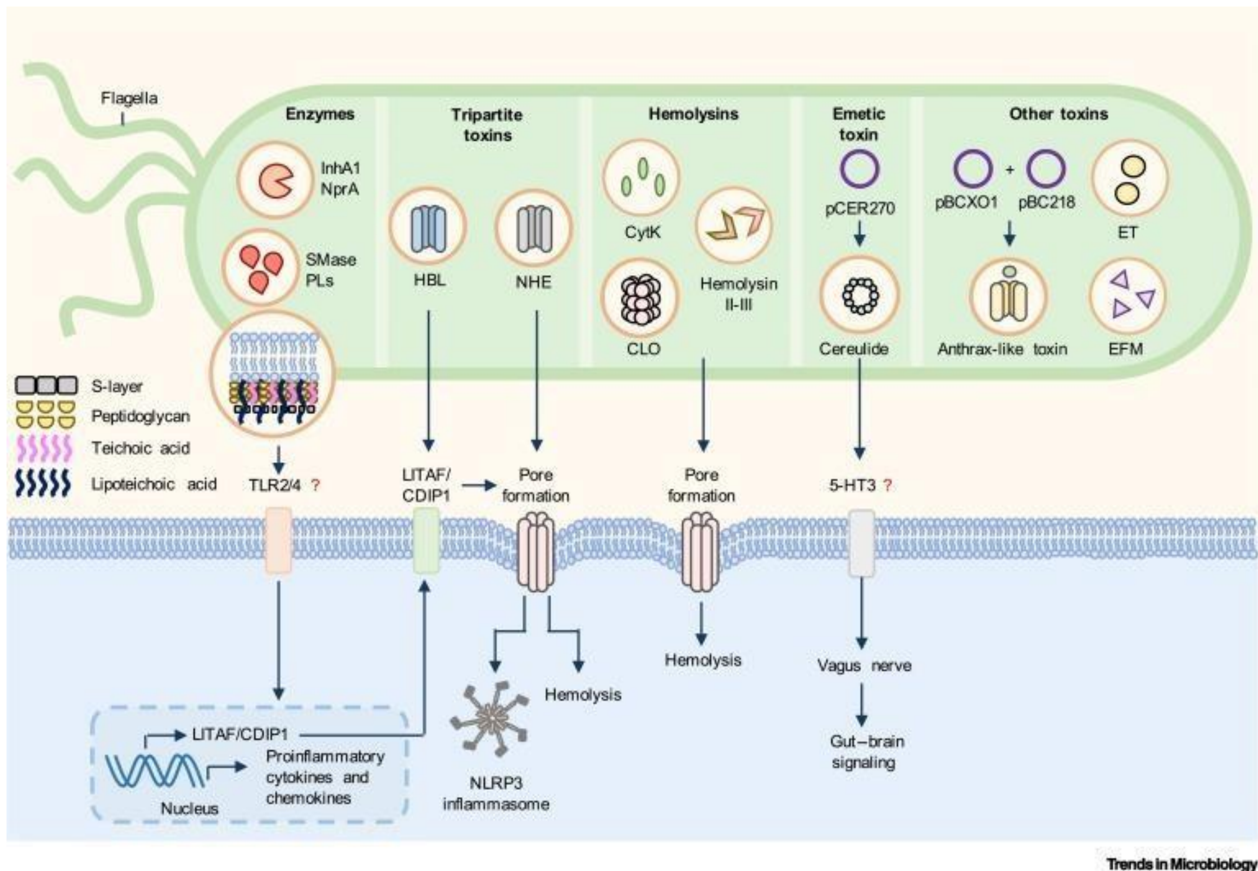

Figure 2. 2: Pathogenesis of *Bacillus cereus* (Tuipulotu *et al.,* 2020)

2.4.7 Treatment of *Bacillus cereus*

Antibiotic therapy is the first line of defense against *B. cereus* infections (Ehling-Schulz *et al.,* 2019). Antibiotic susceptibility profiles for *B. cereus* group species are comparable, and they are susceptible to the majority of first-line antibiotics (Kong *et al.,* 2021). Penicillin was traditionally the antibiotic of choice for the therapy (McDowell *et al.,* 2021). Clinical isolates, on the other hand, have been found to have intrinsic or inducible resistance to Beta-lactam antibiotics. Because *B. cereus* infections are uncommon, generally curable with antibiotics, and, in the case of *B. cereus* food poisoning, self-limiting, vaccines for these infections have not been developed (Bhunia, 2018).

CHAPTER THREE

METHODOLOGY

3.1 SAMPLE COLLECTION

The sampling areas were Oyo, Osun, Ondo, Ogun and Lagos states. The southwestern part of Nigeria has been identified as some of the states with the highest numbers of game-meat consumers. Various game meat was purchased from various open markets (Table 3.1. The purchased samples were placed in sterile food-grade bags and kept on ice packs in cooling boxes and then transported to the laboratory where they were immediately analyzed.

3.2 APPARATUS AND EQUIPMENT

Apparatus used include: stomacher bags, wash bottles, petri-dishes, measuring cylinder, glass pipettes, beakers, conical flasks, glass spreader, inoculating loop, wash bottles, eppendorf tubes, micro pipette (with their tips), test tubes and foil corks (with their racks), PCR tubes, glass slides, oxidase test disc.

Equipment used: Analytical balance, Hot plate stirrer, Autoclave, Vortex mixer, Water distiller, Water bath (set at 80°C), Incubator (37°C), Bunsen burner, Centrifuge, Heating block, Thermal cycler, Gel electrophoresis tanks, Gel documentation system, Microscope.

3.3 MEDIA AND REAGENTS

The work area was sterilized by using 70% ethanol to inactivate any microbial contaminants.

For isolation of Bacillus species:

1% Buffered peptone water (BPW), 0.1% BPW, Nutrient Agar, Brain Hearth Infusion Broth (BHI), 20% Glycerol, and Distilled water.

For isolation of Staphylococcus species:

1% Buffered peptone water (BPW), Blood Agar, Nutrient Agar, Brain Hearth Infusion Broth (BHI), 20% Glycerol, Distilled water, 5% sheep blood.

For molecular identification:

Agarose, 1x TAE buffer, master mix, specific primers, Nuclease free water, Ethidium Bromide.

Table 3.1: Various game-meat sampling locations in Nigeria

LOCATION	GAME-MEAT	NUMBER OF SAMPLES
	Pangolin	
	Bird	
	Deer	
Lagos State	Bush dog	25
	Grasscutter	
	Etu	
	Wild Cat	
	Atika	
	Agbonrin	
	Antelope	
	Monkey	
	Rabbit	
	Porcupine	
	Antelope	
	Grasscutter	
Ogun State	Rabbit	
	Bush rat	12
	Igala	
	Hedgehog	
	Guinea fowl	
	Alligator	
	Civet Cat	
	Rabbit	
Ondo State	Antelope	9
	Grasscutter	
	Grasscutter	
	Guinea Fowl	
	Hare	

Osun State	Sese Antelope	5
Oyo State	Aparo Eta Esii Tuku Guinea Fowl	4
Total		55

For biochemical test:

Crystal Violet, Iodine, alcohol (95%), Safranin, 3% Hydrogen Peroxide.

3.4 PREPARATION OF CULTURE MEDIA

3.4.1 Buffered Peptone Water (BPW)

Peptone water is a microbiological growth medium made up of sodium chloride and peptic digest of animal tissue. The medium has a pH of 7.2 ± 0.2 at 25°C and is high in tryptophan. Buffered Peptone Water is a non-selective broth medium that can be used to grow bacteria as a primary enrichment media.

Preparation

- 10g of the dehydrated medium was dissolved in 1litre of distilled water in a conical flask and was mixed thoroughly. The conical flask is then closed with a foil cork.
- The mixture was then stirred for a while using the magnetic stirrer hot plate to dissolve the powder completely.
- 225ml of the 1% was then dispensed into conical flasks.
- The conical flasks containing the media was then autoclaved at 121°C for 15mins.

3.4.2 Blood Agar

Blood agar is a general purpose, enriched medium often used to grow fastidious organisms and to differentiate bacteria based on their hemolytic properties.

Preparation

- The dehydrated medium was dissolved in the appropriate volume of distilled water i.e. 38g of Mueller Hinton II agar in 1 litre of distilled water based on manufacturer's instructions in a conical flask and mixed thoroughly. The conical flask is then closed using a foil cork (made up of cotton wool wrapped in aluminium foil).

- The mixture was stirred for a while using the magnetic stirrer hot plate to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15mins.

- The medium was then allowed to cool and 5% sheep blood was added to itpoured aseptically into sterile petri dishes and left to solidify.

3.4.3 Nutrient Agar

Nutritional agar is a multipurpose nutrient media for the cultivation of microbes that can sustain the development of a variety of non-fastidious organisms. For the isolation and detection of total count of mesophilic organisms, it was prepared according to the manufacturer's instructions.

Preparation

- The dehydrated medium was dissolved in the appropriate volume of distilled water i.e. 28g of Nutrient agar in 1 litre of distilled water based on manufacturer's instructions in a conical flask and mixed thoroughly. The conical flask is then closed using a foil cork (made up of cotton wool wrapped in aluminium foil).

- The mixture was stirred for a while using the magnetic stirrer hot plate to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15mins.

- The medium was then allowed to cool and poured aseptically into sterile petri dishes and left to solidify.

Note: The medium appears opalescent and is light amber in colour.

3.4.4 Brain Heart Infusion Broth (BHI)

Brain Heart Infusion (BHI) broth is a general-purpose liquid medium for the cultivation and maintenance of a wide range of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria, yeast, and molds from a variety of clinical and non-clinical specimens.

Preparation

- 37 grams of the dehydrated medium was dissolved in 1000 ml of distilled water based on manufacturer's instructions in a conical flask and was mixed thoroughly. The conical flask is then closed using a foil cork (made up of cotton wool wrapped in aluminium foil).
- The mixture was stirred for a while using the magnetic stirrer to completely dissolve the powder.
- 5ml of the media was then dispensed into various test tubes and covered with foil cork and was then sterilized by autoclaving at 121^0C for 15minutes.

3.5 Isolation of ***Bacillus cereus*** and ***Staphylococcus aureus***

3.5.1 Primary Enrichment

Twenty-five grams of each game meat were placed in a sterile stomacher bag containing 225 milliliters of 1% buffered peptone water (enrichment broth) and homogenized for two minutes at 180 revolutions per minute using the stomacher. After homogenization, the material was transferred to conical flasks after which serial dilution was performed and appropriate dilutions were plated on Nutrient agar and Blood agar plates.

3.5.2 Serial Dilution (*Bacillus cereus*)

One milliliter (1 ml) of the samples was pipetted using the micro-pipette (set at 1000 µl) into test tubes containing 9 ml of BPW (0.1%) to obtain 10^{-1} followed by the transfer of 1 ml from 10^{-1}into a new test tube containing 9 ml of 0.1% BPW to create 10^{-2} dilution factor, the diluent is then vortexed. The test tubes were labelled for easy identification. The dilution factor $10^{-2}$was placed in a water bath at 80°C for 10 minutes (for Bacillus spp.)

3.5.3 Spread-Plating

After labelling the agar plates accordingly, 0.1 ml from the appropriate dilutions (10^0 for *Staphylococcus* spp. and $10^{-2}$for *Bacillus* spp.) was plated unto Blood agar and Nutrient agar for the isolation of *Staphylococcus aureus* and *Bacillus cereus*. The spread plate technique was used

to spread the diluents in the plate (the glass rod was dipped in absolute ethanol and then flamed using the Bunsen burner and allowed to cool before spreading in order to maintain aseptic conditions). The plates were incubated at 37ºC for 18-24 hours.

3.5.4 Sub-Culturing

Sub-culturing was done to purify the isolated bacterial colonies from a mixed culture to a new and single culture, the bacterial isolates sub-cultured were those which were differentiated on the basis of their colony morphology, shape, colour, elevation and other physical characteristics. The distinct colonies from the previously incubated Nutrient agar and Blood agar.

A loopful of the isolate was taken using the inoculating loop (the inoculating loop was heated using the Bunsen burner and allowed to cool before taking the loop from the original mixed culture and streaked onto the new petri dish) using the streaking method procedure, the plates were inverted and incubated 37ºC for 18-24 hours.

3.5.5 Cryopreservation of Isolates

A loopful of pure cultured presumptive *Bacillus spp.* and *Staphylococcus aureus* from the incubated nutrient agar was inoculated into 5 ml of BHI broth and incubated at 37ºC for 18-24 hours a sterile. After incubating, 750µl of the inoculum was added into a sterile eppendorf tube containing 750µl of sterile 20% sterile glycerol (duplicated) which serves as cryoprotectant and it was stored in a -4^0C freezer.

3.6 BIOCHEMICAL TEST

3.6.1 Grams Staining

The inoculating loop was sterilized with a bunsen burner flame, and then a pure culture was smeared on a sterile slide and heat fixed by passing it fast across the flame with the smear facing up. For staining, the slides were placed on the staining rack. The smear was covered in crystal violet stain and left for 1 minute before being carefully wiped off under running tap water. The smear was then flooded with Gram's iodine, which was allowed to sit for 1 minute before being drained off under a gentle running tap. The slide was then washed with a decolorizing chemical (70 percent alcohol) and let to stand for 10 seconds. After that, the slide was cleaned under running tap water, drained fully, and counterstained for 30 minutes with safranin. The slide was then blot

dried with filter paper after being washed under gently running tap water until no color appeared in the effluent (Olutiola *et al*., 2000).

3.6.2 Catalase Test

Using a sterilized inoculating loop, the pure culture was smeared on a sterile slide. The smear was then treated with a drop of hydrogen peroxide. The outcome was then examined. The existence of oxygen bubbles indicates that catalase is present, while the absence of bubbles indicates that catalase is absent. (Olutiola *et al*., 2000)

3.6.3 Oxidase Test

The pure culture was smeared on the filter paper, along with a few drops of the oxidase reagent, and the results were examined. Oxidase positive cultures produce a purple tint in less than 10 seconds. Oxidase negative cultures do not develop any purple colour. (Olutiola*et al*., 2000)

3.6.4 Coagulase Test

On a sterile slide, a loopful of natural saline was added and emulsified with a loopful of 24 hour cultures until a homogeneous suspension was formed. The suspension was then given a drop of human plasma and **swirled** for 5 seconds. Clumping that did not re-emulsify reveals a coagulase positive result. The absence of clumping indicates a coagulase negative result (Olutiola *et al*., 2000).

3.6.5 Triple Sugar Iron (TSI) Test

An agar slant of a medium with multiple sugars constituting a medium with multiple sugars with pH-sensitive dye (phenol red), 1% lactose, 1% sucrose, 0.1% glucose, as well as sodium thiosulphate and ferrous sulphate is used for carrying out this test. A straight inoculating loop was used to touch a pure colony and then inoculated into TSI agar by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant. The cap was covered loosely and then incubated at 35-37°C in ambient air for 18-24 hours.

3.6.6 Motility Test

This is used to determine whether an organism is motile or non-motile. The Sulphide Indole Motility (SIM) medium was used. . A straight inoculating loop was used to touch a pure colony and then stabbed once to a depth of only ½ inch in the middle of the tube and removed gently from

the medium. It was then incubated at 35-37°C and examined daily for 7 days for a diffuse zone of growth flaring out from the line of inoculation which indicates motility.

3.7 MOLECULAR IDENTIFICATION

3.7.1 Activation of Isolates

Isolates were taken out of the freezer and allowed to thaw at room temperature. 1ml of BHI was added to Eppendorf tubes and autoclaved. 100µl of *Bacillus and S.aureus* isolates were added to the Eppendorf tubes containing the BHI and incubated at 37◦C for 24 h bringing about the activation of the isolates.

3.7.2 DNA Extraction

The isolates were centrifuged for 5 minutes at 10,000RPM, then the supernatant was decanted. The Eppendorf tube was filled with 1ml of sterile distilled water, vortexed, and centrifuged at 10,000 RPM for 5 minutes, the supernatant was discarded, and the process was repeated. After which , 200 µl of sterile nuclease-free water was pipetted into the Eppendorf tube containing the pellets, vortexed, and then placed in the heating block to boil at 100◦C for 15 minutes, the solution was then placed in ice to cool, and the contents of the Eppendorf tube were centrifuged for 5 minutes at 14,000RPM.

A new set of Eppendorf tubes were labelled with the corresponding codes of the isolates and 150μl supernatant containing the extracted DNA was then transferred into the new Eppendorf tubes and stored in the freezer at -20ºC for further analysis.

3.7.3 Polymerase Chain Reaction (PCR)

The components of the PCR used for *Bacillus* spp. and *S.aureus* identification is shown in Table 3.2 below. The PCR cocktail was made and then transferred to a PCR tube before being placed in the thermocycler (Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany). The PCR was carried out with an initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 2 min; 42°C for 30 s and 72°C for 4 min; and a final elongation step at 72°C for 10 min (Table 3.2). Negative control reactions was included. For negative controls template DNA was replaced with sterile water. The PCR products were confirmed by electrophoresis and visualized under UV light with a Gel Documentation system.

Table 3.2 PCR reaction components used for 16s rRNA amplification

No.	Component	Initial concentration	Final concentration	Volume/rxn
1	Master mix	5x	1x	2ul
2	16sf	20um	0.25um	0.125ul
3	16sr	20um	0.25um	0.125ul
4	DNA			2ul
5	dH_2O			5.75ul
6	Total			10ul

Table 3.3: Protocol for thermal cycler

Analysis	Step	Temperature	Time
1x	Initial denaturation	95^0c	5 min
35x	Denaturation	95^0c	2 min
	Annealing	55^0c	30 sec
	Elongation	72^0c	4 min
1x	Final Elongation	72^0c	10 min

3.7.4 Agarose Gel Electrophoresis

Dry Agarose powder was used to make the agarose, and 1.8g of it was dissolved in 100ml of 1x TAE buffer.The mixture was heated until a clear solution was obtained. A micropipette was used to add 3 µl of ethidium bromide to the mixture. It was then spun, allowed to cool and then transferred to the gel tray which results in a slab with the combs in place, that create wells in the

slab. It was then allowed to solidify before carefully removing the combs. The gel tray containing the slab was placed into the gel tank and 1x TAE buffer was added to the gel tank. 3 µl of DNA ladder was added to the first well, and 4 µl of amplicon (one sample per well) was pipetted into each well that was produced. The tank was attached to the power pack and the gel was ran at 100 volts for 45 minutes. The resulting gel was inspected using the gel documentation system.

3.8 Precautions

- Personal protective equipment, such as a covered shoe, a nasal cover, gloves, and a lab coat, were observed.
- At every stage of the project, aseptic practices were observed.
- There was no cross-contamination of the samples.
- Ensured that the samples were appropriately labeled at all times.
- To avoid killing the organism of interest, the inoculating loop was allowed to cool before making contact with the organism for sub-culturing.

CHAPTER FOUR

RESULTS

4.1 Microbial count

In Lagos state monkey had the highest total viable count (TVC) which is 9.5×10^3 while Quail had the lowest TVC of 4.1×10^3 . In Ogun state antelope had the highest TVC which is 8.6×10^3 while Guinea fowl had the lowest TVC of 4.8×10^3, In Ondo state antelope had the highest TVC which is 8.8×10^3 while Guinea fowl had the lowest TVC of 4.4×10^3, In Osun state antelope had the highest TVC which is 8.6×10^3 while Hare had the lowest TVC of 4.8×10^3 In Oyo state Esii Tuku had the highest TVC which is 7.1×10^3 while Eta had the lowest TVC of 4.3×10^3.

Table 4.1: Results microbial count for *Bacillus cereus*

LOCATION	GAME-MEAT	NUMBER OF SAMPLES	TOTAL VIABLE COUNT (cfu/ml)
	Pangolin		8.6×10^3
	Quail		4.1×10^3
	Deer		8.1×10^3
Lagos State	Bush dog	25	6.4×10^3
	Grasscutter		8.5×10^3
	Etu		5.5×10^3
	Wild Cat		7.3×10^3
	Atika		6.3×10^3
	Agbonrin		4.5×10^3
	Antelope		8.7×10^3
	Monkey		9.5×10^3
	Rabbit		7.5×10^3
	Porcupine		8.3×10^3
	Antelope		8.6×10^3
	Grasscutter		8.4×10^3
Ogun State	Rabbit		7.8×10^3

	Bush rat	12	6.2 x 10^3
	Igala		6.7 x 10^3
	Hedgehog		5.2 x 10^3
	Guinea fowl		4.8 x 10^3
	Alligator		7.3 x 10^3
	Civet Cat		7.2 x 10^3
	Rabbit		7.4 x 10^3
Ondo State	Antelope	9	8.8 x 10^3
	Grasscutter		8.3 x 10^3
	Guinea Fowl		4.4 x 10^3
	Hare		4.8 x 10^3
Osun State	Sese	5	6.8 x 10^3
	Antelope		8.6 x 10^3
	Aparo		5.5 x 10^3
Oyo State	Eta	4	4.3 x 10^3
	Esii Tuku		7.1 x 10^3
	Guinea Fowl		5.0 x 10^3
Total		55	

In Lagos state monkey had the highest total viable count (TVC) which is 9.5 x 10^4 while Quail had the lowest TVC of 4.1 x $10^{4,}$ In Ogun state antelope had the highest TVC which is 8.6 x 10^4 while Guinea fowl had the lowest TVC of 4.8 x $10^{4,}$ In Ondo state antelope had the highest TVC which is 8.8 x 10^4 while Guinea fowl had the lowest TVC of 4.4 x $10^{4,}$ In Osun state antelope had the highest TVC which is 8.6 x 10^4 while Hare had the lowest TVC of 4.8 x $10^{4,}$ In Oyo state Esii Tuku had the highest TVC which is 7.1 x 10^4 while Eta had the lowest TVC of 4.3 x $10^{4.}$

Table 4.2: Microbial count for *Staphylococcus aureus*

LOCATION	GAME-MEAT	NUMBER OF SAMPLES	TOTAL VIABLE COUNT (cfu/ml)

	Pangolin		8.6×10^4
	Quail		4.1×10^4
	Deer		8.1×10^4
Lagos State	Bush dog	25	6.4×10^4
	Grasscutter		8.5×10^4
	Etu		5.5×10^4
	Wild Cat		7.3×10^4
	Atika		6.3×10^4
	Agbonrin		4.5×10^4
	Antelope		8.7×10^4
	Monkey		9.5×10^4
	Rabbit		7.5×10^4
	Porcupine		8.3×10^4
	Antelope		8.6×10^4
	Grasscutter		8.4×10^4
Ogun State	Rabbit		7.8×10^4
	Bush rat	12	6.2×10^4
	Igala		6.7×10^4
	Hedgehog		5.2×10^4
	Guinea fowl		4.8×10^4
	Alligator		7.3×10^4
	Civet Cat		7.2×10^4
	Rabbit		7.4×10^4
Ondo State	Antelope	9	8.8×10^4
	Grasscutter		8.3×10^4
	Guinea Fowl		4.4×10^4
	Hare		4.8×10^4
Osun State	Sese	5	6.8×10^4
	Antelope		8.6×10^4
	Aparo		5.5×10^4
Oyo State	Eta	4	4.3×10^4

	Esii Tuku	7.1×10^4
	Guinea Fowl	5.0×10^4
Total	55	

4.2 Biochemical Tests of *Bacillus* spp.

The results of the biochemical tests indicated that all of *Bacillus* isolates were Gram positive while some appeared positive and negative for oxidase and all were positive for motility test, negative result was also observed in all isolates for catalase and triple sugar iron tests shown in Table 4.1.

Table 4.3: The results of Biochemical test for presumptive identification of *Bacillus* spp.

Isolate codes	Catalase test	Gram staining	Oxidase	Motility	Tripple sugar	Probable organism
ML1B1	+	+	+	+	-	*Bacillus*
G1L1B1	+	+	+	+	-	*Bacillus*
ANS1B1	+	+	+	+	-	*Bacillus*
DL1B1	+	+	+	+	-	*Bacillus*
H1L1B1	+	+	+	+	-	*Bacillus*
GL1B1	+	+	+	+	-	*Bacillus*
SL1B2	+	+	+	+	-	*Bacillus*

4.3 Biochemical Tests of *Staphylococcus* spp.

The results of the biochemical tests indicated that all *Staphylococcus* isolates were Gram positive and also tested positive for oxidase and motility test and tested negative to catalase and triple sugar iron tests shown in Table 4.2.

Table 4.4: The results of Biochemical test for presumptive identification of *S. aureus*

Isolate codes	Catalase test	Gram staining	Oxidase	coagulase	Motility	Tripple sugar	Probable organism
G1L1SA1	+	+	-	+	-	+	*S. aureus*
P1L1SA1	+	+	-	+	-	+	*S. aureus*
HL1SA1	+	+	-	+	-	+	*S. aureus*
GRO1SA1	+	+	-	+	-	+	*S. aureus*
IKSSA1	+	+	-	+	-	+	*S. aureus*
MWSA1	+	+	-	+	-	+	*S. aureus*
APISA1	+	+	-	+	-	+	*S. aureus*

4.4 PCR Amplification result:

The representative visualized result of gel electrophoresis for the detection of 16 rRNA gene from isolates obtained from game-meat using the PCR technique (Figure 4.1). *Bacillus* (n=30) and *S.aureus* (n=30) isolates were randomly selected from the 110 isolates each for the gene confirmation. The gene was then confirmed in 63.3% and 73.3% of *Bacillus* and *S.aureus* isolates, respectively which indicates that

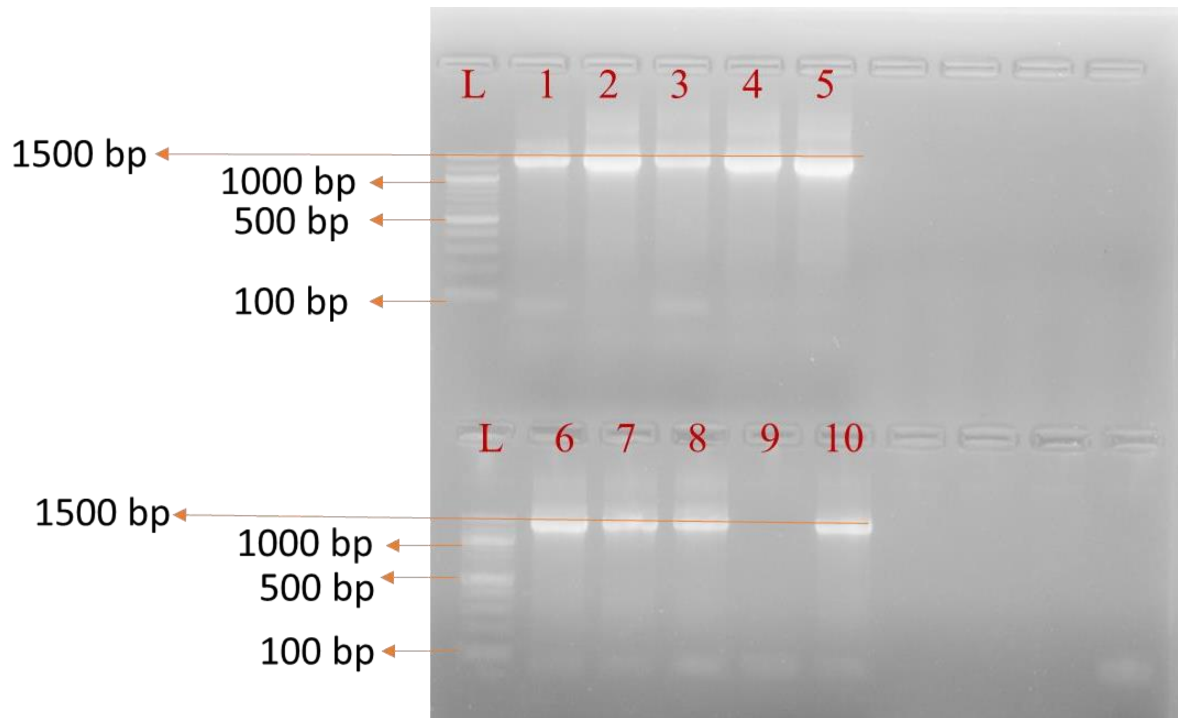

Figure 4. 1: Agarose gel electrophoresis image of PCR amplified products of 16 rRNAstrains. Lane L: molecular weight marker 100 bp (Bio-Mark). Lane 1-5: *Bacillus* spp. strain isolated from game meat. Lanes 6-10: *S. aureus* strain isolated from game meat samples.

CHAPTER FIVE

DISCUSSION, RECOMMENDATION AND CONCLUSION

5.1 DISCUSSION

Fresh meats are sometimes contaminated with bacteria (Burgess *et al.,* 2005), which can be harmful to the human body. The major bacterial pathogens include: *Salmonella, S. aureus, C. botulinum, Clostridium perfringens, B.* cereus and *E. coli* (Hedman *et al*., 2020). The sources of these microbes in meat could be inherent micro-flora in normal tissues of animals, air, environment, or contamination due to unhygienic slaughtering, handling and processing conditions (Pal *et al*., 2018). The morphological characteristics of individual microorganisms serve as preliminary criteria for identification. Of the several morphological properties, colonial characteristics and gram staining reactions were employed in the identification of isolates. In this study, 220 colonies of distinct *Bacillus* spp. and *S.aureus* morphological features, representative of colonies obtained with nutrient agar (*Bacillus* spp.) and blood agar (*S. aureus*), were isolated and selected for further characterization. The selected isolates were bacilli and cocci in shape and were single cells, some were chained in arrangement. Each isolate was subjected to various tests to study their characteristic features in order to identify them.

Biochemical tests carried out in this study indicates presumptive *Bacillus* and *Staphylococcus* spp. These organisms might be pathogenic bacteria and their presence in the game meat samples might be due to contamination from the working environment of the meat preparation and the sale point (Castro *et al.,* 2010). The contamination could also be from the meat handlers. This is because *Staphylococcus* spp. is also present in the membranes of human noses, throats, hair and skin. Among *Staphylococcus* genus, the *S. aureus* isolated in this study is known to be pathogenic to humans while the remaining *Staphylococcus* strains are not necessary pathogenic (Otto, 2013).

In addition isolation of Bacillus species and Staphylococcus aureus from game meat is of public health concern because these pathogens are among the most important enterotoxigenic foodborne pathogens. They are capable of producing a number of enterotoxins that have been implicated in multiple cases of foodborne disease, generally causing either emetic or diarrheal symptoms. Various studies have isolated these pathogens from game-meat (Katani *et al.,* 2019; Oghenekome and Rose, 2020). In the study areas where samples were collected, bush meat were sold to commuters and traders indiscriminately at popular junctions and parks at road sides. This causes

potential exposure of the meat to the sun and dust. Microbial contamination from dust and the environment becomes a possibility. Commuters and locals have been noted to stop at roadsides, major junctions and makeshift kiosk in order to purchase fresh or smoked bush meats from sellers. There are consequences, however of increased interactions between humans and wildlife. Bush meat hunting and consumption creates this bridge between wild life and humans. It had been reported that this had led to increases chances of zoonotic transmission of diseases from animal hosts to humans (Clarence *et al*., 2009).

The presence of high microbial loads might be due to bush meat handling methods, cleanliness of the handling area, management as well as the personal cleanliness of the meat vendors. There is need for public health education for the meat handlers. Members of the public in Nigeria need to ensure meat vendors are neat and the environments the meat is sold in hygienic enough. All the bacterial isolated in this study are potentially pathogens. Microbial growths on food itemsincluding smoked bush meats are usually facilitated by inappropriate preservation procedures. Thus smoking of the meat by the handlers or vendors under poor hygienic conditions may encourage microbial contaminations. Bacteriological quality of food is important parameter for assessing food safety (Clarence *et al*., 2009). High bacterial counts of pathogenic organism is an indication that such food is a potential source of food borne infection (Raji et al., 2006). Proper hygiene is therefore necessary when handling meat and meat products in order to avoid public health hazard.

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5.2 CONCLUSIONS AND RECOMMENDATIONS

The study showed possible public health hazard related with consuming RTE game meat from various states of Nigeria. A high microbial load and the presence of *Bacillus* spp. and *Staphylococcus aureus* in RTE game meat presents a risk to public health and can cause life-threatening foodborne diseases. Therefore, implementation of control measures that aimed at preventing any practice that potentially contaminates game meat should be implemented to prevent any form of public health hazard. Consumers need to be informed about the potential risk of consuming not properly cooked game meat. Regulatory and educational efforts from the government officials are needed to improve the safety of fresh meat that are intended for use as

ready to eat products in Nigeria. Further precautions are needed during the processing and handling of game animals by the hunters, butchers and retailers as the hygienic environment and proper handling can have a greater influence on RTE game meat.

REFERENCES