Mesophilic Bacterial Isolates in Sludge and Waste water from Food Canteens in Tertiary Educational Institutions within Ibadan

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Certification

This is to certify that **Bukola, M. Adeyemi Ekeolu** with matriculation number LCU/PG/001815, carried out this research work titled "**Microbial Profile of Sludge and Waste water from Food Canteens in Tertiary Educational Institutions in Ibadan Metropolis**." in the Department of Biological Science, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Oyo state, for the award of Master Degree (M.Sc.) in Medical Microbiology and that this has not been previously submitted**.**

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Dedication

This research work is dedicated to the Almighty God for making its completion possible**.**

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Abstract

Microbiological safety of the environment around food canteens is of great importance as such environments could be potential sources of food-borne illnesses of epidemic proportions. Food canteens in and around tertiary educational institutions get a lot of patronage and this leads to the generation of a lot of waste water and development of sludge around their environments. The aim of this study is to determine the microbial profile of sludge and waste water generated by ``food canteens in selected tertiary institutions within Ibadan metropolis.36 samples of waste water, sludge and food were collected from three canteens in four tertiary educational institutions. Microbial isolation was carried out using Salmonella-Shigella, Eosine-methylene blue, Mannitol-Salt and Nutrient agars. Incubation was carried out for 24 hours at 37°C. Isolates obtained were subjected to biochemical characterization and molecular identification. Out of the 20 isolates obtained and identified, five were *Pseudomonas aeruginosa*, three *Escherichia coli*, three *Proteus mirabilis*, two *Pectobacterium carotovorum*, one *Acinetobacter baumanni*, two *Bacillus* species, one *Enterobacter cloacae*, one *Providencia vermicola* and one *Enterococcus faecium*. The result obtained shows that the waste water and sludge around the canteens as well as some of the foods sold contain potential pathogens. This appears to be from different potential sources which include raw food materials and fecal contamination. It is therefore of utmost importance to introduce treatment of wastewater to food canteen owners and also proper disposal method so that sludge formation around the canteens is reduced. This will help to reduce the risk of food borne illnesses among people patronising the canteens and subsequent spread to the entire community.

Keywords: Food canteen, tertiary institutions, sludge, waste water, Ibadan

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List of Acronyms

Abbreviation	**Meaning**
ExPEC	extraintestinal *E. coli*
EPEC)	pathovars-enteropathogenic
EHEC	*E. coli* entero-haemorrhagic
ETEC	*E. coli* enterotoxigenic *E. coli*
EIEC	Enteroinvasive *E. coli*
EAEC	enteroaggregative *E. coli*
DAEC	Diffusely adherent *E. coli* are diarrhoe
UPEC	Pathovars-uropathogenic*E. coli*
NMEC	neonatal meningitis *E. coli*
EMB	Eosin Methylene Blue
MSA	Salmonella shigella agar
BLAST	Basic LocalAlignment Sequence Tool
NCBI	National Center for Biotechnology Information
UTIs	Urinary tract infections
PCR	Polymerase chain reaction

Chapter One

Introduction

1.1 Background to the Study

A major public health concern for everyone is the requirement to improve food safety and hygiene standards, particularly in the prevention of food-borne illnesses[1]. The World Health Organization (WHO) defines food as any substance that is meant for human consumption and is either processed, semi-processed, or raw. Similar to this, food is any substance that individuals prepare and consume to meet their physiological demands[2]. It can also be characterized as anything, liquid or solid, that, when consumed and digested, can nourish the body. Food is made up of ingredients that can be eaten, such as meat, bread, and vegetables; it can also be prepared, processed, or semi-processed. Foods that have a limited shelf life and will expire or become contaminated if improperly handled and preserved include perishables such meat, eggs, milk, fruits, and vegetables. Non-perishable foods, including sugar and cereals, do not easily degrade or become contaminated. According to the aforementioned definitions, food is any item consumed that meets the body's nutritional requirements. Therefore, since food is a fundamental human requirement, it must be healthy and secure. This is due to the fact that appropriate nutrition promotes health, but poor nutrition and tainted foods may be harmful to people's health[3].

Literature describes food adulteration as the mixing of food by canteens with additional substances that can be detrimental for human consumption[5]. It is a serious public health issue that has an impact on people's quality of life, particularly in neighborhoods where the majority of residents frequent neighborhood canteens. Food adulteration can lead to food borne illnesses, which pose substantial health risks and are the main causes of morbidity and mortality in underdeveloped nations like Nigeria. According to research, microorganisms like salmonella, campylobacter jejuni, or Norwalk virus can infest food and cause outbreaks of food-borne disorders. For example, a microbiological analysis of a variety of food products served by canteens in different regions of India revealed the presence of microorganisms similar to those stated above[4].

Many urban residents, including construction workers, laborers, market vendors, mechanics, shoemakers, and plumbers, as well as locals who frequent these numerous canteens for the delicacies they serve, depend on food pantries to provide them with basic services. According to several academics, canteens are small-scale businesses that are typically found in plainly furnished facilities or stalls in public spaces where they offer the general public quick and affordable restaurant services6. The canteens operate in both urban and rural areas, where they set up shop next to the communities or carry out their business under a nearby tree or shed. They offer a variety of foods, including rice, beans, okpa, agidi, yam flour, pounded yam, bean-cake, and snacks. People who frequent these food canteens are more concerned with the instant convenience they get than with the cleanliness and safety of the establishments. Canteens are growing in popularity, possibly as a result of certain important functions they perform, such as offering quick and affordable meal services to the public and generating temporary work opportunities for women. While appreciating the crucial function that canteens provide for city dwellers, some point out that because canteen operators don't maintain good food hygiene, these foods might seriously harm people's health[7].

According to the science of food hygiene, individuals, groups, or organizations must maintain a clean environment while taking the essential precautions to prevent dangers and ensure that a food product is fit for human consumption. In terms of food hygiene and safety, contamination with "microorganisms" or "microbes" is typically discussed, however "infectious agents" is preferred when referring to communicable diseases. The methods that avoid microbial contamination of food at all points throughout the supply chain, from farm to table, are specifically referred to as "food hygiene" in this context[9]. For the production and consumption of the food we eat to occur in clean and healthy conditions, food hygiene is essential. Food hygiene's overarching goal is to produce and distribute safe food, which will subsequently support a healthy and prosperous society. Preventing food rotting is one of the specific goals of food hygiene. Foods are organic materials that, over time or when exposed to unfavorable conditions, can alter in chemical makeup and become unfit for eating due to microbial or chemical contamination. Food hygiene also aims to teach and educate people on easy and doable ways to keep food safe so they can safeguard themselves against foodborne illnesses. Additionally, it is to ensure ethical behavior in the food industry and stop the selling of provocative or subpar food. Thus, maintaining a clean environment helps prevent disease and promotes healthy living in society[9].

It is important to remember that neither the national food safety agency nor the local government health agencies have any control over these canteen operators. As a result, the canteens run without any oversight of the food they prepare or how it was made. People who frequent canteens are not only exposed to poor and unhygienic diets and could experience poor health, but they are also potentially experiencing academic development delays, according to some studies, because they are operating in the primitive premises without proper building structures and compartments for various aspects of the restaurant service, like dish-washing and cooking corners. Poor families appear to spend a greater amount of their income on food from canteens because they regard the cost of whole food condiments to be prohibitive. Unfortunately, these less expensive foods tend to be the least nutrient-dense. They are frequently greasy, sugary, salty, and poorly prepared foods, and the food products are polluted as a result of inadequate storage10. The sale of such goods puts consumers' health at risk by causing symptoms including diarrhea, vomiting, and abdominal discomfort, which are all typical in cases of food poisoning[10]. Many factory workers in states with industrial areas that are remote from residential areas depart early for work without eating breakfast, thus they mostly rely on the foods that these canteen operators provide on the factory grounds. The canteens throw unclean water throughout their surrounds and utilize it to wash their plates[11]. Some of them similarly show up with untidy hair and unclean clothes. Food that has been prepared, cooked, served, or stored using less-than-hygienic methods is frequently contaminated with germs and other substances that cause infections and ailments in those who consume it, especially in communities who frequent canteens. Students who consume the food from these filthy community canteens run the danger of contracting illnesses including diarrhea and constipation, among others. This study is intended to investigate the microbial contaminants (profile) of the food sold in the chosen tertiary institutions in the city of Ibadan, Nigeria. This is due to the fact that canteens on tertiary institutions in the city of Ibadan are largely unregulated and there is a lack of data on the prevalence of food-borne illnesses.

1.2 Statement of the Problem

Public health concerns are raised by the practices of canteens, particularly those found on the grounds of tertiary university campuses. Customers of canteens are frequently unaware of the unclean conditions of the food and may not be concerned about the health risks associated with what they purchase and consume there. Many community canteens are discovered to not maintain sufficient hygienic measures, both in the production of their food and in the display of their items for sale[12].

It has been observed that the cooks are usually dirty and unkempt in appearance, negligent about precautions needed to be taken while coughing, sneezing and during toilet use. Display of food is also without regard to environmental contamination, and inadequate supply of water leads to reuse of dirty water in cleaning of wares and plates.

1.3 Justification of the Study

Publication of data obtained from the sampling of foods sold by these canteens will help to call the attention of stake-holders that is, consumers and government agencies to the public health hazard posed by these canteens, and may help to institute the necessary policies needed to effect proper control in this informal sector of the food industry.

1.4 Aim and Objectives of the Study

1.4.1 Aim

The purpose of this study is to identify microbial profile of sludge and wastewater from food canteens in Tertiary institutions in Ibadan metropolis.

1.4.2 Specific Objectives

1. Isolation of bacteria from the food, waste water and sludge collected from the canteens.
2. Biochemical identification of the microorganisms isolated
3. DNA extraction and molecular identification of bacterial isolates obtained
4. Suggest strategies that should be adopted in enhancing the hygienic practices needed by canteens in Ibadan, Oyo State.

1.5 Research Questions

The following research questions guided t``he study:

1. What is the microbial profile of food, sludge and wastewater from food canteens in the chosen tetiary institutions.
2. What is the bacterial load of the samples obtained from the food canteens in these institutions?
3. What is the molecular identities of bacterial isolates obtained from the samples collected

1.6 Significance of the Study

The theoretical relevance of this study is based on the Humoral theory of food and culture as well as social learning, respectively. The two theories' guiding ideas emphasize learning and changing behavior through engagement with the environment. The Ministry of Health, canteens, parents, members of the community who frequent canteens, researchers, and the entire society would all greatly benefit from the study's practical implications. The information would help the Health Ministry officials understand the functions and issues that canteens play in public primary communities. They can use this information to develop effective policy interventions to encourage food safety practices in the various community settings where the canteens operate. The research findings will aid canteens in implementing good hygiene procedures that will reduce the risk of food contamination. It would give them the abilities to assure adequate food hygiene as well as the necessity to keep themselves and the place where they sell their products clean. Environmental health organizations may do this. Those who frequent canteens would also gain financially. This will reassure them that the food served to individuals who frequent community canteens is of a high standard and secure. The results would indeed be helpful to those who use canteens themselves. The managers of the community canteens would provide them with wholesome and secure food. Their nutritional state would be improved by the high-quality food. The results serve as important background information for researchers interested in other areas of food research. The entire society would gain from the discoveries. This would significantly help to stop the spread of food poisoning. Through adopting proper food preparation practices under more hygienic conditions in their homes, it would help the populace nderstand the importance of food safety.

1.7 Scope of the Study

This study will cover the canteens in Tertiary institutions within Ibadan metropolis, Oyo state. The study will examine themicrobial profile of sludge and wastewater from food canteens in selected tertiary educational institutions in Ibadan metropolis, as well as the hygiene practices of the canteens with particular interest on environmental food hygiene practices and sludge studies. The study will also examine strategies for food hygiene practices that canteens would adopt to enhance the hygiene practices in Ibadan, Oyo State.

1.8 Limitation of the Study

This study is limited to investigate the complete identification and phylogenic, biochemical characterization of bacteria associated with sludge and waste water. The study is limited to complete profiling of DNA sequence of bacteria associated with sludge and waste water from selected food canteen in tertiary educational institution in Ibadan metropolis.

1.9 Operational Definition of Terms

Hygiene: conditions or practices conducive to maintaining health and preventing disease, especially through cleanliness.

Canteen: A place in a factory, community, town or communities where meals are provided, usually quite cheaply

Food Services: The food service encompasses any establishment that serves food to people outside their home.

Community: A group of people living in the same place or having a particular characteristic in common.

Fod System: The production, processing, distribution, preparation, consumption, and disposal of food as well as the results of these activities, such as nutritional and food security as well as socio-economic and environmental outcomes, are all included in this. These elements include people, the environment, inputs, processes, infrastructures, institutions, etc.

ood Environment: the social, political, economic, and cultural context in which consumers interact with the food system to decide how to get food and how to prepare it.

Food Availability: the quantity and variey of food items that can be physically accessed by residents of the area and are offered at various food venues (markets, shops, restaurants).

Food Quality and Safety: All the qualities and qualities that customers appreciate, as well as the perceived and actual safety related to food goods.

Healthy Diets: A diet which promotes growth and development, and prevents malnutrition in all its forms for all people.

Bio-fortification: Consumers respect all the traits and qualities, as well as the perceived and actual safety connected with food goods.

Nutrition-sensitive Value Chain: A value chain that focuses on making the food product more nutritious.

Food Security: A situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life

Wasting: low weight-for-height, generally the result of weight loss associated with a recent period of inadequate dietary energy intake and/or disease.

Food Supply Chain: encompasses all activities that move food from production to consumption, including production, storage, distribution, processing, packaging, retailing and marketing.

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Chapter Two

Literature Review

2.1 Conceptual Studies on the Work

Food canteen, sometimes called a canteen, is a type of food service location in which there is little or no waiting staff table service, whether in a restaurant or within an institution such as a large office building or school; a school ining location is also referred to as a dining hall or lunchroom[1]. Food canteens are different from coffee-houses, and it had and still has the meaning "coffee-house”. Instead of table service, there are food-serving counters, stalls or booths, either in a line or allowing arbitrary walking paths.

Customers take the food that they desire as they walk along, placing it on a tray. In addition, there are often stations where customers order food, particularly items such as hamburgers or tacos which must be served hot and can be immediately prepared with little waiting. Alternatively, the patron is given a number and the item is brought to their table. For some food items and drinks, such as sodas, water, or the like, customers collect an empty container, pay at the check-out, and fill the container after the check-out. Free unlimited second servings are often allowed under this system. For legal purposes (and the consumption patterns of customers), this system is rarely, if at all, used for alcoholic drinks in the United States[1].

Customers are either charged a flat rate for admission (as in a buffet) or pay at the check-out for each item. Some self-service food canteens charge by the weight of items on a patron's plate. In universities and colleges, some students pay for three meals a day by making a single large payment for the entire semester.

As food canteens require few employees, they are often found within a larger institution, catering to the clienteles of that institution. For example, schools, colleges and their residence halls, department stores, hospitals, museums, places of worship, amusement parks, military bases, prisons, factories and office buildings often have food canteens. Although some of such institutions self-operate their food canteens, many outsource their food canteens to a food service management company or lease space to independent businesses to operate food service facilities[2].

2.2 Effects of Water Pollution

The presence of pathogenic organisms (infective and parasitic agents), poisonous chemical substances, and industrial or other wastes or sewage in water makes it contaminated or polluted. Polluted surface water can contain a wide variety of pathogenic microorganisms including bacteria and viruses. Unfortunately, clean, pure and safe water can exist only briefly in nature and immediately polluted by prevailing environmental factors aided by human activities. Water from most sources is therefore unfit for immediate consumption without some sort of treatment[3].

The main causes of water pollution are the releases of chemicals and heavy metals from factories and businesses. According to reports, one of the main causes of this is the discharge of untreated sewage into rivers, which eventually has an impact on the environment and ultimately, people[4].

There are a variety of sources from which fecal contamination can originate. Potential sources of fecal coliform contamination include failing sanitary sewer or septic systems, wildlife and waterfowl. Agricultural practices including the spreading of manure and its use as a fertilizer could potentially lead to runoff of fecal-related pathogens. Livestock access to waterways can also lead to direct introductions to streams. Improper disposal of domestic pet wastes is also a potential source of pathogen pollution[4]. Recently, dumpsters have been recognized as a source of pathogens in storm water runoff due to birds using dumpsters as feeding locations; this is also true of rodents. Furthermore, the sources of coastal water contamination are: point discharges of treated and untreated sewage from shoreline outfalls, and non-point discharges. The non-point sources, such as runoff from naturally vegetated areas, discharge pathogens into coastal waters. Besides runoff from vegetated areas, the storm water runoff from urban, commercial, and industrial lands also discharges pathogens into coastal waters[5]. In addition, other sources, such as malfunctioning or poorly sited septic systems, can also introduce significant amounts of pathogens. It was reported that direct deposition of waterfowl feces was a considerable source of pathogens[6]. It was summarized that the transport of various pathogens, such as Giardia, Toxoplasma, and Cryptosporidium (zoonotic parasites) in the coastal environment[7]. Moreover, the presence of sediment in seawater can also increase the survival chance of fecal coliforms, such as *E. coli*. It was alsoshowed that the location and timing of storms off the coastal area in tropical and subtropical environments are also important factors that can potentially influence coastal water quality[8].

Previous studies have shown that the direct discharge of storm water runoff into coastal waters through storm drain systems can cause pathogen contamination, even where separate storm and sanitary sewer systems are in place[9,10]. About 16% of the total fecal coliform inputs were caused by storm water entering Buttermilk Bay in Massachusetts[9]. In addition, coastal rivers draining largely undeveloped watersheds with extensive riparian wetlands can be a natural source of fecal pathogens to coastal waters[10]. On-site septic systems can also contribute significant amounts of fecal pathogens to coastal waters in low-lying fine-grained geological settings where saturated soils enhance pathogen growth. Shoreline wrack deposits could act as a reservoir of fecal bacteria, and the removal of wrack deposits from inter-tidal zones can improve the water quality of adjacent coastal waters[10].

Similar to the castal environment, increasing water-borne pathogen levels in estuaries are a serious threat to public health. Human activities can impact streams as well as estuary pathogen levels when they are adjacent to populated areas, and often provide a means of transportation and substantial recreation. The most common pathogens, previously identified in streams were *Escherichia coli*, *Vibrio cholerae*, Giardia, Cryptosporidium, Salmonella, and *Campylobacter spp*[11]. Urban water disposed through combined sewer outflows is the cause of approximately 12% of estuary impairments in the United States (OECD) [12].

In Abeokuta, Ogun state, Nigeria, a study was done to identify the extended spectrum -lactamase (ESBL) production profiles and antibiotic resistance profiles of Gram-negative bacteria isolated from sludge collected from Lafenwa municipal abattoir and its receiving surface water[13]. Microroganisms isolated included *Salmonella* spp. (9), *Escherichia* coli (15), *Klebsiella* spp. (7), *Shigella* spp. (5), *Pseudomonas* spp. (12), and *Enterobacter* spp., a total of 54 Gram-negative bacteria were isolated (6). All of the chosen antibiotics were resistant to both Enterobacteriaceae and Pseudomonas isolates (31% and 66.6%, respectively), with the exception of ertapenem (98% susceptibility)[13].

Pathogens, including *Vibrio vulnificus* which carries the highest fatality rate of any food-borne pathogen in the U.S., were detected in the Gulf of Mexico Estuary [13]. Several studies discovered that bed sediment plays a vital role (i.e., with the release of particle-attached pathogens from bed sediment to a water column through a re-suspension process) for the persistence and transport of pathogens in the estuaries. Previous studies have shown that pathogen growth and decay are influenced by environmental conditions. For instance, it was revealed that sunlight is a major factor that influences survival of pathogens like *E. coli* and *S. typhimurium* in the streams[14].

Waterborne diseases (Table 2.1) are those transmitted through the ingestion of contaminated water that serves as the passive carrier of the infectious agent. The classic waterborne diseases, cholera and typhoid fever, which have frequently ravaged densely populated areas throughout human history, have been effectively controlled by the protection of water sources and by treatment of contaminated water supplies. In fact, the control of these classic diseases illustrates the importance of water supply treatment which played an important role in the reduction of infectious diseases[13]. Other diseases caused by bacteria, viruses, protozoa and helminths may also be transmitted by contaminated drinking water. However, it is important to remember that waterborne diseases are transmitted by the fecal–oral route, from human to human or animal to human, so that drinking water is only one of several possible sources of infection[14].

Table 2.1: Classification of Water-related Illnesses Associated with Microorganisms[14]

Class	Cause	Example
Waterborne	Pathogens that originate in fecal material and are transmitted by ingestion	Cholera, typhoid fever
Water-washed	Organisms that originate in feces are transmitted through contact because of inadequate sanitation or hygiene	Trachoma
Water-based	Organisms that originate in the water or spend part of their life cycle in aquatic animals and come in direct contact with humans in water or by inhalation	Schistosomiasis, Legionellosis
Water-related	Microorganisms with life cycles associated with insects that live or breed in water	Yellow fever

Water-washed diseases are those closely related to poor hygiene and improper sanitation. In this case, the availability of a sufficient quantity of water is generally considered more important than the quality of the water. The lack of water for washing and bathing contributes to diseases that affect the eye and skin, including infectious conjunctivitis and trachoma, as well as to diarrhea illnesses, which are a major cause of infant mortality and morbidity in developing countries. 2vDiarrheal diseases may be directly transmitted through person-to-person contact, or indirectly through contact with contaminated foods and utensils used by persons whose hands are fecally contaminated. When enough water is available for hand washing, the incidence of diarrheal diseases has been shown to decrease dramatically, as has the prevalence of enteric pathogens such as Shigella[13, 14].

Water-based diseases are caused by pathogens that either spend all (or essential parts) of their lives in water or depend on aquatic organisms for the completion of their life cycles. Examples of such organisms are the parasitic helminth Schistosoma and the bacterium Legionella, which cause schistosomiasis and Legionnaires' disease, respectively. Water-related diseases, such as yellow fever, dengue, filariasis, malaria, onchocerciasis and sleeping sickness, are transmitted by insects that breed in water (e.g., mosquitoes that carry malaria) or live near water (e.g., the flies that transmit the filarial infection onchocerciasis). Such insects are known as vectors[14].

2.3 Importance of Water Quality Parameters

The quality of water influences the health status of any populace, hence, analysis of water for physical, biological and chemical properties including trace element contents are very important for public health studies. Shortage of infrastructure for effective treatment and distribution of water accounts for the incidence of high morbidity and mortality rate associated with waterborne diseases in developing countries[15]. One of the targets of the millennium development goals (MDG) in terms of healthy living for the masses can be achieved through the supply of safe and portable water. The availability of good quality water sources is therefore getting more and more limited, and the effect of water-borne pathogens on human health is expected to be of great concern. It is therefore, important to understand the significance of natural and waste water contribution to transmission of pathogenic microorganisms. The quality of water may be described according to its physicochemical and microbiological characteristics[16].

2.4 Microbiological Quality of Wastewaters

The microbiological quality of treated wastewater is a concern to customers, water suppliers, regulators and public health authorities alike. The increasing industrialization and the growing water demand have led to a global deterioration of surface water quality; thus, the need to assess the microbiological safety of these waters by analyzing them for the presence of specific pathogens and directing efforts to the removal of indicator microbes of faecal origin. According to World Health Organization and European guidelines, *Escherichia coli* indicates faecal contamination of water[18].

Monitoring the microbiological quality of waste water relies largely on examination of indicator bacteria such as *Escherichia coli, Pseudomonas aeruginosa* and other members of the coliform. *E. coli* is a member of the faecal coliform group and is a more specific indicator of faecal pollution than other faecal coliforms. Two key factors have led to the trend toward the use of *E. coli* as the preferred indicator for the detection of faecal contamination, not only in waste water, but also in other matrices as well[19]. Firstly, the findings that some faecal coliformswere non-faecal in origin, and secondly, the development of improved testing methods for *E. coli.* The faecal coliform definition has also been revised to coincide better with the genetic make-up of its members and now includes newly identified environmental species. As a result, faecal coliforms are increasingly being referred to as *thermotolerant* coliforms. This, combined with improved detection methods for *E. coli*, has started a trend toward the use of *E. coli* in place of thermotolerant coliforms as a more reliable indicator of faecal pollution in waste water[20]. At present, *E. coli* appears to provide the best bacterial indication of faecal contamination in waste water. This is based on the prevalence of thermotolerant (faecal) coliforms in temperate environments as compared to the rare incidence of *E. coli*. The prevalence of *E. coli* in human and animal faeces as compared to other thermotolerant coliforms, and the availability of affordable, fast, sensitive, specific and ease of detection further make *E. coli* a suitable indicator organism[21].

2.5 Historic Perspective of Indicator Micro-organisms

Traditionally, indicator micro-organisms have been used to suggest the presence of pathogens. Today, however, we understand a myriad of possible reasons for indicator presence and pathogen absence or *vice versa*. In short, there is no direct correlation between numbers of any indicator and entire pathogens. To eliminate the ambiguity in the term *microbial indicator*, the following three groups are now recognized: i) general (process) microbial indicators, ii) faecal indicators such as *E. coli,* iii) index organisms and model organisms[22].

A direct epidemiological approach could be used as an alternative or adjunct to the use of index micro-organisms. Yet epidemiologic methods are generally too insensitive, miss the majority of waterborne disease transmissions and are clearly not preventative. Nonetheless, the ideal is to validate appropriate index organisms by way of epidemiological studies. A good example is the emerging use of an *enterococci* guideline for recreational water quality. Often epidemiologic studies fail to show any relationship to microbial indicators, due to poor design and/or due to the widely fluctuating ratio of pathogen(s) to faecal indicators and the varying virulence of the pathogens[23].

The use of bacteria as indicators of the sanitary quality of water probably dates back to 1880 when Von Fritsch described *Klebsiella pneumoniae*and *K. rhinoscleromatis*as microorganism characteristically found in humanfaeces. In 1885, Percy and Grace Frankland started the first routine bacteriological examination of water in London, using Robert Koch's solid gelatin media to count bacteria[24]. Also, in 1885, Escherich described *Bacillus coli* and renamed it *Escherichia coli.* In 1891, the Franklands came up with the concept that organism's characteristic of sewage must be identified to provide evidence of potentially dangerous pollution. By 1893, the Wurtz methodof enumerating *E. coli* by direct plating of water samples on litmus lactose agar was being used by sanitary bacteriologists, using the concept of acid from lactose as a diagnostic feature[24]. This was followed by gas production, with the introduction of the Durham tube. The concept of *coliform* bacteria, those bacteria resembling *E. coli,* was in use in Britain in 1901. The colony count for bacteria in water, however, was not formally introduced until the first report. Therefore, the sanitary significance of finding various coliforms along with streptococci and *C. perfringens* was recognized by bacteriologists by the start of the twentieth century[25]. It was not until 1905, however, that MacConkey described his now famous MacConkey's broth, which was diagnostic for lactose-fermenting bacteria tolerant of bile salts. Nonetheless, *coliforms* were still considered to be a heterogeneous group of organisms, many of which were not of faecal origin. The origins of the critical observation that *E. coli* was largely faecal in origin while other coliforms were not could be claimed.

2.6 Characteristics of Indicator Organisms

Indicator organisms are not by themselves usually a health concern for healthy individuals, but their presence in water indicates an increased risk. Historically, faecal indicator bacteria including total and faecal coliforms have been used in many countries as monitoring tools for microbiological impairment of water and for prediction of presence of bacterial, viral and protozoan pathogens. These microorganisms are of faecal origin from higher mammals and birds, and their presence in water may indicate faecal pollution and possible association with enteric pathogens[26].

The indicator organisms presently used for monitoring the efficiency of wastewater treatment facilities and surface-water resources in developing countries are total coliforms and faecal coliforms, although reliance on indicator organisms as the main source of information about the safety of reclaimed water for public health is under review in many jurisdictions. Faecal coliform bacteria include members of the genera *E. coli*, which are faecal in origin as well as organisms that are found in both faecaland non-faecal environments such as *Enterobacter*, *Klebsiella* and *Citrobacter spp*. Heterotrophic plate count bacteria are also used as indicators of the genera microbiological water quality. These organisms use organic compounds for most or all of their carbon requirements[27].

To assure a safe water supply, it is critical to monitor for the presence of possible pathogens. However, it would be expensive and time consuming to check the water supply for all pathogens, instead, an indicator organism is used to assay for fecal contamination. The detection of indicator bacteria is preferred over direct pathogen detection because the former are considered to be normal, non-pathogenic intestinal inhabitants that are present in feces and wastewater in much higher numbers than pathogenic microorganisms and because they are technically easier to detect and quantitate than pathogens[28]. Present standards for the sanitary quality of water, foods and other materials, with respect to fecal contamination, are expressed in terms of concentrations of indicator bacteria. Indicator organisms must have four properties to be useful for water analysis. This includes:

i. The only natural environment of the microbe should be in association with feces and it should always be present.
ii. It should not grow outside of its natural environment.
iii. The bacterium should survive longer than the most viable pathogen, but not so long so that historical events are detected.
iv. It should be easy to detect[29].

2.7 *Escherichia coli* as an Indicator of Faecal Contamination

Various bacteria are found in the digestive tracts and faeces of wild and domestic animals as well as humans. Some of these bacteria, particularly *E. coli* (a predominant member of the faecal coliform group), and *Enterococcus* spp., are used as indicators of faecal contamination in natural waters. Few studies have focused on the identification of specific characteristics of *Escherichia coli* in the flow of bacteriological pollutants. Its presence in humans and animals as a normal inhabitant of the gastrointestinal tract creates opportunities for contamination if proper hygiene is not well practiced. Hence, they only infer that pathogens may be present[30].

Escherichia coli are the predominant member of the facultative anaerobic portion of the human colonic normal flora. *E. coli* with some exceptions generally does not survive well outside of the intestinal tract, its presence in environmental samples, food, or water usually indicates recent faecal contamination or poor sanitation practices in food-processing facilities. The population of *E. coli* in these samples is influenced by the extent of faecal pollution, lack of hygienic practices, and storage conditions[31]. The mere presence of *E. coli* in food or water does not indicate directly that pathogenic microorganisms are in the sample, but it does indicate that there is a heightened risk of the presence of other faecal-borne bacteria and viruses, many of which, such as *Salmonella spp.* or hepatitis A virus, are pathogenic. For this reason, *E. coli* is widely used as an indicator organism to identify food and water samples that may contain unacceptable levels of fecal contamination. *E. coli* is considered a more specific indicator of fecal contamination than fecal coliforms since the more general test for fecal coliforms also detects thermo-tolerant non-fecal coliform bacteria. The *E. coli* test recommended by the United States Environmental Protection Agency (EPA) confirms presumptive fecal coliforms by testing for the lack of an enzyme which is selective for the *E. coli* organism. This test separates *E. coli* from non-fecal thermo-tolerant coliforms[32].

2.8 *Escherichia coli*

Escherichia coli (commonly abbreviated as *E. coli*),is a Gram-negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for product recalls. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2, or by preventing the establishment of pathogenic bacteria within the intestine[33].

The bacterium *Escherichia coli* (*E. coli*), originally known as Bacterium coli commune, belongs to the Family *Enterobacteriaceae* and was first isolated and characterized in 1885 by the German scientist and pediatrician, Theodore Escherich. The bacteria naturally and harmlessly exist in the intestines of all warm-blooded animals, hence the Greek-related root of its Family name, *Enterikos*, meaning “intestine”. It has been stated that the average *E. coli* population in an adult’s intestine is approximately 0.1 percent of the total bacteria. The bacteria are needed by the body to aid in the production of several vitamins, such as Vitamin K and the B-Vitamins. *E. coli* is the major component of normal healthy stool[34]. Some species of *E. coli* co-exist with us nicely; some species cause serious disease. Most of the *E. coli* causes no trouble to humans. Problems arise either if *E. coli* gets somewhere they should not be (such as the urinary tract) or if people are infected by one o the dangerous strains, such as *E. coli* O157:H7. *E. coli* is the major cause of diarrhea (some mild and some severe). It is also a common friendly bacterial inhabitant of the intestinal tract, aiding in the digestion of food. In scientific research, *E. coli* is a standard vector for rapid mass production of biological building blocks, from DNA to protein. Rare would be a biological researcher who has not used *E. coli*[35]. The strain found in healthy intestines and used in the laboratory is different from the dangerous strain being reported in the news. *E. coli* 0157:H7 can be lethal. Its extra genes enable it to stick to the walls of the intestine and produce the virulent Shiga toxin, which injures cells in the intestinal walls as well as blood vessels in the intestine, leading to bleeding. In the bloodstream, these toxins damage other blood vessels, particularly in the kidneys, leading to renal failure[36].

2.8.1 Types of *E. coli*

Certain isolates of *Escherichia coli* have been implicated in a wide range of diseases that affect either animals or humans worldwide. To date, eight pathovars and their mechanisms of disease have been extensively studied. These pathovars can be broadly classified as either diarrhoeagenic *E. coli* or extraintestinal *E. coli* (ExPEC). Six pathovars-enteropathogenic *E. coli* (EPEC), entero-haemorrhagic*E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive*E. coli* (EIEC; including *Shigella*), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC) are diarrhoeagenic and two pathovars-uropathogenic*E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC) are the most common ExPEC isolates[37]. Other pathovars have been identified, but their mechanisms of pathogenesis are not as well-defined.

2.8.1.1 Enteropathogenic *E. coli* (EPEC)

EPEC is a major cause of potentially fatal diarrhea in infants in developing countries. They do not possess any colonization factors and do not produce ST or LT toxins. They produce a non fimbrial adhesin designated intimin, an outer membrane protein that mediates the final stages of adherence[38]. Although they do not produce LT or ST toxins, there are reports that they produce an enterotoxin similar to that of *Shigella*. Other virulence factors may be related to those in *Shigella*. Adherence of EPEC strains to the intestinal mucosa is a very complicated process and produces dramatic effects in the ultra-structure of the cells resulting in rearrangements of actin in the vicinity of adherent bacteria. The phenomenon is sometimes called "attaching and effacing" of cells. EPEC strains are said to be "moderately-invasive" meaning they are not as invasive as *Shigella*, and unlike ETEC or EAEC, they cause an inflammatory response. The diarrhea and other symptoms of EPEC infections probably are caused by bacterial invasion of host cells and interference with normal cellular signal transduction, rather than by production of toxins[38]. They are an important cause of traveler's diarrhea.

2.8.1.2 Enterohemorrhagic *E. coli* (EHEC)

Cattle are a key reservoir for EHEC, which is a highly infectious A/E (human attaching and effacing) pathogen that colonizes the distal ileum and large bowel in humans and is often the causative agent of outbreaks of severe gastroenteritis in developed countries. Transmission to humans usually occurs through contaminated food and water. In North America, Japan and parts of Europe, most outbreaks are due to EHEC serotype O157:H7, whereas other serotypes are important health concerns in other developed countries. Adults and children infected with EHEC suffer from haemorrhagic colitis (bloody diarrhea) and further complications can lead to the potentially fatal haemolyticuraemic syndrome (HUS)[39]. EHEC are also considered to be "moderately invasive". Nothing is known about the colonization antigens of EHEC but fimbriae are presumed to be involved. The bacteria do not invade mucosal cells as readily as *Shigella* but EHEC strains produce a toxin that is virtually identical to the Shiga toxin. The toxin plays a role in the intense inflammatory response produced by EHEC strains and may explain the ability of EHEC strains to cause HUS. The toxin is phage encoded and its production is enhanced by iron deficiency[40].

2.8:1.3 Enterotoxigenic *E. coli* (ETEC)

ETEC is an important cause of diarrhea in infants and travelers in underdeveloped countries or regions of poor sanitation. The disease varies from minor discomfort to a severe cholera-like syndrome. ETEC is acquired by ingestion of contaminated food and water and adults in endemic areas evidently develop immunity. The disease requires colonization and elaboration of one or more enterotoxins[41]. Both traits are plasmid-encoded. ETEC adhesins are fimbriae which are species-specific. For example, the K-88 fimbrial Ag is found on strains from piglets; K-99 Ag is found on strains from calves and lambs; CFA I and CFA II are found on strains from humans. These fimbrial adhesins adhere to specific receptors on enterocytes of the proximal small intestine. Enterotoxins produced by ETEC include the LT (heat-labile) toxin and/or the ST (heat-stable) toxin, the genes for which may occur on the same or separate plasmids. The LT enterotoxin is very similar to cholera toxin in both structure and mode of action. It is an 86 kDa protein composed of an enzymatically active (A) subunit surrounded by 5 identical binding (B) subunits[41,42]. It binds to the same idntical ganglioside receptors that are recognized by the cholera toxin (i.e., GM1) and its enzymatic activity is identical to that of the cholera toxin.

2.8.1.4 Enteroinvasive *E. coli* (EIEC)

EIEC closely resembles *Shigella* in its pathogenic mechanisms and the kind of clinical illness they produce. EIEC penetrates and multiplies within epithelial cells of the colon causing widespread cell destruction. The clinical syndrome is identical to *Shigella* dysentery and includes a dysentery-like diarrhea with fever. EIEC apparently lacks fimbrial adhesions but does possess a specific adhesion that as in *Shigella* and is thought to be an outer membrane protein. Also, like *Shigella*, EIEC strains are invasive organisms. They do not produce LT or ST toxin and unlike *Shigella*, they do not produce the *Shigella* toxin[43].

2.8.1.5 Enteroaggregative *E. coli* (EAEC)

The distinguishing feature of EAEC strains is their ability to attach to tissue culture cells in an aggregative manner. These strains are associated with persistent diarrhea in young children. They resemble ETEC strains in that the bacteria adhere to the intestinal mucosa and cause non-bloody diarrhea without invading or causing inflammation. This suggests that the organisms produce a toxin of some sort[44]. Recently, a distinctive heat labile plasmid encoded toxin has been isolated from these strains, called the EAST (EnteroAggregative ST) toxin. They also produce a hemolysin related to the hemolysin produced by *E. coli* strains involved in urinary tract infections. The role of the toxin and the hemolysin in virulence has not been proven. The significance of EAEC strains in human disease is therefore controversial[44].

2.8.1.6 Diffusely Adherent *E. coli* (DAEC)

DAEC is a heterogenous group that generates a diffuse adherence pattern on HeLa and HEp-2 cells. This pattern is mediated by proteins encoded by a family of related operons, which includes both fimbrial (for example, Dr and F1845) and afimbrial (Afa) adhesins, collectively designated Afa–Dr adhesins (Servin, 2005). DAEC isolates that express any of the Afa–Dr adhesins (which are referred to as Afa–Dr DAEC) colonize the small bowel and have been implicated in diarrhea in children between the ages of 18 months and 5 years, as well as in recurring urinary tract infections (UTIs) in adults[45].

2.9 Other Categories of *E. coli* which are Potentially Pathogenic

The six categories of *E. coli* that are described in the above sections have each been implicated in several diarrhoea studies and are now generally accepted as diarrhoeagenic categories. However, some studies have suggested that there may be still other categories of *E. coli* which are quite distinct from those described above. They include:

2.9.1 Uropathogenic*E. coli* (UPEC)

UPEC infections account for roughly 80% of all UTIs, causing cystitis in the bladder and acute pyelonephritis in the kidneys. UPEC has the challenge of moving from the intestinal tract to establish an infection in the urinary tract, where it uses peptides and amino acids as the primary carbon source for fitness[46]. The ability to ascend the urinary tract from the urethra to the bladder and kidneys reflects exceptional mechanisms for organ tropism, evading innate immunity and voiding clearance by micturition. Several highly regulated virulence factors contribute to this complex pathogenesis, including multiple pili, secreted toxins (for example Sat and vacuolating autotransporter toxin (Vat), multiple iron acquisition systems and a polysaccharide capsule[47].

2.9.2 Neonatal meningitis *E. coli* (NMEC)

NMEC, a common inhabitant of the gastrointestinal tract, is the most frequent cause of Gram-negative associated meningitis in newborns. Fatality rates can approach 40 % and survivors are usually burdened with severe neurological sequelae. The pathogenesis of NMEC is complex, as the bacteria must enter the bloodstream through the intestine and ultimately cross the blood–brain barrier into the central nervous system, which leads to meningeal inflammation and pleocytosis of the cerebrospinal fluid[47]. Recently, a lambdoid phage that encodes O acetyltransferase was discovered, which acetylates the O antigen to provide phase variation and diversity to the capsule and may therefore hide the bacteria from host defenses[47].

2.10 Challenges Associated with the Use of *E. coli* as an Indicator Organism

As soon as the coliform test came into widespread acceptance, complications with its use and interpretation began to emerge. One concern was the discovery that a variety of microorganisms that read positive in the coliform test were not of fecal origin. As a result, the test method has evolved continually to become more specific. Some of the more significant developments were the so-called fecal coliform test which selects for coliforms of fecal origin by using a higher incubation temperature[48]. Though, disease-causing strains of *E. coli* species have been isolated from tap water, waste water sources and mountain streams, examination of pathogenic *E. coli* is not easy due to the uncertainty in determining the pathogenic nature of isolated *E. coli* strains. There is no biochemical marker that can separate pathogenic from non-pathogenic strains and the relationship between serotype and pathogenicity is questionable. The use of *E. coli* as an indicator organism is somewhat restricted by the fact that *E. coli* is not a single species; certain genera of the coliform group such as *Proteus* and *Aerobacter* are normally found outside the human intestinal tract in soil; other organisms found in water that do not represent fecal pollution possess some of the characteristics attributed to *E. coli* and *E. coli* identical to that found in humans is also found in the intestinal tract of other warm-blooded animals[49]. However, primarily, studies have shown that *E. coli* is a much better indicator of disease risk than is faecal coliform, EPA has therefore, recommended that *E. coli* be used as a criterion for classifying waters for fresh water contact recreation. Another weakness of the faecal coliform test and perhaps any indicator organism test geared to human waste is that there are some bacterial pathogens which are unrelated to human wastes [50]. To the degree that naturally occurring microbial pathogens become a significant public health concern, completely new test procedures may have to be developed. Furthermore, while *E. coli* is specific for faecal contamination, there are three inherent problems of using *E. coli* as a confirmation of faecal contamination: i) it is outnumbered by other types of fecal bacteria making it more difficult to find; ii) it does not survive for long outside of the gut; iii) it can be found in pristine environments in the tropics[51]. Therefore, the absence or presence of *E. coli* via a culture test does not absolutely confirm the absence or presence of faecal contamination. The *E. coli* tests used today as an indication of fecal contamination are commonly culture tests although there are PCR tests for the pathogenic strain *E.coli O157:H7* and for enterotoxigenic strains. In addition to the inherent differences in the ecology of the above-mentioned indicator organism, there is also the problem using culturable tests. All culture tests have an inherent bias in that they always underestimate the number of *E. coli* present in the sample[52]. This occurrence happens for a number of reasons, but in the instance of recovering faecal indicators, the bias is primarily for two reasons: i) some healthycoliforms are viable but will not grow in the media prescribed for them; and ii) coliforms found in the environment are often stressed thereby making recovery very difficult despite the growth media used[52].

2.11 Biological Indicators other than *E. coli*

2.11.1 Enterococci

Enterococcus is also known as fecal streptococcus. This group contains a number of species, of which *Enterococcusfaecalis* and *Enterococcus faecium* are predominant[53]. Virtually all mammals carry this organism in the colon at concentrations of approximately 10^6-10^7 g^{-1}. Accordingly, enterococci are approximately 100- to 1,000-fold less numerous than *E. coli.* A particular characteristic of the *Enterococcus* group is that it is quite salt-resistant, which makes it a good indicator of estuarine and ocean waters and the bacterium has a lifespan which approximates that of *E. coli.* A number of methods exist that are both sensitive and specific for the *Enterococcus* group[54]. These include variations of the Multiple-Tube method (such as azide dextrose broth), MF (various fecal-streptococci media), and DST (Enterolert). Like *E. coli,* a sample volume of no more than 100ml is required. Currently, the only regulations in which *Enterococcus* appears concern bathing beaches. *Enterococcus* is strongly being considered as an additional test to *E. coli* or waste water, but not as a replacement[55]. The primary reason for this strategy is that *Enterococcus* is present at lower numbers than *E. coli* in faeces. It is postulated that if a second, highly fecally specific test is performed, public health protection will increase for a modest cost. Reluctance to include an *Enterococcus* test centres on both the added cost and the requirement to put an additional laboratory quality control infrastructure in place. At this time, it is not clear that the additional costs and expertise required would yield sufficient public health protection over and above *E. coli* testing to justify its inclusion[56].

2.11.2 *Clostridium perfringens* Spores

The spores of *Clostridium perfringens* are extremely long lived. In fact, their long life is the major impediment to their use as an indicator of fecal contamination. They simply outlive all known pathogens. In soils and biofilms, they remain quiescent for years. Therefore, many soils with no fecal contamination may still have recoverable *C. perfringens* spores. The methods and expertise required to identify *C. perfringens* spores directly from waste water have been published but have not yet been widely field-tested[57]. It is expected that the cost of performing a *Clostridium* assay will be significantly higher (two to three times that of *Enterococcus)* because of the enhanced technical skill required, anaerobic incubation conditions, and more difficult quality control. *Clostridium perfringens* spores have been shown to be most appealing as indicators of ground water pollution because of their long residency times; however, they have not been adopted by any regulatory body and remain on many lists for discussion with little funding for field work[58].

2.11.3 Bacteriophages

Bacteriophages are viruses, which specifically infect bacteria. Bacteriophages have been suggested as useful indicators to predict the potential occurrence of enteric viruses in water. The survival of bacteriophages is affected by the densities of the host and the bacteriophages in the water sample[59]. In addition, the association of the bacteriophage with solids and the presence of organic matter in the water sample could influence the attachment of the bacteriophages to the host bacterium. Several studies have shown that ultra violet light, temperature, pH of the water, and ion concentrations in the water could affect the survival of bacteriophages in water.

Bacteriophages show higher resistance to environmental stress compared to bacterial indicators such as total coliforms and faecal coliforms and assays for bacteriophages can be conducted quickly, economically and quantitatively. There are several bacteriophages that can be used as indicator organisms which includes; the somatic bacteriophages, *Bacteroides fragilis* HSP40 bacteriophages and male specific F-RNA bacteriophages[60].

On the other hand, microbiologists have studied the efficacy of coliphage utilization as markers of fecal contamination for many years. As of this date, they have not been included in waste water regulations. The primary limitations include the lack of field data, the difficulty of processing the water sample (requiring multiple steps), and the lack of choice of a stable bacterial host[61]. There has also been a lack of association between the detection of bacteriophages in ground water and disease occurrence. Accordingly, the major limitations for acceptance of coliphages for waste water testing include: lack of a standard method, lack of extensive field testing, lack of correlation with disease occurrence, and lack of a stable host. In fact, it is unlikely that somatic colihages would be utilized for testing of waste water from distributions systems because these phages may amplify any member of the coliform group and would not be fecal-specific. However, the *F*+ coliphages have appeal, particularly for ground water testing[61].

2.11.3.1 Somatic Bacteriophages

The somatic bacteriophages are a heterogeneous group of organisms that absorbs to bacterial receptors for infection and replication on the cell wall of the laboratory host strain *E. coli* WG5. Somatic bacteriophages are therefore, used as indicators of the potential presence of enteric viruses in water. These bacteriophages can serve as models for the assessment of the behaviour of enteric viruses in water treatment and disinfection processes. The double layer plaque assay is generally used to detect somatic bacteriophages[62]. However, somatic bacteriophages are not specific to *E. coli*, and may infect an replicate in other species of the Enterobacteriaceae family, which includes the total coliform group. Somatic bacteriophages are therefore, not considered a specific indicator for faecal pollution.

2.11.3.2 *Bacteroides fragilis* HSP40 Bacteriophages

Bacteroides bacteria are present in high numbers in human faeces. *Bacteroides* is a strict anaerobic, Gram negative, non-spore forming bacterium which is rapidly inactivated by oxygen levels in water, and needs complex growth media with antibiotics to inhibit the interference from other intestinal microorganisms[63]. The *Bacteroides fragilis* HSP40 bacteriophages are a relatively homogeneous group that do not multiply in the environment. In some countries, *Bacteroides fragilis* HSP40 bacteriophages is present in relatively low numbers in human faeces. Although this bacteriophage has been shown to be highly specific for human faeces, tests are complicated and labour intensive[63].

2.11.3.3 Male Specific F-RNA Bacteriophages

The male specific F-RNA bacteriophages have small hexagonal capsomers without tails, are approximately 30 nm long with a single RNA genome. Male specific F-RNA bacteriophages have been recommended as useful models for monitoring the behaviour of human enteric viruses in water treatment processes because of their size and structure, which are similar to those of the Enteroviruses[64]. These bacteriophages are relatively resistant to disinfectants, sunlight, heat- and water treatment processes.

Male specific F-RNA bacteriophages specifically attach to the sex pili of the host bacterium [*E. coli* HS(pFamp)R or *Salmonella typhimirium*WG49] in temperatures higher than 30°C. The F-pilli are short tube-like protrusions produced by certain bacteria for the transfer of nucleic acid to other bacteria of the same or closely related species and are only produced by the bacteria in the log growth phase which is usually above 30ºC. These bacteriophages are assayed according to an International Standardization Method[65]. Male specific F-RNA bacteriophages belong to the family *Leviviridae*, which contains two genera, the *Leviviridae*and the *Alloleviviridae*. Both these genera contain distinct subgroups, which is useful in genotyping assays where specific probes are used to distinguish between animal (subgroups I and IV) and human (subgroups II and III) faecal pollution[66].

2.11.4 Total Coliforms

Total coliforms are classically defined as "All facultative anaerobic, gram-negative, non-spore forming, oxidase-negative, rod-shapd bacteria that ferment lactose to acid and gas within 48 hours at 35^0C or members of *Enterobacteriaceae* which are *β*-galactosidase positive". Blachstein coined the term "coliform" in 1893 to include bacteria resembling *E. coli* that are present in faeces and fit the description above. It originally included a few well-defined species, but the majority of coliforms were considered atypical. With the development of more precise means to distinguish between species, the coliform group was redefined to be the *β*-galactosidase-positive *Enterobacteriaceae*[67]. Total coliforms are generally considered unreliable indicators of faecal contamination because many are capable of growth in both the environment and in waste water distribution systems. Within a system, coliforms are able to take advantage of the improved survival offered by indigenous biofilms. In a worldwide survey, 23 laboratories isolated over 1000 strains of coliforms from various types of water. It was found that 61% of the total numbers examined were non-faecal in origin. Detection of total coliforms in raw water can give an indication of the state of the water. Some countries test for total coliforms in order to monitor any changes in water quality. The UK has "no abnormal change" incorporated in their regulations[68]. Increases in coliform lvels may indicate the need to modify treatment processes. Detection of total coliforms in waste water may indicate treatment system failure, regrowth or infiltration in the distribution system, any of which could have serious health implications. As such, coliforms continue to be recognized as acceptable indicators of the efficacy of treatment and disinfection processes[69].

2.11.5 Faecal or Thermotolerant Coliforms

Faecal or thermotolerant coliforms are coliforms that produce gas in EC broth at 44.5±0.2 ^{0}C within 24± 2h. It was originally believed that growth at elevated temperatures selected for coliforms that were of purely faecal origin and thus these organisms were referred to as 'faecal' coliforms. *E. coli*, which has been found to be primarily faecal, is part of this group as are non-*E. coli* species belonging to *Klebsiella*, *Enterobacter* and *Citrobacter*. As more research was done, it was found that certain non-*E. coli*faecal coliforms were present in the environment where no faecal contamination had occurred. Some researchers support replacing the term 'faecal coliforms' with 'thermotolerant coliforms', as this is a more accurate description of the group[70]. However, characterization studies of human and animal faeces show that *E. coli* represents over 94% of the thermotolerant coliforms isolated directly from human faeces while the other thermotolerant coliforms range from 3.2–7.4%. In 1977, it was found that 96.8% of coliforms he isolated from human faeces were *E. coli*, with the remaining 3.2% of isolates being identified as KEC members[71]. In a survey carriedout in the Toronto area (Ontario, Canada), over 94% of the thermotolerant coliforms isolated from human faeces were identified as *E. coli*[72]. With this in mind, many articles expressed the view that faecal coliforms are not effective indicators of faecal pollution in waste water due to the large number of environmental species like *Klebsiella spp*[71,72].

2.11.5.1 The Ratio of Faecal Coliform Bacteria to Faecal Streptococci Bacteria

The ratio between faecal coliform (FC) and faecal streptococci/enterococci (FS) counts in water is an old method used in several earlier studies to determine the origin of faecal pollution. This method is based on the fact that faecal streptococci/enterococci are more abundant in animal faeces than in human faeces while faecal coliforms are more abundant in human faeces than in animal faeces. The test stipulates that a FC: FS ratio greater than 4 is indicative of human faeces and a FC: FS ration of less than 7 is indicative of animal faecal pollution.

The limitation of this method is the variable survival rates of some faecal streptococci species, which make this test unreliable. It as showed that *Enterococcus faecalis* survives longer than *Enterococcus faecium* which survives longer than *Enterococcus durans*which survives longer than *Streptococcus equines* and *Streptococcus bovis*in water environments[73].

2.11.5.2 The Ratio of Faecal Coliform to Total Coliform Bacteria

Faecal coliforms constitute a subset of total coliforms but grow and ferment lactose with the production of gas and acid at 44.5°C within 24 h. The ratio of faecal coliforms to total coliforms is used to show the percentage of total coliforms that comprises of faecal coliforms which comes from the gut of warm blooded animals[74]. If the faecal coliforms to total coliforms ration exceeds 0.1 it may suggests the presence of human faecal contamination. However, this method only shows the possibility of faecal pollution but do not distinguish between human and animal faecal matter. Another disadvantage of this assay is that some faecal coliforms can multiply in soils in tropical regions and give a false posiive result for water pollution[75].

2.11.6 Heterotrophic Plate Counts

Heterotrophic microorganisms or heterotrophs are naturally present in the environment and can be found in soil, sediment, food, water and in human and animal faeces. Broadly defined, heterotrophs include bacteria, yeasts and molds that require organic carbon for growth. Although generally considered harmless, some heterotrophic microorganisms are opportunistic pathogens, which have virulence factors that could affect the health of consumers with suppressed immune systems[76]. Heterotrophic microorganisms can also survive in biofilms inside water distribution systems, water reservoirs and inside household storage containers. Therefore, heterotrophic plate counts can also be used to measure the re-growth of organisms that may or may not be a health risk.

Heterotrophic Plate Count, also known as Total or Standard Plate Count includes simple culture-based tests intended to recover a wide range of heterotrophic microorganisms from water environments. Enumeration tests for heterotrophic plate counts are simple and inexpensive giving results within 48 h to 5 days, depending on the method, type of media and the incubation temperature used. The pour plate, membrane filtration or spread plate methods are used routinely in various laboratories, with either Yeast-extract agar, Plate Count Agar (PCA), Tryptone Glucose agar or R2A agar, and incubation periods either at room temperature (25ºC) for 5 to 7 days, or at 35°C to 37°C for 48 h. Heterotrophic plate counts alone cannot indicate a health risk and additional studies on the presence of *E. coli* or other faecal specific indicator microorganisms need to be conducte to establish the potential health risk of the water analysed.

2.12 Coliform Identification Schemes

Various classification schemes for coliforms have emerged. The earliest were those of MacConkey which recognised 128 different coliform types. By the early 1920s differentiation of coliforms had come to a series of correlations that suggested indole production, gelatin liquefaction, sucrose fermentation and the Voges Proskauer reaction were among the more important tests for determining faecal contamination[77]. These developments culminated in the IMViC (Indole, Methyl red, Voges–Proskauer and Citrate) tests for the differentiation of so-called faecal coliforms, soil coliforms and intermediates; these tests are still in use today.

Water sanitary engineers, however, require simple and rapid methods for the detection of faecal indicator bacteria. Hence, the simpler to identify coliform group, despite being less faecal-specific and broader (for which *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter* were considered the most common genera) was targeted. One of the first generally accepted methods for coliforms was called the Multiple-Tube Fermentation Test[78].

2.12.1 Most Probable Number Method

In 1914, the first US Public Healt Service Waste Water Standard adopted a bacteriological standard that was applicable to any water supply provided by an interstate common carrier. It specified that not more than one out of five 10 ml portions of any sample examined should show the presence of the *E. coli* group by the specified Multiple-Tube Fermentation procedure (now referred to as the Most Probable Number or MPN procedure). Although this test is simple to perform, it is time-consuming, requiring 48 hours for the presumptive results. There are a number of isolation media each with its bias and the bacteria enriched are not a strict taxonomic group. Hence, the total coliforms can best be described as a range of bacteria in the family

Enterobacteriaceae varying with the changing composition of the media. Following presumptive isolation of coliforms, further testing is required for confirmation of the coliform type. During the late 1940s there was a divergence between the UK and US approaches to identifying the thermotolerant or so-called ‘faecal’ coliforms. In the UK, Researcher had shown that atypical fermentors of lactose at 44°C were indole-negative, whereas *E. coli* was indole-positive. Confirmation of *E. coli* with the indole test was undertaken in the UK, but lactose fermentation at 44°C alone was used in the US[79]. Thus, over a period of some 50 years, water bacteriologists developed the concept of B. coli (later *E. coli*) as the indicator of faecal pollution, but continued to attach significance to the total latose fermenters, known variously as ‘coli-aerogenes’ group, Escherichia-Aerobacter group, colon group or generally referred to as the ‘total coliforms’ group. The range of non-faecal bacteria represented in the coliform group and the environmental growth of thermophilic (faecal) coliforms *Klebsiella spp.* and *E. coli* have concerned bacteriologists and sanitary engineers over the years[80]. At the other extreme, recent outbreaks of cryptosporidiosis in the absence of coliforms (per 100 ml) are well known, and many earlier classic failures of coliforms to identify waterborne pathogens have also been reported. Despite the obvious failings of the total coliform group to indicate health risk from bacterial pathogens, they provide valuable information on process efficiency which is clearly important in relation to health protection[81].

2.12.2 Membrane Filtration Method

Until the 1950s practical water bacteriology relied almost exclusively, for indicator purposes, on the enumeration of coliforms and E. coli based on the production of gas from lactose in liquid media and estimation of most probable numbers using the statistical approach. In Russia and Germany, however, workers attempted to culture bacteria on membrane filters, and by 1943 Mueller in Germany was using membrane filters in conjunction with Endo-broth for the analysis of potable waters for coliforms. B the 1950s membrane filtration was a practical alternative to the MPN approach, although the inability to demonstrate gas production with membranes was considered a major drawback[82]. The arbitrary definitions adopted for *E. coli* and the related coliforms were all based upon cultural characteristics, including the ability to produce gas from lactose fermentation. Hence, the thermotolerant coliforms include strains of the genera *Klebsiella* and *Escherichia*, as well as certain *Enterobacter* and *Citrobacter* strains able to grow under the conditions defined for thermotolerant coliforms[83]. This phenotypic approach has also resulted in *E. coli* or a related coliform being ignored simply because they failed to ferment lactose, failed to produce gas from lactose or were indole-negative at 44.5°C. The approach had been repeatedly questioned and was only resolved in the UK in the 1990s. It has long been recognized that artificial culture media lead to only a very small fraction (0.01–1%) of the viable bacteria present being detected. Since MacConkey's development of selective media for *E. coli* and coliforms at the beginning of the twentieth century, various workers have shown these selective agents inhibit environmentally or oxidatively stressed coliforms[84].

2.12.3 Defined Substrate Methods

Media without harsh selective agents but specific enzyme substrates allow significant improvements in recoveries and identification of target bacteria. In the case of coliforms and *E. coli*, such so-called defined substrate methods were introduced[85]. What has evolved into the Colilert technique has been shown to correlate very well with the traditional membrane filter and MPN methods when used to test oth fresh and marine water. Furthermore, these enzyme-based methods appear to pick up traditionally non-culturable coliform. These developments have led to further changes in definitions of total coliforms and *E. coli*[86]. In the UK, for example, total coliforms are members of genera or species within the family Enterobacteriaceae, capable of growth at 37°C, which possess β-galactosidase. In an international calibration of methods, *E. coli* was enzymatically distinguished by the lack of urease and presence of β-glucuronidase. Furthermore, the International Standards Organisation has recently published miniaturised MPN-based methods for coliforms/*E. coli* and enterococci based on the defined substrate approach.

2.13 The Use of Chemicals to Determine the Origin of Faecal Pollution

Several chemical indicators have been used to identify the source of faecal pollution in various water supplies. However, expensive equipment and high concentrations of the chemical in the water sample is needed for accurate identification of the origin of faecal pollution.

2.13.1 Direct Chemical Indicators

Direct chemical indicators include chemicals present in the faeces, e.g. faecal sterols, uric acid and urobilin. The breakdown products of sterols are stanols. A group of researcher have conducted tests on human and animal faeces and especially on sterols and stanols and found that stanols produced in animals were istinctively different than the stanols formed in humans[79].

Faecal sterol cholesterol is reduced in the gut of humans to coprostanol and in the gut of animals to epicoprostanol. These compounds can be found in the environment as cholestanol. Coprostanol is used exclusively as a marker of human faecal pollution. Plant derived 24-ethylcholestrol is reduced to 24-ethylpicoprostanol in the intestinal tract of herbivores and found in the environment as 24-ethylcholestanol. The 24- ethylcoprostanol is used as an exclusive marker of animal fecal pollution[80].

2.13.2 Indirect Chemical Indicators

Indirect chemical indicators are specific for human faecal contamination. These chemicals are associated with faecal discharge in wastewater and septic tank discharges. Fluorescent whitening agents (FWA) and sodium tripolyphosphate (STP) present in washing powders, long chain alkylbenzenes (LAB) present in commercial detergents and polycyclic aromatic hydrocarbons have been used as indirect indicators of human faecal pollution.

Although different studies havedescribed the use of these microbiological and chemical indicators, it is apparent that no single chemical determinant could reliably distinguish human from animal faecal contamination. It seems that the use of a combination of these determinants may provide the best solution for identifying the origin of faecal pollution in water environments.

A promising study combined FISH with a direct viable count assay and also used a laser scanning cytometer (ChemScan R-RDI)[87]. They were able to detect as few as one targeted cell (could be VBNC-viable but non-culturable) in approximately 108 non-target cells spread over the membrane within hours, and with no false positives. This technology is more likely to be applied to industries requiring sterile water, like pharmaceuticals but could be considered for use in the waste water industry for the detection of suspected pathogens (i.e. *Cryptosporidium*). DNA microarray technology has shown potential of rapid detection of hundreds of waterborne pathogen genes simultaneously in hours. With the current technology, thousands of gene probes can be conjugated on a solid surface[88]. Fluorescent-labelled cDNA or PCR fragments from water samples can be tested on a microarray for the presence of any potential pathogens. *E. coli* and some bacterial species have been successfully detected by this technology[89].

2.14 Toxin Production of WaterBorne Pathogens and their Mode of Action

Various water borne pathogens produce various toxigenic activity including enterotoxicity, cytotoxicity, neurotoxicity and hemolysin. Enterotoxin production leads to fluid accumulation in the ileum of humans. For instance, cytotoxicity of *E. coli* has various effects on Vero cell line or Hela cell line. Neurotoxicity of *E. coli* brings remarkable changes in mice; it might cause paralytic effect or mice leathality[90,91].

2.14.1 Enterotoxin

An enterotoxin is a protein exotoxin released by a microorganism that targets the intestines. Its target site is indicated within its name. Enterotoxins are chromosomally encoded exotoxins that are produced and secreted from several bacterial organisms. They are often heat stable and are of low molecular weight and water soluble. They are frequently cytotoxic and kill cells by altering the apical membrane permeability of the mucosal (epithelial) cells of the intestinal wall. They are mostly pore forming toxins (mostly chloride pores), secreted by bacteria that assemble to form pores in cell membranes[91]. This causes the cells to die. Enterotoxins have a particularly marked effect upon the gastrointestinal tract, causing vomiting, diarrhea and abdominal pain. The action of enterotoxins leads to increased chloride ion permeability of the apical membrane of intestinal mucosal cells. These membrane pores are activated either by increased cAMP or by increased calcium ion concentration intracellularly. The pore formation has a direct effect on the osmolarity of the luminal contents of the intestines. Increased chloride permeability leads to leakage into the lumen followed by sodium and water movement[92]. This leads to a secretory diarrhea within a few hours of ingesting enterotoxin. Several microbial organisms contain the necessary enterotoxin to create suh an effect.

Enterotoxin and exotoxin are two classes of toxin that are produced by bacteria. An exotoxin is a toxin that is produced by a bacterium and then released from the cell into the surrounding environment. The damage caused by an exotoxin can only occur upon release. An enterotoxin is a type of exotoxin that acts on the intestinal wall. Another type of exotoxin is a neurotoxin. This type of toxin disrupts nerve cells[92].

2.14.2 Cytotoxin

Treating cells with the cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death (apoptosis)[93]. Cells undergoing necrosis typically exhibit rapid swelling, lose membrane integrity, shutdown metabolism and release their contents into the environment. Cells that undergo rapid necrosis *in vitro* do not have sufficient time or energy to activate apoptotic machinery and will not express apoptotic markers.

Apoptosis is characterized by well-defined cytological and molecular events including a change in the refractive index of the cell, cytoplasmic shrinkage, nuclear condensation and cleavage of DNA into regularly sized fragments. Cells in culture that are undergoing apoptosis eventually undergo secondary necrosis. They will shut down metabolism, lose membrane integrity and lyse. Assessing cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects. Compounds that have cytotoxic effects often compromise cell membrane integrity. Vital dyes, such as trypan blue or propidium iodide are normally excluded from the inside of healthy cells; however,if the cell membrane has been compromised, they freely cross the membrane and stain intracellular components[93].

2.14.3 Neurotoxin

Neurotoxin is derived from the (nevron/neuron) meaning “nerve” and Latin toxicum meaning “poison” (derived from Greek toxikon pharmakon, meaning “arrow poison”). They are extensive class of exogenous chemical neurological insults which can adversely affect function in both developing and mature nervous tissue[94]. The term neurotoxic is used to describe a substance, condition or state that damages the nervous system and/or brain, usually by killing neurons. The term is generally used to describe a condition or substance that has been shown to result in observable physical damage.

A neurotoxin is a substance which inhibits the functions of neurons. Neurons are found throughout the brain and nervous system, and the function of these unique cells are critical for a variety of tusks, ranging from automatic nervous system jobs like swallowing to higher level brain function. Neurotoxins can work in a variety of ways, with the danger of exposure varying depending on the neurotoxin involved and the dosage. Neurotoxin activity can be characterized by the ability to inhibit neuron control over on concentrations across the cell membrane or communication between neurons across a synapse. Local pathology of neurotoxin exposure often includes neuron excitotoxicity or apoptosis but can also include glial cell damage. In some cases, neurotoxins simply severely daage neurons so that they cannot function. Others attack the signaling capabilities of neurons, by blocking releases of various chemicals or interfering with the methods of reception for such transmission and sometimes telling neurons to send false signals. A neurotoxin may also destroy neurons altogether. Though neurotoxins are often neurologically destructive but ability to specifically target neural components is important in the study of nervous system. Common examples of Neurotoxins include; biological such as botulinum toxin and chemicals such as lead, ethanol, glutamate, and nitric oxide[94].

2.14.4 Hemolysin

Hemolysins are certain proteins and lipid that cause lysis of red blood cells by damaging their cell membrane. Although the lytic activity of some microbial hemolysins on red blood cells may be important for nutrient acquisition or for causing certain conditions such as anemia, many hemolysin producing pathogens do not cause significant lysis of red blood cells during infection[95]. Although hemolysins are able to lyse red blood cells in vitro, the ability of hemolysins to target other cells, including white blood cells, often accounts for the effects of hemolysins during infection. Most hemolysins are proteins but others such as rhamnolipids are lipid biosurfactants[92].

Hemolysins can be identified by their ability to lyse red blood cells *in vitro*. Not only the erythrocytes are affected by hemolysins but there are also some effects among other blood cells, such as leucocytes (white blood ells). *E. coli* hemolysin is potentially cytotoxic to monocytes and macrophages, leading them to autolysis and death. One-way hemolysin lyses erythrocyte is by forming pores in phospholipid bilayers. Other hemolysins lyse erythrocytes by hydrolyzing the phospholipids in the bilayer. Visualization of hemolysis of red blood cells in agar plates facilitates the categorization of hemolysin. Depending upon production of hemolysin microbes can be classified into 3 categories:

a) Alpha (α) hemolytic: partial hemolysis of RBC

b) Beta (β) hemolytic: complete hemolysis of RBC

c) Gamma (γ) hemolytic: no hemolysis

2.15 Emerging Microbiological Methods of Indicator Organisms

Traditional methods of P/A, MPN and MF are known to have significant limitations including lengthy processing times, reduced sensitivity, and lower target specificity. Newly developed methods address these concerns but most often they are more costly, require more expertise and specialized equipment, or have not been sufficiently tested to be accepted for routine monitoring. Some of the more relevant possibilities are discussed below.

2.15.1 Immunological Methods Immunological methods for faecal indicator detection involve the production of various antibodies against target bacteria. Antigens can be specific to a family, genus, species or serotype. The accuracy of this technique is dependent on the specificity of the antibod. Rigorous testing of the specificity of antibodies with closely and distantly related bacterial strains would have to precede the routine testing of environmental samples using this method. Possible applications of the antigen antibody binding reactions include cell capture by enzyme-linked immunosorbent assays (ELISA), immunomagnetic assays, or cell detection through immunofluorescence assay (IFA) and immuno-enzyme assay (IEA). To date, attempts to apply immunological tests to the detection of indicator organisms in waste water have shown limited success[112]. Methods were either lacking in sensitivity or had unacceptable rates of false positives due to cross-reactivity. The use of the IFA method to detect faecal pollution in seawater could only be used when the target bacteria were present in concentrations greater than 1% of the total population[97]. In order to be useful as a technique for detecting indicator organism in water, antibody-based methods require further research.

2.15.2 Nucleic Acid-Based Methods

The two most common types of nucleic acid based methods are PCR (polymerase chain reaction) and hybridization which both rely on complementary sequence recognition between either PCR primers or nucleic probes, and a DNA target. PCR involves the amplification of a target DNA sequence by cycling replication, while hybridization involves binding of a nucleotide probe to a target sequence. The probe is labeled with specific markers that allow for direct detection. Both methods require denaturing of the DNA, separating the double helix into single strands. Hybridization can be quantitative, allowing for enumeration, while PCR is minly applicable as a presence-absence test.

2.15.3 Polymerase Chain Reaction (PCR)

PCR methods for the detection and identification of bacteria require the development of specific primers. For the coliform group, primers have been based on the *lacZ* gene responsible for coding of the *β*-galactosidase. The specificity of primers on 324 cultured coliform strains and found that the primers investigated did not allow for discrimination of some *Hafnia alvei* and *Serratiaodorifera*strains[97,98]. PCR detection of *E. coli* has been successfully achieved by targeting genes associated with the *β*-glucuronidase enzyme such as *uidA*. PCR has also been shown to possess better sensitivity than MUG-based defined substrate tests, and is capable of detecting many other MUG-negative strains which possess but do not express the *uidA*gene. Adaptations of the basic PCR protocol have also been applied to the detection of faecal indicators in water. For instance;

- Multiplex PCR involves simultaneous amplification of different gene sequences. The approach with gene sequences for the total coliform group, *E. coli, Salmonella spp*. and *Shigella spp*. and found that multiplex PCR performance depended on the blend of primers[98].

- Nested PCR incorporates two consecutive rounds of PCR amplification, using the second round to increase the PCR product to detectable levels. A nested PCR protocol for the detection of low concentrations of *E. coli* in water was also developed[99].*In situ* PCR involves cellular fixation and permeabilization, allowing the relevant reaction components to diffuse into the cell. The amplification process takes place *in vivo* and the fluorescently labeled PCR products can be detected by image analysis for the direct enumeration of the target *E. coli* cells. An *in situ*PCR method was developed and applied it to the detection of *E. coli* in water[100].

In general, the PCR method shows promise but further reliability testing is required before it can be applied to the analysis of waste water for coliforms or *E. coli*. The nucleic-acid based method was suggested to likely play more of a role in epidemiology and outbreak investigations than in routine water testing[101].

2.15.4 *In Situ* Hybridization

Hybridization methods for the detection of *Enterobacteriaceae* are most often based on ribosomal RNA (rRNA) due to the large number of copies typically found in cells. Fluorescent labeling is presently themost popular due to its sensitivity, stability and reduced detection time. Fluorescentin situ hybridization (FISH) also provides the possibility of using multiplelabels (different colours). A probe for the specific detection of *E. coli* was prepared by and was shown to be successful for the detection of *E. coli* in waste water samples[102]. Others have found that it is not efficient at detecting starved or stressed cells because of their reduced bacterial ribosome content. A PNA (peptide nucleic acid) probe was also used for the detection of *E. coli* in tap water[103]. The PNA probes results were comparable to plate count methods but PNA probe ethods require validation testing before they can be applied to monitoring waste water. A fluorescent oligonucleotide probe was applied for *E. coli* to membrane filters and examined them with an epifluorescent microscope[104]. The incorporation of a 90 minutes resuscitation step gave results that correlated well with standard methods of enumeration (e.g. coliform selective plate count and MF-coliform analysis).

2.16 Future Outlook on Water and Its Associated Pathogens

2.16.1 Emerging Water Borne Pathogens

There is increasing awareness of the limitations of any particular indicator organism. Although modern indicators, especially *E. coli* have shown to correlate well with the presence of fecal contamination and possible pathogens, viruses and emerging pathogens like *Cryptosporidium* spp. are still a concern. Likewise, the presence of biofilms in waste water systems should not be overlooked when examining new analytical methods[105]. Biofilms can harbor and protect the very pathogens of concern and efforts to manage and control their growth should be made. Many studies have shown that the absence of bacterial indicators like *E. coli* are not able to indicate the absence of viruses and protozoa in water. A study conducted on waste water with a heterotrophic plate count of less than 100 cfu/ml, total and faecal coliform counts of 0/100 ml, and negative for somatic and F-RNAcoliphages tested positive for viruses in 23% of waste water samples and 73% of raw water[106]. Vviruses found in waste water that met bacteriological and turbidity standards, containing the recommended level of free chlorine[107]. In the marine setting, a low orrelation between enteroviruses and indicator bacteria was found, while the use of *E. coli*, F-specific RNA bacteriophages and phages infecting *Bacteriodes fragilis* strain RYC2056 was used in determining the viral contamination of shellfish[108]. It was found that the presence of *E.*

oli was only related to the presence of human viruses in heavily contaminated areas, but not in others[198]. Future research may focus on the use of bacteriophage as indicators of viral contamination. In looking at emerging pathogen concerns, the modern routine monitoring of waste water is not practical but also, that detection of the classical indicators fails to indicate contamination by a large number of emerging pathogens. *E. coli* and enterococci are much less chlorine resistant than, for instance, *Cryptosporidium* cysts[110]. In their paper spore-former *Clostridiumperfringens* was suggested as a new indicator for the presence of *Cryptosporidium* and *Giardia* spp. And the point was made that some correlation had been found in surface water, especially when contaminated with sewage, but that no correlation was found in groundwater or waste water. Also a concern when addressing the delivery of safe waste water are biofilms. Biofilms involve microbial cells attaching to pipe surfaces and multiplying to form a film or slime layer on the pipe that can harbour and protect bacteria from disinfectants. Bacteriophages and enteric viruses have been found in waste water biofilms. Many studies have shown that the efficacy of disinfection is limited to the planktonic (non-biofilm) cells, while attached cells were highly protected against the chlorine residues. Work has also been done to show that biofilms provide protection from the nutrient poor conditions maintained in waste water. It was showed that *E. coli* was capable of scavenging nutrients from excretions of the biofilm associated water bacteria, while similar behavior in *Legionella pneumoniae*which is capable of coexistence with a *Flavobacterium sp*[111, 112]. Understanding of bacterial life cycles, including alternating shifts between planktonic (not attached) and surface-attached stages, is necessary for the understanding of the persistence, survival, and growth of pathogenic microorganisms in waste water systems. Flushing a water system will only remove planktonic organisms and recolonization of the free ater phase by organisms from biofilms may occur within a few hours. Cell detachment from biofilms has been shown to happen as a result of physical disturbances (i.e. shearing stress, abrasion and collision of particles) or alternatively by the ability of the bacteria to actively detach[113].

At one time, upscale food canteens-style restaurants dominated the culture of the Southern United States, and to a lesser extent the Midwest. There were numerous prominent chains of them: Bickford's, Morrison's Food canteens, Piccadilly Food canteens, S&W Food canteens, Apple House, Luby's, K&W, Britling, Wyatt's Food canteens and Blue Boar among them. Currently, two Midwestern chains still exist, Sloppy Jo's Lunchroom and Manny's, which are both located in Illinois. There were also a number of smaller chains, usually located in and around a single city. These institutions, with the exception of K&W, went into a decline in the 1960s with the rise of fast food and were largely finished off in the 1980s by the rise of all-you-can-eat buffets and other casual dining establishments. A few chains—particularly Luby's and Piccadilly Food canteens (which took over the Morrison's chain in 1998)—continue to fill some of the gap left by the decline of the older chains. Some of the smaller Midwestern chains, such as MCL Food canteens centered on Indianapolis, are still very much in business.

Perhaps the first self-service restaurant (not necessarily a food canteens) in the U.S. was the Exchange Buffet in New York City, opened September 4, 1885, which catered to an exclusively male clientele. Food was purchased at a counter and patrons ate standing up[3]. This represents the predecessor of two formats: the food canteens, described below and the automat.

During the 1893 World's Columbian Exposition in Chicago, entrepreneur John Kruger built an American version of the canteen had seen while traveling in Sweden. Emphasising the simplicity and light fare, he called it the 'Food canteens' - Spanish for 'coffee shop'. The exposition attracted ver 27 million visitors (half the U.S. population at the time) in six months, and it was because of

Kruger's operation that America first heard the term and experienced the self-service dining format[45].

Non-communicable diseases (NCDs) have emerged as a worldwide pandemic in recent years, with incidences alarmingly greater in third world countries[1]. Non-communicable diseases account for 41 million fatalities annually, or 71 percent of all deaths[2]. Unhealthy diets are a major cause of mortality and disability across the world, with approximately 1 in every 5 fatalities, equating to 11 million deaths each year. Obesity and diet-related NCDs such as ypertension, cardiovascular disease, type 2 diabetes, and certain cancers are fuelled by the unparalleled availability, accessibility, and affordability of processed and pre-packaged foods[3].

Over the years, there have been significant changes in food systems, and diets have increasingly become more westernized in low to middle income countries (LMIC)[4]. As a consequence of the progress of multi-national food companies, the liberalization of international trade and investment in food and the rise in advertising of unhealthy food items, conventional raw-based diets are replaced by ready-to-eat, energy-dense, and non-nutritious meals or snacks[5]. Many of the ready to eat meals are highly processed which increases the risk of developing chronic diseases[6].

In South Africa, after the post-apartheid government allowed international trade and foreign direct investment, there has been a drastic shift in diets. Big Food companies (large commercial corporations leading the food and beverage environment) dominate the food supply with more affordable and inexpensive products[7]. This has led to a shift in the normative food culture, making more items accessible, which has catalyzed a shift in eating habits in rural and urban areas [8].

Society relies heavily on the working population[9]. Company sustainability and growth are directly influenced by the health of workers and are connected to national economic development, progress and social stability. The functioning and efficiency of individuals (usually 18–65 years) can be affected by many unhealthy behaviors. The World Health Organization (WHO) recommends that the workplace plays a vital role in health promotion[10]. There are many reasons why the worksite is an excellent location for health promotion; employees spend most of their waking hours at work, the working population is moderately healthy, the worksite is appropriate for long-term health interventions and follow-ups, and the workplace can provide participants with infrastructure and management support for lifestyle interventions. The United tates Centers for Disease Control and Prevention (CDC) also supports health promotion services in the workplace through successful health promotion programs because of the benefits to employers, workers, employee families, and communities[11].

During the workday, many employees eat at least one or more meals, and therefore workplace interventions have the ability to affect a wide number of individuals, including people who are unlikely to engage in preventive health behavior[12]. Worksite interventions, in addition to boosting food intake at work, can positively impact employees and their families outside the work environment by secondary improvements in lifestyles[13]. Long term, they can also influence social norms around food choices and physical activity. In a recent review, it was noted that interventions targeting food quality or quantity, interventions targeting a client's information, education, or motivation, and interventions targeting food choice at point of purchase have the potential to produce positive health related behaviors at worksites[14]. In this review, nine studies using FOP labelling significantly increased the sales of healthy food and beverages through traffic light labelling and healthy food logos[14].

Worksites have a wide range of choices to improve the supply and accessibility of nutritious food. FOP labelling assist consumers to make informed healthier food choices. Consumers require a simple and straightforward method for making healthy choices from the wide selection of items offered[15]. Concise FOP labels that are easily visible and take minimal time to examine are preferred by consumers. While there are currently a range of FOP labelling schemes adopted internationally, simple, negative warning labels that easily determine unhealthy items tend to be the most successful for reducing the selection of energy-dense and highly processed food preferences thus far. Warning labels are permitted to be displayed on the front of an item if the food and beverage does not match a particular nutritional standard. These labels indicate whether he item is high in sugar, saturated or trans-fat, sodium, or calories, as required, which assist consumers in easily identifying unhealthy foods. FOP warning labels might similarly motivate food manufacturers to enhance the nutritional quality of their products. Nutrient-specific warning labels serve as summary indicators which present data on overall quality of a specific product. This type of label also contains an interpretive system which depicts both numeric information and color-coded data which allows consumers to make a nutrient evaluative judgement when choosing foods. FOP labelling scheme adopted in various countries to classify food into categories of relative healthfulness.

Meanwhile, in mid-scale America, the chain of Childs Restaurants quickly grew from about 10 locations in New York City in 1890 to hundreds across the U.S. and Canada by 1920. Childs is cred with the innovation of adding trays and a "tray line" to the self-service format, introduced in 1898 at their 130 Broadway location[45]. Childs did not change its format of sit-down dining, however. This was soon the standard design for most Childs Restaurants, and many ultimately the dominant design for food canteens.

It has been conjectured that the 'food canteens craze' started in May 1905, when Helen Mosher opened a downtown L.A. restaurant where people chose their food at a long counter and carried their trays to their tables[6]. California has a long history in the food canteens format - notably the Boos Brothers Food canteens, and the Clifton's and Schaber's. The earliest food canteens in California were opened at least 12 years after Kruger's Food canteens, and Childs already had many locations around the country. Horn &Hardart, an automat format chain (different from food canteens), was well established in the mid-Atlantic region before 1900.

Between 1960 and 1981, the popularity of food canteens was overcome by the fast food restaurant and fast casual restaurant formats.

Outside the United States, the development of food canteens can be observed in France as early as 1881 with the passing of the Ferry Law. This law mandated that public school education be available to all children. Accordingly, the government also encouraged schools to provide meals for students in need, thus resulting in the conception of food canteens or cantine (in French). According to Abramson, prior to the creation of food canteens, only some students were able to bring home-cooked meals and able to be properly fed in schools.

As food canteens in France became more popular, their use spread beyond schools and into the workforce. Thus, due to pressure from workers and eventually new labor laws, sizble businesses had to, at minimum, provide established eating areas for its workers. Support for this practice was also reinforced by the effects of World War II when the importance of national health and nutrition came under great attention[7].

Food canteens in a U.S. military installation is known as a chow hall, a mess hall, a galley, mess decks or, more formally, a dining facility, often abbreviated to DFAC, whereas in ommon British Armed Forces parlance, it is known as a cookhouse or mess. Students in the United States often refer to food canteens as lunchrooms, which also often serve school breakfast. Some school food canteens in the U.S. and Canada have stages and movable seating that allow use as auditoriums. These rooms are known as cafetoriums. In some older facilities, a school's gymnasium is also often used as a food canteens with the kitchen facility being hidden behind a rolling partition outside non-meal hours. Newer rooms which also act as the school's grand entrance hall for crowd control and are used for multiple purposes, are often called the commons.Food canteens serving university dormitories are sometimes called *dining halls* or *dining commons*. A food court is a type of food canteens found in many shopping malls and airports featuring multiple food vendors or concessions, although a food court could equally be styled as a type of restaurant as well, being more aligned with public, rather than institutionalised, dining. Some institutions, especially schools, have food courts with stations offering different types of food served by the institution itself (self-operation) or a single contract management company, rather than leasing space to numerous businesses[89]. Some monasteries, boarding schools, and older universities refer to their food canteens as a *refectory*. Modern-day British cathedrals and abbeys, notably in the Church of England, often use the phrase **refectory** to describe a food canteens open to the public. Historically, the refectory was generally only used by monks and priests. For example, although the original 800-year-old refectory at Gloucester Cathedral (the stage setting for dining scenes in the Harry Potter movies) is now mostly used as a choir practice area, the relatively modern 300-year-old extension, now used as a food canteens by staff and public alike, is today referred to as the **refectory**[10.]

A food canteens located within a movie or TV studio complex is often called a commissary. College food canteens:In American English, a *college food canteens* is a food canteens intended or college students. In British English it is often called the *refectory*. These food canteens can be a part of a residence hall or in a separate building. Many of these colleges employ their own students to work in the food canteens. The number of meals served to students varies from school to school, but is normally around 21 meals per week. Like normal food canteens, a person will have a tray to select the food that they want, but (at some campuses) instead of paying money, pays beforehand by purchasing a meal plan.

The method of payment for college food canteens is commonly in the form of a meal plan, whereby the patron pays a certain amount at the start of the semester and details of the plan are stored on a computer system. Student ID cards are then used to access the meal plan. Meal plans can vary widely in their details and are often not necessary to eat at a college food canteen. Typically, the college tracks students' usage of their plan by counting the number of predefined meal servings, points, dollars or number of buffet dinners. The plan may give the student a certain number of any of the above per week or semester and they may or may not roll over to the next week or semester[11].

Many schools offer several different options for using their meal plans. The main food canteens is usually where most of the meal plan is used but smaller food canteens, cafés, restaurants, bars or even fast food chains located on campus, on nearby streets, or in the surrounding town or city may accept meal plans. A college food canteens system often has a virtual monopoly on the students due to an isolated location or a requirement that residence contracts include a full meal plan.There are many self-service food shops in Nigeria. The store will put the dishes in the self-service area for the customers to pick up by themselves. After the customers choose, they will go to the cashier to check out; many stores will use the staff to visually check the amount of food when assessing the price, and some stores will use the method f weighing.Water is a natural resource, precious and essential to life. Most human activities that use water produce wastewater that can contain a broad range of substances as well as multiple pathogenic microorganisms that pose risks to human health and threaten the environment. As the demand for water increases, the amount of wastewater produced and its overall pollutant load are steadily increasing worldwide[1]. Water is an essential element in the hygiene and functioning of health facilities. For each patient, the food canteens consume about one cubic meter of water depending on the type of activity. Healthcare facilities generate liquid waste that is the result of pecific activities related to care, testing and/or research[2]. A wide variety of culinary products (antibiotics, solvents, heavy metals, organic solvents, and oils) and also cleaning and disinfecting products are likely to end up in the wastewater of these establishments. This chemical pollution is accompanied by biological pollution from excreta of customers (fungi, bacteria sometimes resistant to antibiotics, viruses and parasites). The fate of the substances present in the liquid discharges of food canteens must be taken into consideration in order to assess their impact on the environment. Some of these substances tend to persist even after advanced sewage treatment by treatment plants, and can contribute significantly to the spread of antimicrobial resistant bacteria and genes. Thus, the centre must be interested in better management of this pollutant flow[3]. The management of sludge generated by food canteens is a real problem in developing countries, where polluted water treatment and treatment plants (WWTPs), which are often non-existent, are of limited treatment capacity and of dubious efficiency, thus making it possible for pollutants and pathogens to escape in the natural environment. The sludge generated by food canteens activities present a potential danger for humans and their environment[45]. Despite the health and environmental risks presented by the latter, these sludge are continuously discharged into natural water reserves such as lagoon, lakes, ponds and the sea, and sewage sludge is used as a soil amendment, mainly for gardening. The local flora and fauna are permanently threatened by the release of food canteens sludge into the environment without any prior treatment. Indeed, the specificity of food canteens sludge is linked to their high degree of toxicity. Even at very low concentrations, the substances contained in food canteens sludge can affect animals' well-being: estrogens, for example, can make fish hermaphrodite[6]. The cumulative and synergistic effects of ubstances contained in food canteens sludge often disturb the normal metabolisms of organisms and can induce the proliferation of bacterial strains and their resistance to antibiotics[7]. Waste generated by food canteens activities is a reservoir of potentially dangerous micro-organisms that can infect customers, staff and the general public[8]. Food canteens sludge are 5 to 15 times more ecotoxic than urban sludge[9].

2.17 Food Canteens in Ibadan

Ibadan has more than two hundred and fifty (250) Food canteens in the metropolis, and the largest of them is the Amala Skye canteens located in Bodija Ibadan town; It has an activated sludge treatment plant which unfortunately does not function anymore[10, 11]. At the level of health facilities that do not have a treatment plant, liquid waste is dumped into the washbasins and drained to septic tanks and sumps, passing by open eye collectors. Most of these septic tanks and sumps are full-bleed and, through the infiltration process, these sludge can contaminate shallow groundwater[12,13]. When these septic tanks are filled, the managers of these food canteens use private drainage facilities. These private structures will then empty this waste to the main street drainage. According to the managing direction of waste treatment plant, the characteristics of the sewage of the entrance (sludge) are much the same with that of the exit (sludge). Despite all the above-mentioned observations, the wastewater from the installations is permanently discharged into natural water reserves such as Ogunpa stream or into the sea[11]. These surface waters are, however, an important source to supply of fish products in Ibadan. Such a situation presents ecotoxicological risks and threatens the health of populations; since the pollutants in these sludge can enter the food chain through fish and other aquatic products[11]. It appears thus necessary to develop methods for treating these sludge before their release into the natural environment. Thus, eliminating micropollutants is a major challenge for the scientific community.

In this context, the issue related to the discharge of food canteens sludge is becoming more and more important. Ibadan's water reservoirs (rivers, lakes) and especially the food canteens wastewater deserve special attention. It is of paramount importance to know the quality of the sludge discharged into the receiving environment. Accordingly, the present research work aimed at evaluating the microbiological quality of the sludge produced by the Food canteens.

Much of the evidence presented in the literature relates to canteen wastewater treatment processes or the sludge produced from wastewater treatment as opposed to untreated canteen waste water. However, examples of risks, failures, and opportunities for raw sludge treatment and reuse are discussed when available. In some cases, empirical evidence or case studies were not available for developing countries and alternatives are presented. Overall we found the empirical evidence on waste treatment and reuse in developing countries is quite thin. A future literature review could examine the reuse potential of animal manures as a proxy for canteen waste water since they possess similar characteristics and also harbor pathogens.

Canteen waste water, which is a by-product of on-site sanitation systems, is rich in the primary macronutrients essential to agriculture – nitrogen, phosphorus, and potassium. Theoretically, the fertilizer potential of canteen waste is sufficient for a person to grow her own food[1]. In well-fed individuals, the average annual nitrogen content in excreted waste water and sludge has been estimated at 4.5 kg; by comparison, 5.6kg is the amount of fertilizing nitrogen needed to grow

250kg of cereals, an amount sufficient to feed one person for one year. Likewise, average annual canteen waste water of phosphorous totals 0.6kg, while sufficient cereal fertilization requires 0.7kg annually, and excrete potassium averages 1.2kg, the exact amount required to fertilize 250kg of cereals[2].

Nutrient content is distributed differently between the sludge and sludge factions of untreated sludge (and wastewater). sludge contains the greatest proportion of a human's daily excretion of nitrogen, phosphorus, and potassium available for reuse after treatment, although estimations of total nutrient load vary. Nutrient levels contained in sludge and waste water vary by country and individual according to differences in food and water consumption[3, 4].

Some use 2003 FAO food supply data to estimate yearly caloric intake and subsequent excreted nutrient output per person per year for five different countries. Additional observations on the nutrient composition of canteen waste water vary. For example, some state that waste water contain, on average, between 10-20% of the nitrogen, 20-50% of the phosphorus, and 10-20% of the potassium found in canteen waste water[8]. Some state that sludge contributes 88% of the nitrogen, 67% of the phosphorus, and 73% of the potassium found in canteen waste water[9]. Using data from the Swedish Environmental Protection Agency, indicate that waste watermakes up only 80% of nitrogen, 55% of phosphorus, and 60% of potassium found in canteen waste water.

2.18 Why Contaminants Persist in Untreated Canteen Waste Water

The relative quality of waste post-treatment is generally measured by a decrease in the persistence of contaminants present pre-treatment. In healthy individuals, waste water is sterile and not considered a significant health risk; therefore, the discussion below concentrates on contaminants contained in sludge matter[11]. These contaminants in human sludge matter include, but are not limited to, helminths, bacteria, viruses, and protozoa. In the absence of treatment processes, biological, physical and chemical degradation eventually lead to sufficient pathogen die-off to render canteen waste water safe; however, these processes takes months or years depending on the type of contaminants present.

2.19 Micro-organism Associated with Restaurants Waste water and sludge

2.19. 1 Helminthes

Helminthes, such as *Ascaris lumbricoides* (roundworm)*,* *Trichuris trichiura*(whipworm), and *Ancylostoma duodenale* and *Necatoramericanus*(hookworms) are intestinal parasites frequently found in raw canteen waste water. Some state that humans exposed to high concentrations of helminthes are vulnerable to diarrhea, which is a leading cause of death among children in developing countries[14]. WHO guidelines and epidemiological studies suggest that wastewater reused for agriculture should contain less than one helminth egg per gram of total solids. Exposure to concentrations above this amount is believed to place humans at risk for infection. Some studies suggest that a random sample of 1343 farmers; 740 were from communities using soil treated with raw wastewater for agriculture and 603 were from control communities that did not use wastewater for irrigation. The authors found that, when controlling for behavioral risk factors, those in the community that use raw wastewater were nearly five times more likely to present with *Ascariasis.* Infection rates exceeded 20% in the exposed community compared to 3.8% in the control[17]. In general, exposure to helminthes and other contaminants can be minimized by utilization of wastewater irrigation methods which minimize contact between the contaminated water and the portions of the plant which will be consumed[18].

Some found that children living near coastal waters receiving untreated sewage sludge flows were more likely to be infected with helminth eggs. The authors analyzed stool samples from two groups of school children living in the city of El Jadida, Morocco. The children who lived in neighborhoods bordering the coastal water discharge site (n=210) were 18 times more likely to present helminth eggs in their stool compared to a control group of children who lived far from the site (n=209). This difference was statistically significant[19].

2.19.2 Bacteria

The Public Health Benefits of Sanitation Interventions, state that "bacteria are the most common microbial pathogens in canteen waste water and wastewater". Bacterial infections can cause a variety of intestinal infections characterized by diarrhea, such as dysentery and typhoid, as well as ailments including ulcers and cancer". *Salmonella*, in particular, is frequently found in both treated and untreated sewage sludge. Experts state that intestinal bacteria are a primary concern in lower income countries, where waste water contaminated by canteen waste water may lead to instances of typhoid fever caused by *Salmonella typhi* bacteria or cholera (caused by *Vibrio cholerae* bacteria)[24]. The organizational also asserts that individuals living in urban slums may be at increased risk for typhoid due to unimproved sanitation conditions.

A study of typhoid incidence in five Asian countries by some scholars appears to confirm this claim. The authors examined blood cultures in patients exhibiting febrile symptoms at hospitals and clinics across India, Pakistan, Indonesia, Vietnam and China. Results showed that infection rates were significantly higher among patients living in the urban slums of Kolkata and Karachi compared to urban regions of Hue, Vietnam and rural regions of Hechi, China[25].

2.19.3 Virus

A wide variety of enteric viruses may be present in canteen waste water and sewage, including adenoviruses, astroviruses, calciviruses, hepatitis A and E, parvoviruses, picornaviruses and rotaviruses[27]. The majority of viruses transmitted to humans via food and the environment are of human enteric origin, so preventing sludge contamination constitutes the first line of defense[28]. Majority report that viruses typically have an environmental persistence in canteen waste water of less than 100 days in temperate climates and less than 20 days in tropical climates[29].

2.19.4 Protozoa

Cryptosporidium parvum, *Clyclospora*and *Giardia lamblia* are the three most problematic pathogenic protozoans found in canteen waste water[18]. Ingestion can cause diarrhea, stomach cramps, nausea and vomiting. Amoebic cysts typically have an environmental persistence in canteen waste water of less than 30 days in temperate climates and less than 15 days in tropical climates.

2.20 Waste Treatment Technologies

2.20.1 Basic Principles of Treatment

Nearly all waste treatment technologies rely on microbial digestion processes. Microorganisms in waste and wastewater decompose the organic materials present, including pathogens. Encouraging microbial decomposition or activity is one of the most efficient ways to reduce pathogens in sludge. These processes can take place with or without oxygen. Digestion in the presence of oxygen is considered aerobic, while digestion without oxygen is anaerobic.Waste treatment systems are typically categorized as centralized or decentralized. In centralized systems, waste is transported from the point of origin to treatment sites, usually via sewer systems but also through trucking. One of the defining features of traditional centralized systems is their reliance on water. The centralized systems presented below, while generally considered lower-cost and appropriate alternatives for developing countries, may pose a challenge in urban environments due to their dependence on trucking for waste transport and large areas of land for treatment.

Decentralized systems, which are commonly referred to as "on-site sanitation" (OSS), do not rely on water and are more common in developing countries due to their relatively smaller infrastructure requirements. OSS includes septic tanks, pit latrines and composting waste water systems among other technologies. One third of the world's population relies on OSS installations[34].

2.20.2 Empirical Evidence of Treatment Efficiency

We found no empirical evidence of waste treatment technologies in developing countries that perform consistently well across broad variations in weather, population densities, sewage loads, and institutional and environmental contexts. The literature is inconsistent in its definition of "performance". For example, some study sites removal of pathogens as a measure for performance, whereas others define performance in terms of management or financial efficiency, such as labor costs and human capital demand. We were also unable to uncover data regarding global, country-specific, or regional failure rates based on sludge treatment type. There is an extensive literature on efficiencies of resource-intense, conventional treatment systems more common in developed countries. Many authors explicitly cite the lack of scientific research conducted within lower income countries as a major limitation to making recommendations for cost-effective and technologically feasible strategies for treating canteen waste water[35].

Efficiency is defined in terms of pathogen deactivation or suitability for reuse. Treatment options are categorized as either centralized or decentralized.

2.20.3 Centralized Waste Treatment Systems

2.20:3.1 Constructed Wetlands (Planted Drying Beds)

Constructed wetlands, also known as planted drying beds, are man-made wetlands that separate waste into two factions-dewatered solids and liquid - via planted vegetation, soils, and anaerobic and aerobic digestion. Several studies suggest that constructed wetlands are a lower cost, technically feasible approach for wastewater stabilization in developing countries because they have low energy requirements. However, they do require significant land area.This treatment option may only function well with small sludge loads during dry seasons[40]. Further treatments to both the liquid and solids factions may also be necessary to ensure sufficient pathogen removal.

Some studies suggest there is economic value – apart from improved sanitation – from use of constructed wetlands. A researcher conducted an experiment in Cameroon to test the efficiency of constructed wetlands without an initial separation of liquids and solids. The authors vegetated six dewatering beds with antelope grass, a valuable local plant. They speculated that the byproducts from the constructed wetlands–both biosolids and the emergent plants used in treatment – could be considered valuable for soil enhancement and forage to satisfy local farming needs. After six months of testing varying mixed canteen waste water and wastewater loads on the beds, the authors found the biosolids to be high in nutrients. Antelope grass yields from the treatment beds were two to three times higher than yields on natural wetlands during the same time period.The biosolids did require further treatment prior to widespread application since heminth egg concentrations exceeded WHO safety standards for reuse (79 eggs per gram of total solids compared to WHO recommendations of less than 1 helminth egg/g for unrestrictive use in agriculture) [44].

Some reviewed a pilot constructed wetland project supported by the Asian Institute of Technology (AIT). The planted drying beds used gravel and sand filters and narrow-leaved cattails to separate and treat raw septage produced by 3,000 people. Dewatered biosolids extracted from the bed showed favorable nitrogen and phosphorus levels. Nematode egg concentrations were high, but did not pose a health threat due to deactivation[45].

Some studied the treatment efficiencies of an 80-hectare constructed wetland in the Shandong Province of China. The authors sampled inflowing waste and constructed wetland sludge (after treatment) for a period of five years from 1999 to 2004. Results showed that treatment via constructed wetlands decreased the persistence of sludge coliforms by 99.6%, on average[46].

2.20.3.2 Water Stabilization Ponds

Water stabilization pond systems typically consist of several engineered ponds that operate in a series. Sludge accumulates in the bottom of the first pond during treatment while sludge flows through a series of secondary and tertiary treatment ponds. Accumulated sludges are left to dewater until total solids concentrations are high enough to facilitate shoveling. The US EPA suggests that low-tech solutions, such as stabilization ponds and lagoons, are well-suited for developing countries due to their low operations and management costs in comparison to mechanized systems. However, the agency cautions that large land requirements and elevated salinity concentrations may mean they are not suitable for urban populations.

Majority consider water stabilization ponds to be favorable for developing countries since they require less energy, operations and maintenance compared to conventional mechanical systems[50]. However, some research suggests that this system may be subject to failure if periodic desludging is not practiced[51]. Additionally, a 2007 report by Ammary indicates that treated sludge from water stabilization ponds increased soil salinity in Jordan, which consequently reduced crop productivity.

According to research, water stabilization ponds have the capacity to remove parasites associated with sludge waste mixed with wastewater sludge. Over a period of two years, the authors collected samples from two adjoining ponds in Marrakech, Morocco to determine the protozoan cyst and helminth egg removal efficiency. The authors measured *Ascaris* eggs and *Giardia* cysts since they are known to be resistant to “hostile environments” and are common in urban settings. The first pond basin retained the mixed canteen waste water and wastewater for 9.5 days after which point the solids settled and the remaining sludge was transferred to a second pond for 6.5 days of treatment. After two years of testing (monthly) samples from different depths in both ponds, the authors found the following:

1. Concentrations of both eggs and cysts were significantly higher during the warmer seasons ($p<0.05$).
2. Humidity and rainfall influenced performance.
3. No helminth eggs were detected in the treated wastewater after the full retention period (16 days) whereas 39.5% of raw sludge and wastewater samples presented eggs.
4. Only two of the 48 samples had protozoan after 16 days compared to 50% of the raw samples.
5. Parasites removed from the sludge were absorbed into the settled solids, which required additional treatment prior to reuse. Overall, the water stabilization ponds performed well and sludge was deemed suitable for reuse in irrigation after secondary treatment[55].

A comparison of contaminant removal efficiencies between water stabilization ponds and constructed wetlands is presented in Table 2.2. Removal efficiencies are compared with US EPA reuse standards, which state that an acceptable range of sludge coliforms is less than $1\text{-}10^6$ colony forming unit per 100 milliliters and less than one active helminth egg per liter of sludge after secondary treatment. These standards are consistent with WHO guidelines.

Table 2.2: Estimated sludge Coliforms and Helminth Egg Persistence after Treatment System;

	sludge Coliforms	**Helminth eggs**	**EPA Treatment Standard**
Stabilization Pond + maturation pond	1 x 10^3 FC/100ml	< 1 egg/L	Meets standard for both sludge coliforms and helminth eggs
Stabilization Pond + high rate pond	1 x 10^5 FC/100ml	> 1 egg/L	Meets standard for sludge coliforms only
Stabilization Pond + algae removal	1 x 10^5 FC/100ml	> 1 egg/L	Meets standard for sludge coliforms only
Constructed Wetlands	1 x 10^5 FC/100ml	< 1 egg/L	Meets standard for both sludge coliforms and helminth eggs

Source[55].

2.20.4 Decentralized (On-Ste) Waste Treatment Systems

2.20.4.1 Pit Latrines

Pit latrines rely on the creation of anaerobic conditions to stimulate microbial digestion of pathogens. They have low energy and water requirements, but can lead to significant public health and environmental impacts if collections are not well management or if incompletely digested waste leaches into surrounding soil[58]. EPAR Brief 104: the Public Health Benefits of Improved Sanitation presents a comparison of unimproved pit latrine use to improved latrine technologies. The study is summarized below.

Many studied the frequency of disease across eight communities in El Salvador to determine the effectiveness of improved pit latrines on health outcomes. The sample included 449 people in households. Four of the communities used pit latrines or no latrine, while the other four communities used Ecological Sanitation latrines. Ecological sanitation, or EcoSan, is based on four primary principles according to research: water conservation, canteen waste water containment to prevent contamination, canteen waste water treatment to inactivate contaminants and nutrient recycling for agricultural use. Of the EcoSan latrine communities, two featured solar-augmented latrines, and two featured double-vault desiccating latrines without a solar heating component.After regression analysis to control for confounding variables including pig ownership, dirt floors, medication and agricultural employment, the study found that compared to traditional latrines, users of double-vault EcoSanwaste water systems had lower rates of hookworm, *giardia*, and *E. histolytica*, but higher rates of *Ascaris* and *Trichuri*s helminth prevalence. In contrast, users of solar latrines had lower rates than controls of Ascaris, but higher rates of *E. histolytica* infection. The authors suggested that solar EcoSanwaste water systems may have been more effective than the double-vault EcoSanwaste water systems at heating the

Ascaris eggs in EcoSansludge containers to intolerable levels, reducing pathogen risks. The authors also suggested that the primary health risks to users of both types of EcoSanwaste water systems might be from exposure to pathogens during the process of emptying supposedly-neutralized biosolids from the waste water systems[64].

Dry sanitation systems, such as composting waste water systems and dehydrating waste water systems, are a viable alternative to waste management in developing countries. Composting occurs if canteen waste water is kept below a certain moisture level, either by separating sludge and waste water (for example, by sludge diverting waste water systems) or by mixing dry carbon sources such as ash, sawdust, or straw into the combined sludge/sludge sludge. Pathogen removal is highly dependent on composting temperatures, pH levels and time. If the canteen waste water or sludge is removed too early or if temperatures do not reach 50-60 °C, handlers may be at risk – especially if the canteen waste water were originally contaminated with *Ascaris* or schistomsomiasis.

Agricultural reuse of sludge captured from sludge diverting waste water systems has been tested for over 15 years. Storage time and temperature have been shown to have the greatest impact on pathogen removal efficiencies. Some conducted bacteria and virus survival experiments on source-separated sludge to determine potential health risks for reuse. The author found that bacteria such as *Salmonella* were inactivated quickly and presented a low risk of transmitting gastrointestinal infections via crops. Viruses, such as *Rotavirus*, were removed after one month of storage at 20° Celsius. Ninety percent of protozoa were eliminated after six months of storage at 4° Celsius.

2.21 Risks of Reusing Untreated or Undertreated Waste

In addition to presenting public health risks, the agricultural reuse of untreated waste can have negative consequences on soil and water quality. The excessive application of biosolids, as with petroleum-based fertilizers, can produce an oversaturation of nitrogen and other nutrients that is beyond the capacity of plants to utilize and receiving environments to absorb. Reuse of canteen waste water can also poison the soil and groundwater with concentrations of salts and heavy metals, especially when industrial wastes are commingled.

Scientists conducted an assessment of the quality of soil, surface water, ground water, and crops surrounding two sewage treatment plants in Varnasi and Kanpur, India. The goal of the study was to determine whether exposure to treated and untreated wastewater for agriculture presented a health risk to farmers. Seventy-four samples were drawn from farms that use treated and untreated wastewater for irrigation as well as biosolids for soil amendment. Seventy-five samples were drawn from farms not exposed to wastewater or biosolids. Results showed that soils receiving wastewater or biosolids for fertilizer contained high concentrations of nickel, cadmium and pesticides – all of which have been associated with neurobehavioral and gastrointestinal disorders. A significant number of individuals in the exposed group exhibited gastric symptoms, decreased concentration, depression and irritability, which the authors attributed to the high concentrations of contaminants present in surface waters, soils, and foods. Some report that the groundwater beneath wastewater irrigated agricultural fields in Pakistan had higher concentrations of contaminants (coliform bacteria, helminthes, heavy metals) and fertilizers (nitrates) than did the groundwater in areas not utilizing wastewater irrigation. The level of total nitrogen contained in the wastewater was 78.3 mg/l, which exceeded the FAO irrigation water quality standard of 5.0 mg/L[78].

2.21.1 Reusing Waste water for Agriculture

The reuse of treated wastewater, biosolids and sludge in agriculture could reduce the dependency on chemically manufactured fertilizer, improve soil productivity, reduce pollution, and conserve water.

2.21.2 Reuse of Biosolids and Treated Wastewater

Some report that the International Fertilizer Industry Association estimates that nearly 170 million tons of chemical fertilizer is produced annually. The authors suggest that nitrogen, potassium, and phosphorus recovered from canteen waste water could provide a low-cost alternative to expensive inputs in developing countries. They state that biosolids are preferable to inustrial fertilizers because they contain organic carbon, which improves soil texture, enables aeration, and promotes root development. Some agrees, stating that properly composted canteen waste water is an excellent soil conditioner because it replenishes the humus layer, which improves water retention. According to some, treated wastewater that is reused for irrigation has the potential to improve crop yields, conserve water, and offset the demand for (and thus costs of) chemical fertilizer. The authors used FAO crop, farmer survey, and Agricultural Bureau data from Pixian[86], China (a peri-urban district of nearly 500,000 people) to model the output potential of various crop yields based on water consumption, cropping patterns, and two alternatives irrigation strategies. The first model simulated expanded irrigation from canal and river sources, largely believed to be polluted. The second model simulated replacing existing irrigation sources with treated wastewater. Their results indicate that a wastewater reuse strategy could boost productivity by an additional US$20M annually and would conserve approximately 35 million cubic meters of surface water. Examined farmers' perceptions of the economic benefit of canteen waste water use in the ManyaKrobo district of Ghana. They hypothesized that the negative nutrient balances in the soil could be remedied by the proper and safe application of raw canteen waste waters from un-seweredwaste water systems. They also believed that the decision to use canteen waste water would be influenced by factors such as access to extension services, land tenure, education, and age. The authors interviewed 30 farmers who used canteen waste water and 30 who did not. Perceived agronomic benefit had the strongest influence on the probability a farmer would use canteen waste water[90]. The differences among the groups were significant. Users believed the canteen waste water were good for soil structure and an important source of nutrients compared to non-users. Non-users believed the canteen waste water deposited on farms had low quality, based on visual appearance. Net income for canteen waste water users was US$412.47 compared to $147.35 for non-users. Non-users cited the foul smell, distance for canteen waste water delivery, poor road conditions, and low quantity as major constraints to canteen waste water adoption. Some used a Monte Carlo simulation to estimate the benefits of improved waste reuse in Hanoi, Vietnam, a city of approximately 3 million people. The authors estimate that replacing septic tanks with sludge diversion latrines could recover anywhere between 18-45% of the phosphorus currently "lost" in the landfill or co-composting process 70% of the city's solid waste is collected and deposited in landfills.

2.21.3 Reuse of sludge as a Fertilizer

Scientists conducted an analysis of several studies that estimate the efficiency of crop yields after human-sludge fertilizer application. The authors found that waste water could outperform other forms of fertilizer, such as animal manure, when other factors such as rainfall were taken into consideration. Over-application of sludge-based fertilizer can, however, damage the salinity and electrical conductivity of soils.

Some believe that the benefits of sludge reuse are great since the cost of treatment is relatively low and usage is believed to reduce dependency on chemical fertilizers that potentially pollute surface and ground waters. The World Health Organization appears to agree with other researchers, stating that the fertilizing capacity of sludge compares well with ammonium and urea-based chemical fertilizers due to its naturally high nitrogen and urea content.

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Chapter Three

3.0 Methodology

3.1 Collection of Samples

sludge and waste water samples were collected from three different food canteens amongst selected tertiary institution; namely Leadcity University, University of Ibadan, The Technical University and The Polytechnic of Ibadan within Ibadan metropolis.

3.1.1 Equipment

Beakers (Pyrex), Conical flasks (Pyrex), Autoclave, Petri-dishes, Forceps, Cotton wool, Spirit lamp, McCartney bottles, Filter paper, Measuring Cylinder, Refrigerator, Spatula, Test tubes(Pyrex), Incubator, Inoculating Loop, Cock borer, Hand gloves, Nose masks, Compound Microscope, Slides, Pippete, Forcepts, Spatula, Durham tubes, Water bath (CB.2Sq2) (Gallenkampe England), Oven (Gallenkampe England), Pasteur Pipettes (Pyrex),Pcr Machine,,Agarose gell elecrtophoresis machine, uv transillumination (Edvotek MD 20827-1232), Centrifuge, vortex shaker.

3.1.2 Chemicals/ Reagents

Crystal Violet, Lugol's Iodine, Immersion Oil, Safranin, Hydrogen peroxide, Amyl alcohol, Starch, Cysteine, P-Dimethylaminobenzaldehyde, Hydrochloric acid, Potassium hydroxide, Sodium hydroxide, Methyl red, Lead acetate, Ethidium bromide, TAE buffer, Agarose powder.

3.1.3 Sterilization of Work bench and Apparatus /Materials Used

Laboratory Benches top were swapped using cotton wool and Methylated spirit to ensure clean working environment that is free of bacterials and fungi, this is done by soaking cotton wool inside methylated spirit and used to clean the bench before and after each day work in the laboratory.

Sterilization of glasswares was done using dry heat method of sterilization, The glass wares were wrapped with aluminium foil and put inside hot air oven at 170°c for 30min. Sterilization of Incubator Used was done by soaking the trays in warm water with mild bleach/disinfectant and all the adhering dirts were scrubbed off with brush.

3.1.4 Media Used

Nutrient Agar, Agar Agar, Nutrient Broth, Peptone Water, MacConkey Agar, Eosin Methylene Blue (EMB) agar, Mueller-Hinton agar and Salmonella Shigella agar (SSA). The media were prepared according to manufacturer's instructions.

3.1.5 Preparation of Media

A. Preparation of Eosin Methylene Blue (EMB) agar

Eosin Methylene Blue (EMB) agar was prepared by weighing 35.9 and dissolved in one thousand millilitre (1000ml) of sterilized distilled water and was mixed until suspension was uniform. It was later transferred into autoclave at 15ibs of pressure (120°C) for 15minutes, the conical flask rmoved and allowed to cool before pouring inside the petri dishes.

B. Preparation of Nutrient agar

Nutrient agar was prepared by weighing 28.0g and dissolved in one thousand millilitre (1000ml) sterilized distilled water, it was mixed until the suspension was uniformed, It was later transferred into autoclave at 15ibs of pressure (120°C) for fifteen minute (15min),The conical flask was removed ans allowed to cool before pouring into petri dishes.

C. Preparation of salmonella shigella agar (MSA)

Salmonella shigella agar was prepared by weighing 63.02g and dissolved in one thousand millilitre (1000ml) of sterilized distilled water, it was mixed until the suspension was uniformed and was later transferred into autoclave at 15ibs of pressure (120°C) for fifteen minutes (15min), The conical flask was removed and allowed to cool before pouring into petri dishes.

D. Preparation of Mannitol salt agar

Mannitol salt agar was prepared by weighing 111.02g and was dissolved into one thousand millilitre (1000ml) of sterilized distilled water, it was mixed until the suspension was uiformed and was later transferred into autoclave at 15ibs of pressure (120°c) for fifteen minutes (15min),The conical flask containing the solution of mannitol salt agar was allowed to cool before pouring into the petri dishes.

E. Preparation of Nutrient Broth for the Biochemical parameters

130g of nutrient broth was weighed and dissolved into one thousand millilitre(1000ml) of sterilized distilled water using one thousand millilitre capacity of conical flask, It was well mixed untill the suspension was uniform, it was later transferred into the autoclave at 15ibs of pressure

(120°c) for fifteen minutes (15min) ,The conical flask was removed and allowed to cool before pouring into the petri dishes.

3.2 Isolation of Bacteria

A serial dilution is a series of sequential dilutions used to reduce a dense culture of cells to a more countable concentration. Each dilution will reduce the concentration of bacteria by a specific amount. Serial dilution was carried out to 10^{-6} for all the samples. In brief, six serial dilutions were made of 1 gram of sample in 9 ml sterile distilled water ($10^{-1} – 10^{-6}$). 1ml Each dilution was inoculated into four different agar plates by pour plate techniques, allowed to set before being incubated in air at 37^{o}C for 24 hrs.

The bacteria isolated, counted and reported as CFU/ml (colony forming units). Pure culture was isolated from these plates by streak plate method using inoculating loop on nutrient agar[1].

3.2.1 Characterization of Bacterial isolates

The bacterial were characterized based on colonial appearances such as shape, colour, elevation and Gram reaction. In addition to morphological characterization the isolated bacterial cultures were cultivated in selective media and certain biochemical test were performed[1].

3.3 Gram Staining

3.3.1 Gram's Staining Procedure

Thin smears of pure bacterial cultures were made on clean glass slide air dried and heat fixed. mear was covered with crystal violet for 30 seconds, rinsed with distilled water and smear was ooded with Grams iodine solution for 60 seconds, destainedwith 70% ethyl alcohol and then distilled water again, the smear was covered with safranin for 30 seconds, washed with distilled water and blot driedair dried and observed under microscope[2].

3.3.2 Biochemical Identification of the Isolates

The biochemical properties of identified isolates were confirmed through standard biochemical methods[3].

The used biochemical tests are Catalase test, Indole test, Citrate test, Hydrolysis of starch, Production of hydrogen sulphide, Gas production, Acid production, MR, VP, Hemolysis test.

3.3.3 Catalase Test

Catalase test is used to identify microrganisms that produce the enzym catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into oxygen and water. $2H_2O_2 \rightarrow 2H_2O + O_2$

This test was carried out in a lamina flow hood. Small inoculum of bacteria isolate was mixed into two to three drops of hydrogen peroxide solution (3 %) and observed for the rapid elaboration of oxygen bubbles. The lack of catalase was observed by a lack of bubbles production[4].

3.4 Indole Test

This test is important in the grouping and identification of anaerobic bacteria. The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. Tryptophan is an amino acid that can undergo deamination and hydroysis by bacteria that express tryptophan enzyme.

24hrs young culture media was inoculated into 10ml of tryptophan broth which was already sterilized at 121°C for 15 minutes and allow to cool before the inoculation, incubated at 37°C for 24-48 hours. After 48 hours of incubation 5 drops of 0.5mL of Kovac's reagent was added to the broth culture, shaken gently allow to stand for 20 minutes. Formation of a deep red colour (ring) at the top layer indicate a positive and yellow coloration indicates negative result[4].

3.5 Citrate Test

This test was carried out to study the ability of an organism to use citrate as a sole source of carbon and energy. 24g of citrate agar was dissolved in 500ml of distilled water followed by sterilization in autoclave. Dispense into the petri dishes allow to cool, aseptically inoculate by streaking the organisms once across the surface. The inoculated medium was incubated for 24 to 48 hours, the colour of the medium indicates the result. A change from green to blue indicates utilization of the citrate which is positive but if the media retain the green color after incubation period then the bacteria is citrate negative[4].

3.5.1 Hydrolysis of Starch Test

This test was done to identify bacteria that can hydrolyse starch (amylose and amylopectin). Starch agar plates were inoculated with the selected isolates and incubated at 35 °C for 2 days. After incubation each plate was flooded with aqueous iodine for 30 seconds. The iodine reacts with the starch to give a dark brown colour. Hence a clear zone around the bacteria growth indicates positive results, while blue black colouration indicates a negative result[4].

3.5.2 Hydrogen Sulphide Utilization Test

An iron compound and a sulfur compound are included in the test medium to test for the production of hydrogen sulfide gas. Indicator paper was used (filter paper) cut in strips and sterilised, soaked in saturated lead acetate solution, the soaked paper was dried.

The strip was inserted inside the inoculated sample bottles between the plug with the lower end above the medium. Incubated at optimum temperature for 2-7days, together with an uninoculated control. Production and liberation of hydrogen sulphide causes blackening which occured by the end of the incubation period given a positive result, when there are no changes on the filter paper it indicate a negative result[4].

3.5.3 Hemolysis Test

Blood agar is an enriched bacterial growth medium used to culture those bacteria that do not grow easily. Preparation of Blood Agar was done as instructed by the manufacturer. Sterilize by autoclaving at 121°C for 15 minutes. Transfer thus prepared blood agar base to a 50°C water bath.

When the agar base was cooled to 50°C, sterile human blood aseptically was added and mixed well gently. Formation of air bubbles was avoided, the blood was warmed to room temperature at the time of dispensing to the molten agar base. 15 ml was dispensedto sterile Petri plates aseptically. Inoculated the isolates on the plates. Incubate at 35-37°C overnight. Growth were checked for characteristics of each species.

To know the type of hemolysis, the blood agar plate must be held up to a light source and observed with the light coming from behind (transmitted light). If either type of hemolysis is present, then one will observe a zone of hemolysis surrounding a growing colony.

Alpha (α) hemolysis: Partial lysis of the RBC (red blood cell) to produce a greenish-grey or brownish discoloration around the colony.

Beta (β) Hemolysis: Beta-hemolysis is the complete lysis of RBCs, resulting in a distinct, clear,colorless zone surrounding and under the colony.

Gamma (γ) or non-hemolysis: Gamma-hemolysis indicates no hemolysis of RBCs. There is no change in the medium under and surrounding the colonies[5].

3.5.3 Methyl Red Test

5mililiter of glucose phosphate broth (1g glucose 0.5% KH_2PO_4, 0.5% peptone) and 100ml of distilled water were dispensed in clean test tubes and sterilized. Tubes were inoculated with the test organisms and incubated at 37^0C for 48hrs. At the end of incubation, few drops of methyl red solution were added to each test tubes and colour change was observed. A red colour indicates a positive reaction[6].

3.5.4 Voge's - Proskaeur Test

5mililiter of glucose phosphate broth (1g glucose 0.5% peptone and 100ml of distilled water) were dispensed in clean test tubes and sterilized. The tubes were then inoculated with the test organisms and incubated at 37^0c for 48hrs.

After incubation, 6%α-naphtol and 6% sodium hydroxide were added to about 1ml of the broth culture. A strong red colouration formed within 30mins indicates positive reaction[6].

3.5.5 Gas Production Test

The organism's capacity to ferment glucose and transform pyruvic acid, the result of glycolysis, into gaseous byproducts are being tested. Clean test tubes were filled with 4-5 ml of glucose phosphate broth (1g glucose 0.5% peptone and 100ml of distilled water), which was then autoclaved at 121°C for 15 minutes to sanitize. Durham tubes were then placed inside the test tubes upside down. The broth will be pushed into the upside-down Durham tube during the sterilizing procedure. Use an inoculating loop to aseptically dispense the test microorganism into each test tube. For seven days, tubes were incubated at 35–37°C. increased incubation times to confirm a bad outcome. Positive results will result in an inverted Durham tube producing a bubble, which may be little or large depending on the amount of gas produced[7].

3.6 Molecular Identification of Bacteria

3.6.1 DNA Extraction using NIMR-BIOTECH Genomic DNA Extraction Kit

The strains were identified using 16SrRNA amplification.

NIMR Kit contains Lysis Buffer, Wash Buffer 1, Wash Buffer 2, Elution Buffer, Spin Column and Collection tubes.

Step by step Procedure for extraction is as described: 100µl of sterile water was added into a well labelled eppendorf tube containing harvested cells. 500µl of the lysis buffer was added to each tube containing the samples, the tubes were vortexed and incubated at 56ºC for 10 min, after which they were centrifuged at 10,000 RPM for 1 min. This was followed by the addition of 200µl of Absolute ethanol to the centrifuged tube, the mixture was transfered into the spin column placed inside a collection tube and then centrifuged at 10,000 RPM for 30 sec. The flow-through was discarded while the collection tube was blotted on a tissue paper. Each of the spin column was returned into their respective collection tubes and then 500µl of Wash Buffer 1 was added to each spin column containing the samples and then centrifuged at 10,000 RPM for 30 sec. The flow-through was again discarded and the collection tube was blotted on a tissue paper. 500µl of Wash Buffer 2 was added to each of the spin column and then centrifuged at 10,000 RPM for 1 min. The flow-through was again discarded and the collection tube was blotted on a tissue paper. The spin column in the collection tube was Centrifuged again at 12,000 to 14,000rmp for 3 minutes to remove all traces of ethanol. The Spin column was placed in a well labelled 1.5mL Microcentrifuge tubes and then 50µl of Elution Buffer was added to the centre of each Spin column, this was incubated at room temperature for 1 to 2 minutes, centrifuged at 10,000 RPM for 1 min to elute the DNA. The eluted DNA was stored at -20.

3.6.2 DNA Quantification

3.6.2.1 Spectrofluoremetry

Qubit fluorometer was used to measure DNA quantity. 199µl aliquot of the elution buffer was added into a sterile Qubit tube followed by addition of 1µl of the extracted DNA, this was votexed and placed in the Qubit fluorometer to read the DNA concentration. The readings were measured in ng/µl and it ranges from 5-150ng/µl to 10-200ng/µl.

3.6.2.2. Gel Electrophoresis for Extracted DNA

It is a technique used to separate DNA, RNA or protein molecules based on their size and electric charge. 1gram of agarose powder was measured for the electrophoresis was used for DNA quantification, 1g agarose powder was mixed with 100mL 0.5xTAE(Buffer TAE and EDTA) in a microwavable flask and microwaved for 3 minutes until the agarose solution is boiled. The agarose solution was left to cool for 5 minutes to about 60^{o}C before adding 30 µl of ethidium bromide (0.1% w/v) the agarose was poured into a gel tray with the well comb in place and kept at room temperature for 15 minutes until it has completely solidified.

3.6.2.3 Loading Samples and Running an Agarose Gel

Solidified agarose gel tray was placed into the gel box (electrophoresis unit) and filled with 0.5xTAE until the gel was coveredand the comb gently removed. A 3µl molecular weight ladder was carefully loaded into the first lane of the gel followed by negative control, continued with 3µl of each PCR product into the wells that were created by removal of the comb The gel was run at 80-120 V for about 1 hour, then was carefully removed from the gel box. The gel was thereafter viewed under UV transillumination (Edvotek MD 20827-1232)

3.6.3 Polymerase Chain Reaction

3.6.3.1 Polymerase Chain Reaction Mix Components

The PCR mix was made up of 20µl of Taq 5X FIREPol® Master Mix from Solis BioDyne Estonia. 0.5µl each of forward(799F-5'AACACGGATTAGATACCC-3') and reverse (1193R-5'ACGTCATCCCCACCTTCC-3') primer, 4µl of the Master mix, 2µl of DNA template and then made up with 13µl Nuclease free water, the mixture was mixed gently with the aid of micropipete tip and vortexed briefly. The tubes were placed in the PCR machine.

3.6:3.2 Cycling Conditions

The PCR thermal cycler used was Gene Amp PCR system 9700. The cycling parameters include: Initial denaturing at 94ºC for 2 minutes, followed by 35 cycles of denaturing at 94ºC for 2 minutes, annealing at 53ºC for 1 minutes and elongation at 72ºC for 1 minutes. Followed by a final elongation step at 72ºC for 10 minutes and hold temperature at 4ºC forever. Amplified fragments were visualized on safe view stained 0.5g agarose electrophoresis gels.

3.6.3.3 Gel Electrophoresis for PCR Product

1.5gram of agarose powder was measured for the electrophoresis was used for polymerase chain reaction (PCR) product, 1.5g agarose powder was mixed with 100mL 0.5xTAE(Buffer TA and EDTA) in a microwavable flask and microwaved for 3 minutes until the agarose was completely dissolved, 30µl of ethidium bromide was added. The agarose solution was left to cool for 5 minutes, the agarose was poured into a gel tray with the well comb in place and kept at 4ºC for 15 minutes until it has completely solidified.

3.6.4 Loading Samples and Running an Agarose Gel

Solidified agarose gel tray was added into the gel box (electrophoresis unit) and filled with 0.5xTAE until the gel was covered. A 3µl molecular weight ladder was carefully loaded into the first lane of the gel followed by negative control, continued with 3µl of each PCR product into the wells that was created. The gel was ran at 80-120 V for about 1 hour, then was carefully removed from the gel box. To visualize the DNA fragments, the gel was viewed under UV transillumination (Edvotek MD 20827-1232)

3.6.5 Gene sequencing

The PCR products were subjected to sanger sequencing and the data uploaded on Basic LocalAlignment Sequence Tool (BLAST) algorithm in the GenBank of the National Center for Biotechnology Information (NCBI). The best matched data in the GenBank with the highest identities will be selected.

Endnotes

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Chapter Four

Presentation of Results and Discussions of Findings

4.1 Results

Table 4.1 shows the morphological and biochemical results of the tests done on the isolates gotten different water, sludge and food samples from lead city university food canteens. Out of 13 isolates 4 were Gram positive 9 where Gram negative. Out of the 4 Gram positive ones, 3 were bacilli, and 1 was Rod, Out of 9 Gram negative, 3 were cocci, 4 were bacilli and 2 were cocci/bacilli. The result of the various biochemical tests shows that 8 were catalase positive, 5 were catalase negative. The whole 12 isolates were negative to Methyl red. 12 isolate were also negative to Voges-Proskauer Test, 7 were indole positive, 6 were indole negative.

Table 4.1: Morphological and Biochemical Characteristics of Isolates Obtained from Waste water, Sludge and Food Sample from Different Canteen within Lead City University

Isolate	Gram Staining	Catalase	MR	VP	Indole
1 A^5	+ve Bacilli	+	-	-	-
2 A^5	+ve Bacilli	-	-	-	+
3 A^5	+ve Bacilli	-	-	-	-
$4A^5$	-ve Bacilli	+	-	-	-
$5A^4$	-ve Bacilli	+	-	-	-
$6A^2$	-ve Bacilli	+	-	-	+
$7A^3$	-ve Bacilli	-	-	-	-
$8A^5$	-ve Bacilli	+	-	-	+
$9A^4$	-ve Cocci	+	-	-	+
$10A^5$	-ve Cocci/Bacilli	-	-	-	-
$11A^1$	-ve Cocci	+	-	-	+
$12A^3$	-ve Cocci	-	-	-	+
$13A^3$	+ve Rod	+	-	-	+

Source: Author's Field Work, 2022

Key:

-ve : Negative

+ve : Positive

MR : Methyl Red Test

VP : Voges-Proskauer Test

Table 4.2: shows the morphological and biochemical results of the tests done on the isolates gotten from different food canteen in The polytechnic Ibadan. Out of 13 isolates 8 were Gram positive, 5 were Gram negative. Out of 8 Gram positive,7 were cocci, 1 were bacilli. Out of 5 Gram negative 2 were cocci and 3 bacilli. The result of the various biochemical test shows that 9 were catalase positive, 3 were catalase negative. 11 were negative to Methyl red test while 1 were positive. The whole 13 isolates were negative to Voges-Proskauer test, 5 were indole positive, 7 were indole negative.

Table 4.2: Morphological and Biochemical Characteristics of Isolates Obtained from Different Canteen at the Polytechnic Ibadan

Isolate code	**Gram staining**	**Catalase**	**MR**	**VP**	**Indole**
1B^1	+ve Cocci	+	-	-	-
2B^1	+ve Cocci	+	-	-	-
3B^4	-ve Rod	-	-	-	+
4B^4	-ve Cocci	+	-	-	-
5B^3	-ve Cocci	-	+	-	-
6B^4	-ve Bacilli	+	-	-	+
7B^2	+ve Cocci	+	-	-	+
8B^5	-ve Bacilli	+	-	-	+
9B^4	+ve Cocci	-	-	-	+
10B^1	+ve Cocci	+	-	-	-
11^3	+ve Cocci	+	-	-	-
12B^3	+ve Cocci	+	-	-	-
13B^2	+ve Cocci	+	-	-	+

Source: Author, Field Work, 2022

Key :

-ve : Negative

+ve : Positive

MR : Methyl Red Test

VP : Voges-Proskauer Test

Table 4.3 shows the morphological and biochemical results of the tests done on the isolates gotten from different canteens at Technical University. Out of 13 isolates 4 were Gram positive, 9 were Gram negative. The whole Gram positive were Bacilli, Out of 8 Gram negative, 2 were cocci and 6 were Bacilli. The result of the various biochemical test shows that 5 were catalase positive, 8 were catalase negative. The isolates were negative to both Methyl red and Voges-proskauer Test, 11 were negative to Indole Test while just 2 were positive to indole Test.

Table 4.3: Morphological and Biochemical Characteristics of Isolates Obtained from Different Food Canteens at the Technical University Ibadan

Isolate code	**Gram staining**	**Catalase**	**MR**	**VP**	**Indole**
$1C^5$	+ve Bacilli	+	-	-	-
$2C^5$	+ve Bacilli	-	-	-	-
C^2	-ve Cocci	+	-	-	+
$4C^4$	+ve Bacilli	-	-	-	-
$5C^3$	-ve Cocci	-	-	-	-
$6C^4$	-ve Bacilli	+	-	-	-
$7C^3$	-ve Bacilli	-	-	-	-
$8C^4$	-ve Bacilli	+	-	-	-
$9C^3$	-ve Bacilli	-	-	-	-
$10C^5$	-veBacilli	+	-	-	-
$11C^4$	-ve Bacilli	-	-	-	-
$12C^4$	+ve Bacilli	-	-	-	-
$13C^1$	-ve Cocci	+	-	-	+

ource: Author, Field Work, 2022

Key:

-ve : Negative

+ve : Positive

Mp : Methyl Red

Vp : Voges-proskauer

Table 4.4 shows the morphological and biochemical results of the tests done on the isolates gotten from different Canteens at the University of Ibadan. Out of 14 isolates 6 were Gram positive, 6 were Gram negative. Out of 6 Gram positive 1were rod, 3 were cocci and 3 were Bacilli, Out of 6 Gram negative, 3 were cocci/Bacilli, 1 Cocci, 1 diplococci and 1 rod. The result of the various biochemical test shows that 3 out of 12 isolates were positive to catalase Test, The whole 12 isolates were negative to Methyl red test, 2 were positive to Voges-Proskaver Test,6 were indole positive,6 were indole negative.

able 4.4: Morphological and Biochemical Characteristics of Isolates Obtained from Different Food Canteen at the University of Ibadan

Isolate code	Gram Staining	Catalase	MR	VP	Indole
1D^2	-ve diplococci	-	-	-	+
2D^3	-ve Bacilli	-	-	-	+
3D^2	+ve Rod	-	-	-	-
4D^1	+be Cocci	+	-	+	-
5D^3	+ve Bacilli	-	-	+	-
6D^1	+ Cocci	+	-	-	-
7D^2	+ve Bacilli	-	-	-	-
8D^1	-ve Rod	-	-	-	+
9D^2	-ve Cocci	+	-	-	-
10D^2	-ve Cocci	-	-	-	-
11D^5	+ve Bacilli	-	-	-	-
12D^3	-ve Cocci	-	-	-	-
13D^2	-ve Cocci	-	-	-	+

Source: Author, Field Work, 2022

Key

-ve : Negative

+ve : Positive

Mp : Methyl Red

V : Voges-proskauer

Table 4.5: The Isolate Code and the Probable Identity Based on the Gram Stain that was done

Isolate code	Gram staining result	Propable Organisms Identity
1A^5	+ve cocci in clusters	*Staphylococcus aureus*
2A^5	+ve Bacilli	*Staphylococcus aureus*
3A^5	+ve Bacilli	*Staphylococcus aureus*
9A^4	-ve Cocci	*Shigellaspp*
10A^5	-ve bacilli	*Salmonellasp*
1A$_1$	-ve Cocci in chains	*Shigellaspp*
12A^3	-ve Cocci	*Salmonellasp*
2B^4	-ve Bacilli	*Pseudomanaeruginosa*
3B^4	-ve Rod	*E.coli*
6B^3	+ve cocci	Pediococcus
11B^3	+ve Cocci	*Microbacterium flavum*
12B^3	+ve Cocci in chains	*Streptococcussp*
3C^2	+ve Rod	*Bacillus subtillis*
7C^2	+ve Bacilli/cocci	*Pediococcussp*
8C^1	-ve Rods	*Enterobacter aerogene*
12C^3	-ve Bacilli	*Salmonellasp*
3D^2	-verods	*Shigellasp*

Source: Author, Field Work, 2022

Table 4.6: **The Isolates that were selected for Molecular Analysis for Identification based on their Morphological Characteristics**

Serial Number	**Isolate Code**	**Gram Staining**	**Identified Bacteria**
1.	$4B^4$	-ve Cocci	*Escherischia coli*
2.	$3B^4$	-ve Rod	*Escherichia coli*
3.	$6C^4$	+ve Cocci	*Proteus mirabilis*
4.	$11C^4$	-ve Bacilli	*Pectobacterium carotovorum*
5.	$11B^3$	+ve Cocci	*Acinetobacter baumanni*
6.	$7A^3$	-ve Bacilli	*Pectobacterium carotovarium*
7.	$2D^2$	+ve Rod	*Pseudomonas aeruginosa*
8.	$13A^3$	+ve Rod	*Bacillus cereus*
9.	$8A^5$	-ve Bacilli	*Pseudomonas aeruginosa*
10.	$11A^1$	-ve Cocci in Chains	*Bacillus safensis*
11.	$2B^1$	+ve Cocci	*Escherichia coli*
12.	$3C^2$	+ve Bacilli	*Proteus mirabilis*
13.	$1D^2$	-ve Diplococci	*Proteus mirabilis*
14.	$8D^1$	-ve Rod	*Pseudomonas aeruginosa*
15.	$1B^1$	+ve Cocci/Bacilli	*Enterobacter cloacae*
16.	$13C^2$	-ve Bacilli	*Providencia vermicola*
17.	$3A^2$	+ve Bacilli	Nil
18.	$13B^2$	+ve Cocci	*Pseudomonas aeruginosa*
19.	$13D^2$	-ve Cocci	*Psedomonas aeruginosa*
20	$13C^1$	-veCocci	*Enterococcus faecium*

Source: Author, Field Work, 2022

4.2 Molecular Identification and Phylogenetic Analysis of Bacteria with Desired Traits

4.2.1 Molecular Identification of Bacteria with Desired Traits

Isolates coded 4B^4, 3B^4, 2B^1 were identified as *Escherichia coli,* 2D^2, 8A^5, 8D^{1,} 13B^2, 13D^2 were identified as *Pseudomonasaeruginosa*, 11C^4,7A^3 were identified as *Pectobacteriumcarotovorum*, 6C^4, 3C^1, 1D^2, were identified as *Proteus mirabilis,* 11B^3 was identified as *Acinetobacter baumannii*, 13A^3 was identified as *Bacillus cereus,* 11A^{1,} wasidentified as *Bacillus safensis,* 1B^1 was identified as *Enterobacter cloacae,* 13C^2 was identified as *Providencia vermicola* and 13C^1 was identified as *Enterococcus faetum*respectively after subjecting their isolated DNA to 16S ribosomal RNA gene amplification by polymerase chain reaction (PCR), also confirming it by subjecting it to gel electrophoresis and analyzing the base sequences of the amplified strand by Sanger sequencing following the manufacturer's instruction.

Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Pectobacteriumcarotovorum, Acinetobacter baumannii, Bacillus cereus, Bacillus safensis, Enterobacter cloacae, Providencia vermicola and Enterococcus faetum. The identified strainshas a 100%, 99.74%, 99.73%, 99.48%, 99.47%, 99.46%, 99.21%, 99.20%, 98.69%, 98.40%, 95.79% and 93.68% identified respectively using BLAST 2.10.0N+. The 16S ribosomal RNA gene of each of the isolates was submitted to GenBank and each was assigned the following accession numbers: ON856201 *Escherichia coli,* ON856202 *Escherichia coli,* ON856203 *Proteus mirabilis*, ON856204 *Pectobacteriumcarotovorum*, ON856205 *Acinetobacter baumannii,* ON856206 *Pectobacteriumcarotovorum,* ON856207 *Pseudomonas aeruginosa,* ON856208 *Bacillus cereus,* ON856209 *Pseudomonas aeruginosa,* ON856210 *Bacillus safensis,* ON856211 *Escherichia coli,* ON856212*Proteus mirabilis,* ON856213 *Proteus mirabilis,* ON856214 *Pseudomonas aeruginosa,* ON856215 *Enterobacter cloacae,* ON856216 *Providencia vermicola,* ON856217 *Pseudomonas aeruginosa,* ON856218 *Pseudomonas aeruginosa,* ON856219 *Enterococcus faecium* ON856219

Table 4.7: Characteristics and Properties of Partial 16S Ribosomal RNA Sequences from the Samples using nBLAST on GenBank

Sample No	Sample ID	Accession Number	Length	Identified species	Details	E-value	Alignment score	Highest query coverage (%)
11	LCU-IBD-4B4	ON856201	374 bp	*Escherichia coli*	99.20% similarity using BLAST 2.10.0N+	0.0	>200	100
12	LCU-IBD-3B4	ON856202	377 bp	*Escherichia coli*	100% similarity using BLAST 2.10.0N+	0.0	>200	100
13	LCU-IBD-6C4	ON856203	380 bp	*Proteus mirabilis*	100% similarity using BLAST 2.10.0N+	0.0	>200	100
14	LCU-IBD-11C4	ON856204	375 bp	*Pectobacteriumcarotovorum*	99.47% similarity using BLAST 2.10.0N+	0.0	>200	100
15	LCU-IBD-11B3	ON856205	379 bp	*Acinetobacter baumannii*	99.21% similarity using BLAST 2.10.0N+	0.0	>200	100
16	LCU-IBD-7A3	ON856206	381 bp	*Pectobacteriumcarotovorum*	99.47% similarity using BLAST 2.10.0N+	0.0	>200	100
17	LCU-IBD-2D2	ON856207	378 bp	*Pseudomonas aeruginosa*	99.74% similarity using BLAST 2.10.0N+	0.0	>200	100

18	LCU-IBD-13A3	ON856208	374 bp	*Bacillus cereus*	99.47% similarity using BLAST 2.10.0N+	0.0	>200	100
19	LCU-IBD-8A5	ON856209	375 bp	*Pseudomonas aeruginosa*	100% similarity using BLAST 2.10.0N+	0.0	>200	100
20	LCU-IBD-11A1	ON856210	375 bp	*Bacillus safensis*	98.40% similarity using BLAST 2.10.0N+	0.0	>200	100
21	LCU-IBD-2B1	ON856211	380 bp	*Escherichia coli*	95.79% similarity using BLAST 2.10.0N+	0.0	>200	100
22	LCU-IBD-3C1	ON856212	382 bp	*Proteus mirabilis*	98.69% similarity using BLAST 2.10.0N+	0.0	>200	100
23	LCU-IBD-1D2	ON856213	363 bp	*Proteus mirabilis*	93.68% similarity using BLAST 2.10.0N+	0.0	>200	100
24	LCU-IBD-8D1	ON856214	373 bp	*Pseudomonas aeruginosa*	99.46% similarity using BLAST 2.10.0N+	0.0	>200	100
25	LCU-IBD-1B1	ON856215	373 bp	*Enterobacter cloacae*	99.73% similarity using BLAST 2.10.0N+	0.0	>200	100
26	LCU-IBD-13C2	ON856216	368 bp	*Providencia vermicola*	100% similarity using BLAST	0.0	>200	100

					2.10.0N+			
8	LCU-IBD-13B2	ON856217	374 bp	*Pseudomonas aeruginosa*	99.73% similarity using BLAST 2.10.0N+	0.0	>200	100
29	LCU-IBD-13D2	ON856218	372 bp	*Pseudomonas aeruginosa*	99.46% similarity using BLAST 2.10.0N+	0.0	>200	100
30	LCU-IBD-13C1	ON856219	382 bp	*Enterococcus faecium*	99.48% similarity using BLAST 2.10.0N+	0.0	>200	100

Source: Author's Field Work, 2022

P

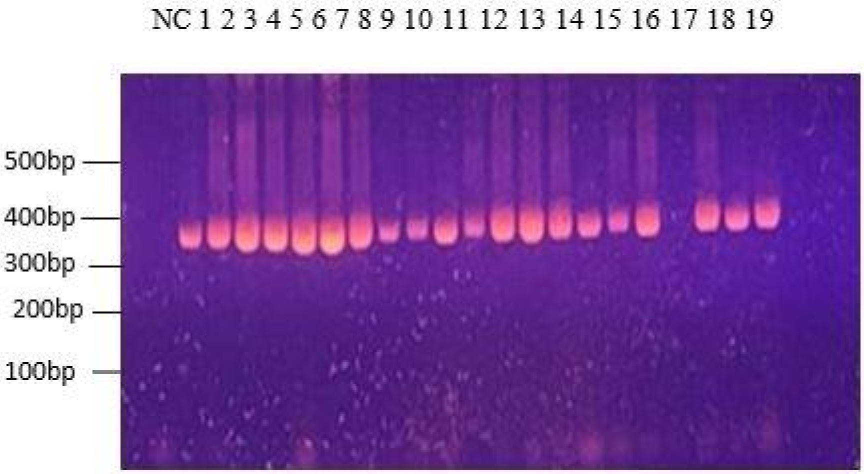

Figure 4.1: Result of the Gel Electrophoresis Done After Genomic

Source: Author's Field Work, 2022

The gel image obtained after running the amplified 16SrRNA gene of the nineteen isolates with the molecular marker is shown in Figure 4.1. DNA isolation from the selected samples.Gel image for PCR of the nineteen isolates with molecular marker/ladder. The DNA samples loaded in the gel showing amplification of the amplicon in 394bp-400bp.

4.2.2 Phylogenetic Analysis of Bacteria with Desired Traits

The DNA sequencing of the nineteen isolates are listed below**.**

>ON856201|*Escherichia coli* strain LCU-IBD-4B4 16S ribosomal RNA gene, partial sequence
GTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGC
CTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAA
CTTTACAGAGATGGATTGGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCA
GCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCA
GCGGTCCGGACGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATG
ACGT

>ON856202|*Escherichia coli* strain LCU-IBD-3B4 16S ribosomal RNA gene, partial sequence

AATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGAC
CGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGC
GGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACG
GAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCG
TCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTG
CCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGG
ATGACGT

>ON856203|*Proteus mirabilis* strain LCU-IBD-6C416S ribosomal RNA gene, partial sequence

ACGATGTCGATTTATAGGTTGTGGTCTTGAACCGTGGCTTCTGGAGCTAACGCGTTAAATCG
ACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAA
GCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCA
GCGAATCCTTTAGAGATAGAGGAGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTG
TCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTG
TTGCCAGCACGTAATGGTGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTG
GGGATGACGT

>ON856204|*Pectobacterium carotovorum* strain LCU-IBD-11C416S ribosomal RNA gene, partial sequence

GTCGATTTGTGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCG
CCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGA
ACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTC
AGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCC
AGCGATTCGGTCGGGAACTCAAATGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATG
ACGT

>ON856205|*Acinetobacter baumannii* strain LCU-IBD-11B316S ribosomal RNA gene, partial sequence

ACGATGTCTACTAGCCGTTGGGGCCTTTGAGGCTTTAGTGGCGCAGCTAACGCGATAAGTAC
ACCGCCTGGGGAGTACGGTCGCAAGACTAAAACTCAAATGAATTGACGGGGGCCCGCACAA
GCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATACT
ACAAACTTTCCAGAGATGGATTGGTGCCTTCGGGAATCTAGATACAGGTGCTGCATGGCTGT
CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTTTCCTTACT
TGCCAGCATTTCGGATGGGAACTTTAAGGATACTGCCAGTGACAAACTGGAGGAAGGTGGG
GATGACGT

>ON856206|*Pectobacterium carotovorum* strain LCU-IBD-7A316S ribosomal RNA gene, partial sequence

GATGGTCGATTTGTGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCG
ACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAA
GCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCA
GAGAATTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGT
CGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGT
GCCAGCGATTCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGG
ATGACGTAA

>ON856207|*Pseudomonas aeruginosa* strain LCU-IBD-2D216S ribosomal RNA gene, partial sequence

ATGGTCGGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGA
CCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAG
CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTG
AGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTC
GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGT
TACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGG
GATGACGT

>ON856208|*Bacillus cereus* strain LCU-IBD-13A316S ribosomal RNA gene, partial sequence

GACTGCTAGTGTTAGGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCG
CCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGA
AAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTC
AGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGC

CATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATG
ACGT

>ON856209|*Pseudomonas aeruginosa* strain LCU-IBD-8A516S ribosomal RNA gene, partial sequence

AGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCG
CCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGA
ACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTC
AGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTAC
CAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT
GACGT

>ON856210|*Bacillus safensis* strain LCU-IBD-11A116S ribosomal RNA gene, partial sequence

GACTGCTAGTGTTAGGGGGTTTCCAGCCCTCTTAGTGCTGCAGCTAACGCATTAAGCACTCC
GCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCG
GTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTG
ACAACCCTAAAGATAGGGGTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGC
CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTG
CCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT
GACGT

>ON856211|*Escherichia coli* strain LCU-IBD-2B1 16S ribosomal RNA gene, partial sequence

ACGATGTTCTACTAGCTGGTGGTGCCTTTGAGGCTTGACTACCGGAGCTAACGCGTTAAGTC
GACCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAATGAATTGACGGGGGCCCGCACA
AGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCC
TCGAAACTTTTCAGAGATGAAATTGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTG
TCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTG

TTGCCAGCGTTTCGGCCGGGAACTCTAAGGATACTGCCAGTGATAAACTGGAGGAAGGTGG
GGATGACGT

>ON856212|*Proteus mirabilis* strain LCU-IBD-3C1 16S ribosomal RNA gene, partial sequence

AACGTAGTCGATTTAGAGGTTGTGGCCTATGAGCCGTGGCTTCTGGAGCTAACGCGTTAAAT
CGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCAC
AAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATC
AGCGAATCCTTTAGAGATAGAGGAGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCT
GTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTT
GTTGCCAGCACGTCATGGTGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGT
GGGGATGACGT

>ON856213|*Proteus mirabilis* strain LCU-IBD-1S2 16S ribosomal RNA gene, partial sequence

AGGTGGGGGCCTTGAGCCGTGGCTTCTGGATCTAACGCGTTAAATCAACCGCCTGGGGAGTA
CGGCCGCAAGGTTAAAACTCAATGAATTGACGGGGGCCCGCACAAGCGGGGGAGCATGGGG
TTTAATTCAATGCAACGCAAAAAACCTTACCTACTCTTGACATCCTGGGAATCCTTTAAAAA
TAGGGGAGGGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGT
GAAATGTTGGGTTAAGTCCCCCAACGAGCGCAACCCCTTATCCTTTGTTGCCACCACGTAAT
GCTGGAAACTCAAAGGAGACTGCCGACGATAAACCGGAGGAAGGTGGGTATGAC

>ON856214|*Pseudomonas aeruginosa* strain LCU-IBD-8D1 16S ribosomal RNA gene, partial sequence

GACTAGGGCGTTGTGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCC
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GAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAAC
TTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAG
CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCA

GCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT

>ON856215|*Enterobacter cloacae* strain LCU-IBD-1B1 16S ribosomal RNA gene, partial sequence

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>ON856216|*Providencia vermicola* strain LCU-IBD-13C2 16S ribosomal RNA gene, partial sequence

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>ON856217|*Pseudomonas aeruginosa* strain LCU-IBD-13B2 16S ribosomal RNA gene, partial sequence

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GCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT

>ON856218|*Pseudomonas aeruginosa* strain LCU-IBD-13D2 16S ribosomal RNA gene, partial sequence

GAcTaGgcGTtGtAGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT

>ON856219|*Enterococcus faecium* strain LCU-IBD-13D2 16S ribosomal RNA gene, partial sequence

TAACGGATAGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTCCCCTTCGGGGGCAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTGTTAGTTGCCATCATTCAGTTGGGCACTCTAGCAAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACG

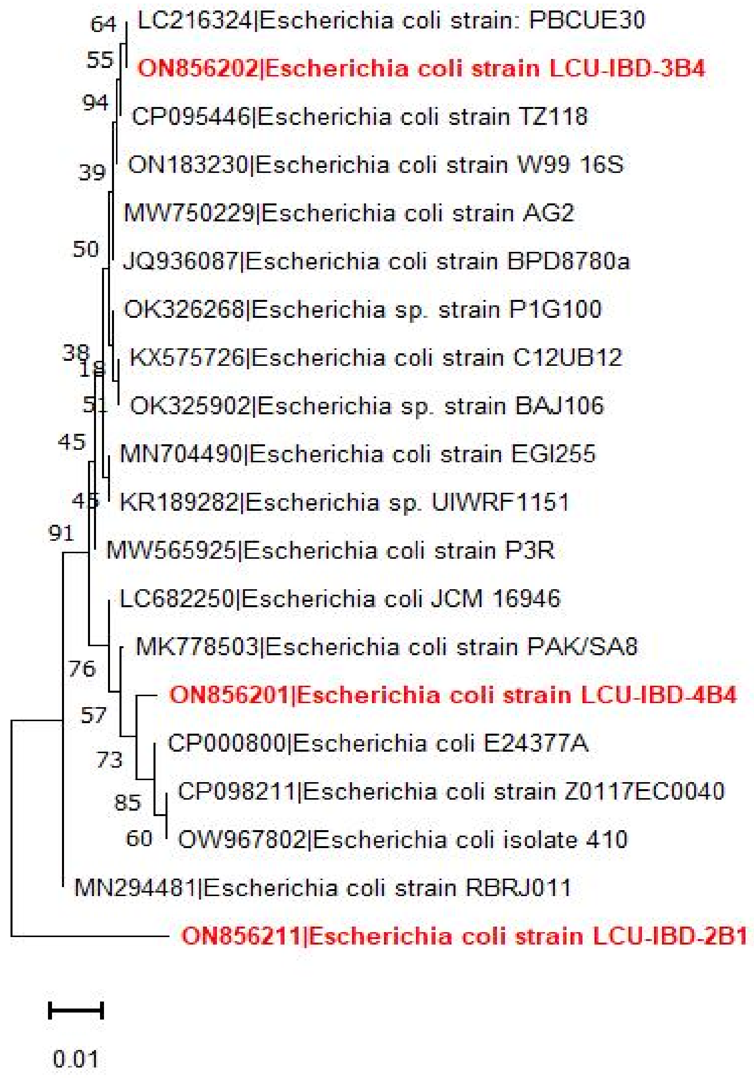


Figure 4.2: Phylogenetic Tree Showing Evolutionary Relationship of *Escherichia coli* Obtained from Ibadan, Nigeria (in red) with others stains available in the GenBank database

Source, Author Field Work, 2022

The evolutionary history was inferred using the Neighbor-Joining method[1]. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches[2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor methodand are in the units of the number of base substitutions per site[3]. This analysis involved 20 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1437 positions in the final dataset. Evolutionary analyses were conducted in MEGA11[4].

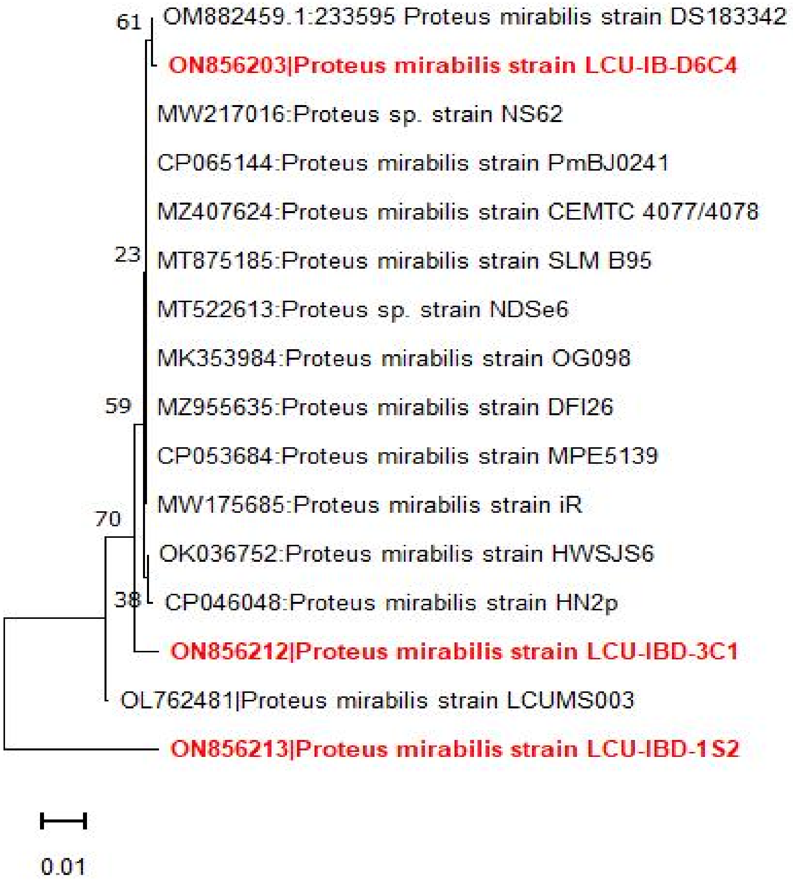


Figure 4.3: **P**hylogenetic tree showing evolutionary relationship of *Proteus mirabilis* obtained from in Ibadan, Nigeria (in red) with others stains available in the GenBank database.

Source: Author's Field Work, 2022

The Neighbor-Joining approach was used to infer the evolutionary history[1]. The ideal tree is displayed. In the bootstrap test (1000 repetitions), the proportion of duplicate trees in which the linked taxa clustered together is displayed next to the branches[2]. With branch lengths in the same units as the evolutionary distances used to estimate the phylogenetic tree, the tree is rendered to scale. The Jukes-Cantor method was used to calculate the evolutionary distances, which are expressed in terms of the number of base substitutions per site[3]. There were 16 nucleotide sequences in this investigation. For each sequence pair, all unclear places were eliminated (pairwise deletion option). The final dataset contained 1481 locations altogether. In MEGA11, evolutionary analyses were carried out[4]

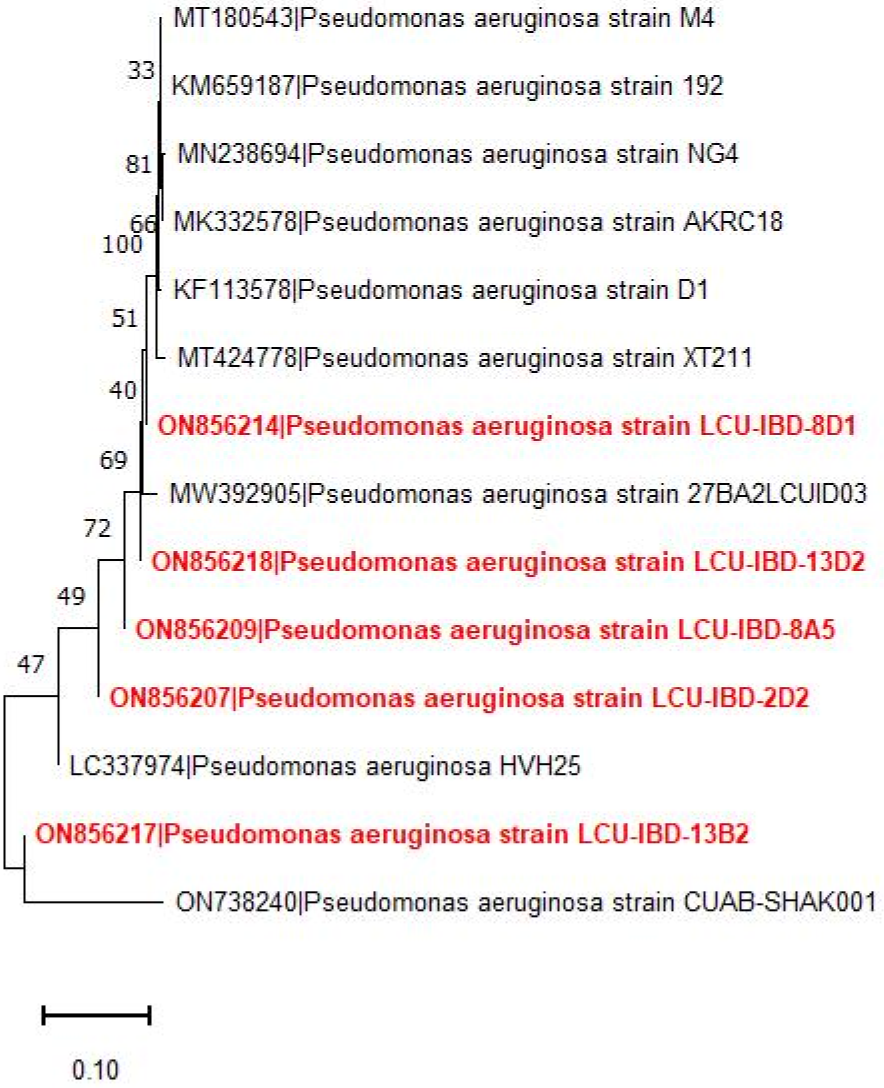


Figure 4.3: Phylogenetic tree showing evolutionary relationship of *Pseudomonas aeruginosa* obtained from in Ibadan, Nigeria (in red) with others stains available in the GenBank database

Source: Author's Field Work, 2022

The Neighbor-Joining approach was used to infer the evolutionary history[1]. The ideal tree is displayed. In the bootstrap test (1000 repetitions), the proportion of duplicate trees in which the linked taxa clustered together is displayed next to the branches[2]. With branch lengths in the same units as the evolutionary distances used to estimate the phylogenetic tree, the tree is rendered to scale. The Jukes-Cantor technique was used to calculate the evolutionary distances, which are expressed in terms of the number of base substitutions per site[3]. There were 14 nucleotide sequences in this investigation. For each sequence pair, all unclear places were eliminated (pairwise deletion option). The final dataset contained 1488 locations altogether. In MEGA11, evolutionary analyses were carried out[4].

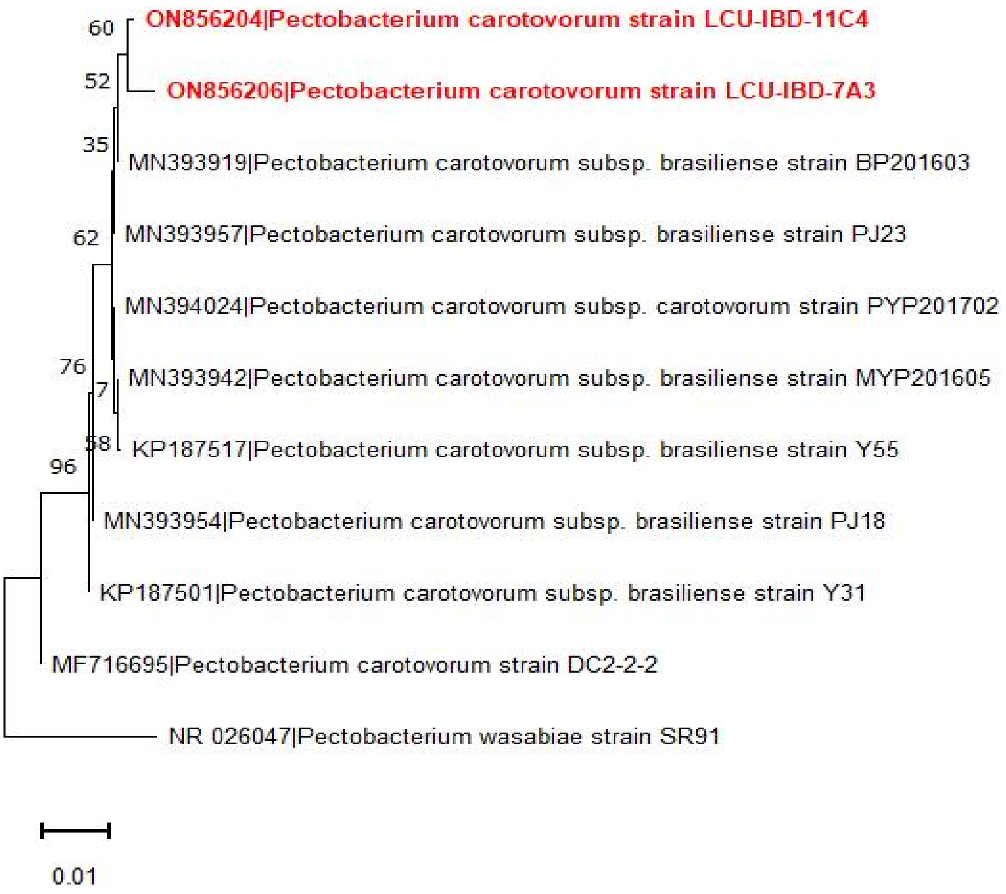


Figure 4.4: Phylogenetic Tree Showing Evolutionary Relationship of *Pectobacteriumcarotovorum*obtained from in Ibadan, Nigeria (in red) with others stains available in the GenBank database.

Source: Author's Field Work, 2022

The Neighbor-Joining approach was used to infer the evolutionary history[1]. The ideal tree is displayed. In the bootstrap test (1000 repetitions), the proportion of duplicate trees in which the linked taxa clustered together is displayed next to the branches[2]. With branch lengths in the same units as the evolutionary distances used to estimate the phylogenetic tree, the tree is rendered to scale. The Jukes-Cantor method was used to calculate the evolutionary distances, which are expressed in terms of the number of base substitutions per site[3]. There were 11 nucleotide sequences in this investigation. For each sequence pair, all unclear places were eliminated (pairwise deletion option). The final dataset contained 1472 locations altogether. In MEGA11, evolutionary analyses were carried out[4].

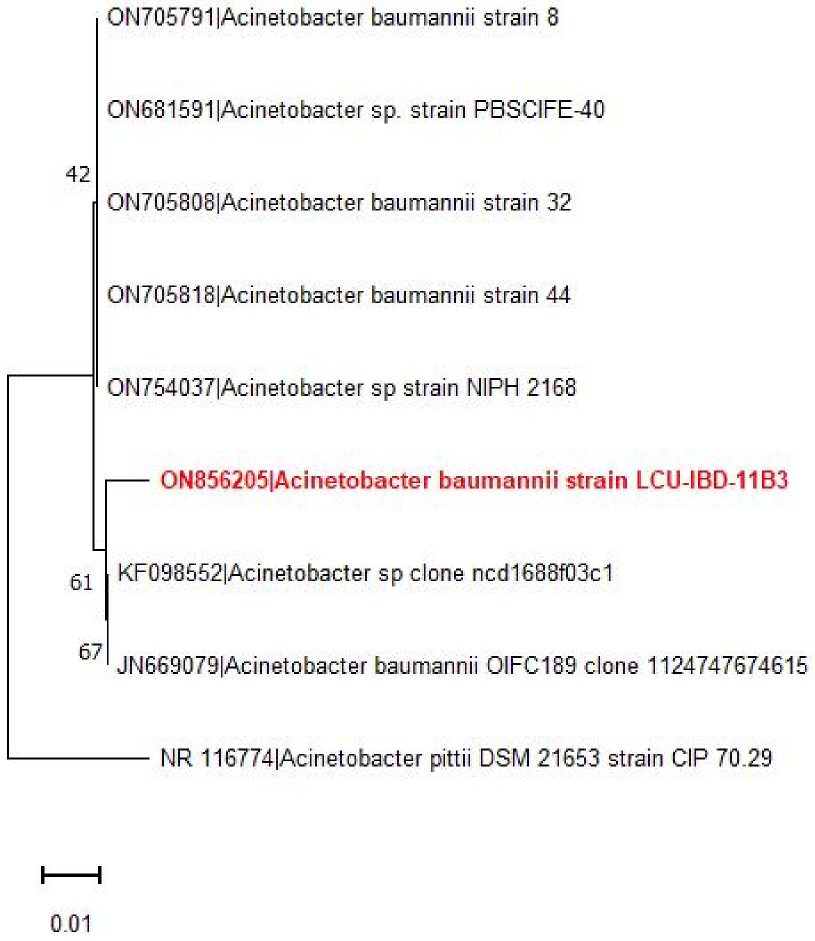
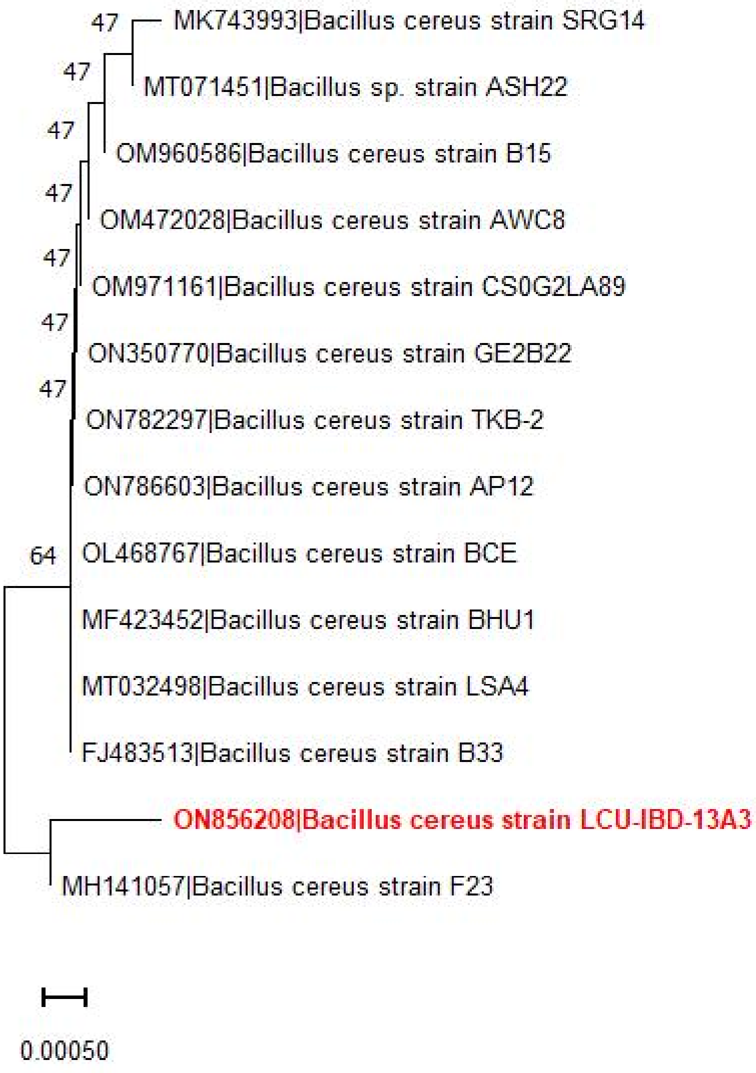


Figure 4.5: Phylogenetic Tree Showing Evolutionary Relationship of *Acinetobacter baumannii* Obtained from in Ibadan, Nigeria (in red) with others stains available in the GenBank database

Source: Author's Field Work, 2022

The Neighbor-Joining approach was used to infer the evolutionary history[1]. The ideal tree is displayed. In the bootstrap test (1000 repetitions), the proportion of duplicate trees in which the linked taxa clustered together is displayed next to the branches[2]. With branch lengths in the same units as the evolutionary distances used to estimate the phylogenetic tree, the tree is rendered to scale. The Jukes-Cantor method was used to calculate the evolutionary distances, which are expressed in terms of the number of base substitutions per site[3]. There were 9 nucleotide sequences in this analysis. For each sequence pair, all unclear places were eliminated (pairwise eletion option). The final dataset contained 1533 locations altogether. In MEGA11, evolutionary analyses were carried



out[4].

Figure 4.6: Phylogenetic tree showing evolutionary relationship of *Bacillus cereus* obtained from in Ibadan, Nigeria (in red) with others stains available in the GenBank database

Source: Author's Field Work, 2022

The Neighbor-Joining approach was used to infer the evolutionary history[1]. The ideal tree is displayed. In the bootstrap test (1000 repetitions), the proportion of duplicate trees in which the linked taxa clustered together is displayed next to the branches[2]. With branch lengths in the same units as the evolutionary distances used to estimate the phylogenetic tree, the tree is rendered to scale. The Jukes-Cantor method was used to calculate the evolutionary distances, which are expressed in terms of the number of base substitutions per site[3]. There were 14 nucleotide sequences in this investigation. For each sequence pair, all unclear places were eliminated (pairwise deletion option). The final dataset contained 1453 locations altogether. In MEGA11, evolutionary analyses were carried out[4].

Figure 4.7: Phylogenetic Tree Showing Evolutionary Relationship of *Bacillus safensis* obtained from in Ibadan, Nigeria (in red) with others stains available in the GenBank database

Source: Author’s Field Work, 2022

The Neighbor-Joining approach was used to infer the evolutionary history[1]. The ideal tree is displayed. In the bootstrap test (1000 repetitions), the proportion of duplicate trees in which the linked taxa clustered together is displayed next to the branches[2]. With branch lengths in the same units as the evolutionary distances used to estimate the phylogenetic tree, the tree is rendered to scale. The Jukes-Cantor method was used to calculate the evolutionary distances, which are expressed in terms of the number of base substitutions per site[3]. There were 11 nucleotide sequences in this investigation. For each sequence pair, all unclear places were eliminated (pairwise deletion option). The final dataset contained 1437 locations altogether. In MEGA11, evolutionary analyses were carried out[4].

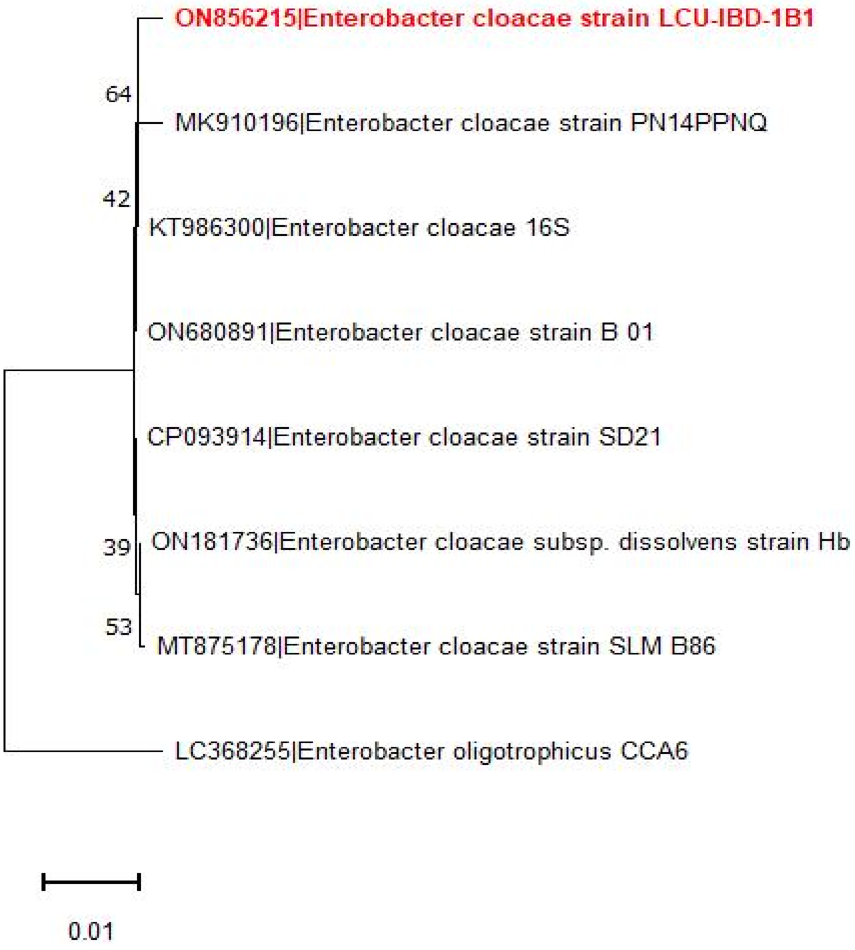


Figure 4.8: Phylogenetic tree showing evolutionary relationship of *Enterobacter cloacae* obtained from in Ibadan, Nigeria (in red) with others stains available in the GenBank database

Source: Author's Filed Work, 2022

The Neighbor-Joining approach was used to infer the evolutionary history[1]. The ideal tree is displayed. In the bootstrap test (1000 repetitions), the proportion of duplicate trees in which the linked taxa clustered together is displayed next to the branches[2]. With branch lengths in the same units as the evolutionary distances used to estimate the phylogenetic tree, the tree is rendered to scale. The Jukes-Cantor method was used to calculate the evolutionary distances, which are expressed in terms of the number of base substitutions per site[3]. There were 8 nucleotide sequences in this investigation. For each sequence pair, all unclear places were eliminated (pairwise deletion option). The final dataset contained 1538 locations altogether. In MEGA11, evolutionary analyses were carried out[4].

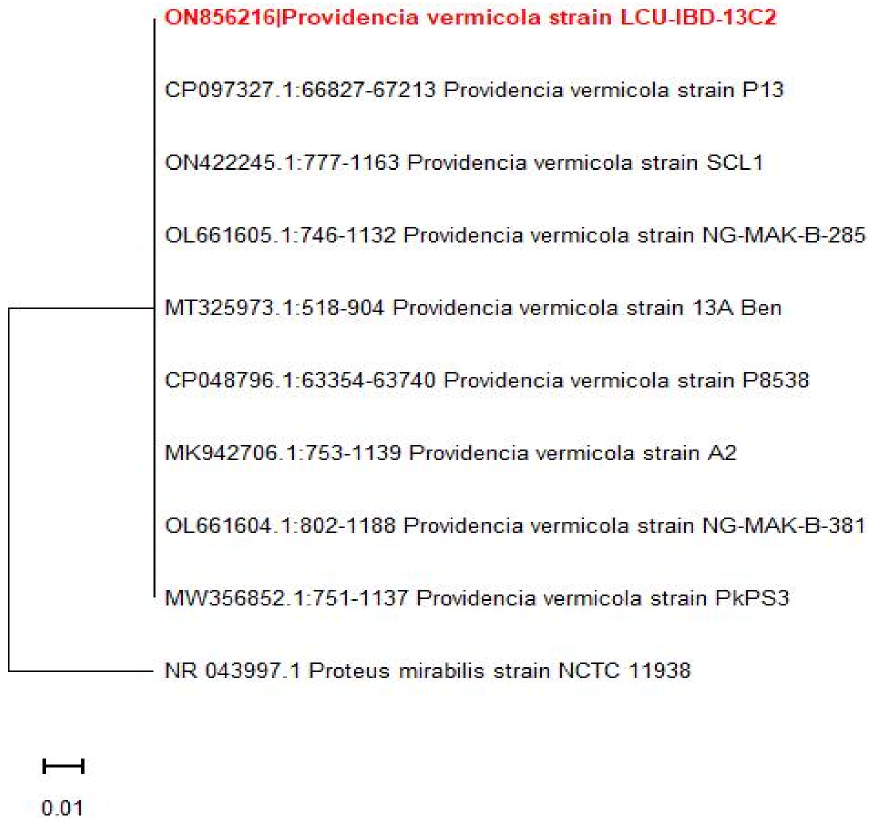


Figure 4.9: Phylogenetic Tree Showing Evolutionary Relationship of *Enterobacter cloacae* obtained from in Ibadan, Nigeria (in red) with others stains available in the GenBank database.

Source: Authors's Field Work, 2022

The Neighbor-Joining approach was used to infer the evolutionary history[1]. The ideal tree is displayed. In the bootstrap test (1000 repetitions), the proportion of duplicate trees in which the inked taxa clustered together is displayed next to the branches[2]. With branch lengths in the same units as the evolutionary distances used to estimate the phylogenetic tree, the tree is rendered to scale. The Jukes-Cantor method was used to calculate the evolutionary distances, which are expressed in terms of the number of base substitutions per site. Ten nucleotide sequences were subject to this investigation. For each sequence pair, all unclear places were eliminated (pairwise deletion option). The final dataset contained 1503 locations altogether. In MEGA11, evolutionary analyses were carried out[4].

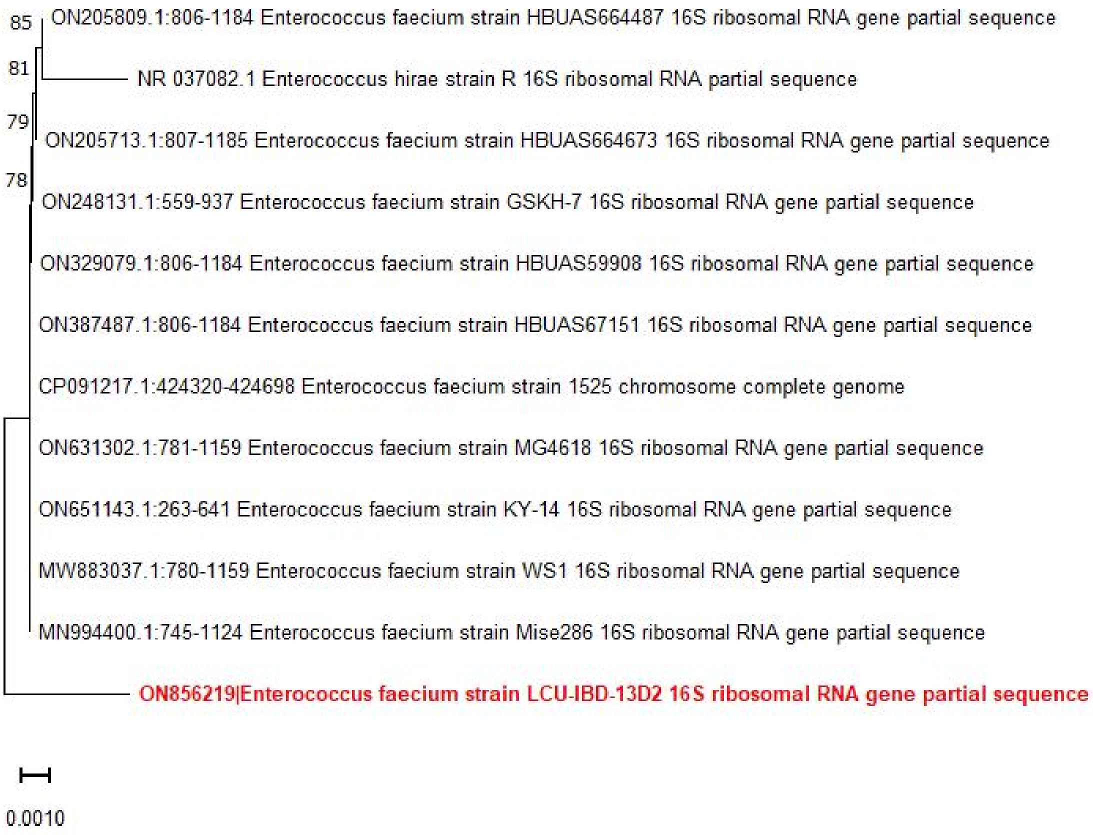


Figure 4.10: Phylogenetic Tree Showing Evolutionary Relationship of *Enterococcus faecium* obtained from in Ibadan, Nigeria (in red) with others stains available in the GenBank database

Source: Author's Field Work, 2022

The Neighbor-Joining approach was used to infer the evolutionary history[1]. The ideal tree is displayed. In the bootstrap test (1000 repetitions), the proportion of duplicate trees in which the linked taxa clustered together is displayed next to the branches[2]. With branch lengths in the same units as the evolutionary distances used to estimate the phylogenetic tree, the tree is rendered to scale. The Jukes-Cantor method was used to calculate the evolutionary distances, which are expressed in terms of the number of base substitutions per site[3]. Twelve nucleotide sequences were subject to this investigation. For each sequence pair, all unclear places were eliminated (pairwise deletion option). The final dataset contained 1537 locations altogether. In MEGA11, evolutionary analyses were carried out[4].

4.3 Discussion of the findings

Water bodies that receive wastewater are polluted and contaminated with compounds that are offensive and potentially harmful. The microbiological contamination of water, particularly with pathogenic bacteria, is one of the most significant causes of water pollution. Many watery illnesses are typically caused by enteric bacteria. Water contamination is a severe environmental issue because it has a negative impact on both human health and the biological richness of the aquatic ecosystem. According to the study's findings, several bacteria of concern were isolated from the samples that were gathered and examined[5].

It was observed for the isolates selected for identification that the identity obtained via biochemicalcharacterisation didn’t match up with the molecular identity when the 16srRNA gene of the organisms where amplified, sequenced and blasted on NCBI database. The phenotypic identity of the 20 isolates selected were *Staphylococcusaureus, Pseudomonas aeruginosa, Escherichia coli, Enterobacter aerogene,Shigella sp, Pediococcus, Streptococcus, Bacillus* *subtillis,Microbacterium flavum, and Salmonella.* Using the molecular techniques, their identities were*Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Pectobacteriumcarotovorum, Acinetobacter baumannii, Bacillus cereus, Bacillus safensis, Enterobacter cloacae, Providencia vermicola* and *Enterococcus faetum*.This trend was also reported by some researchesand it may be due to the fact that the basis of biochemical characterisation is generally not as accurate as for identification like genotypic methods[6].

In the culture - dependent assessment of some ready - to - eat foods like African Salads (Enugu), Fried rice, Jollof rice, oil beans e.t.c. (Benin city), organisms like *E. coli, Enterobacter* spp, *Enterococcus* spp, *Enterobacter* spp were isolated. These are some of the microorganisms also isolated in this research. These microorganisms are coliforms and are indicative of fecal contamination which could be as a result of unhygienic practices along the production chain of the food, or even the use of contaminated water in washing and cleaning of plates[7,8].

Pectinolytic bacteria and fungi that break down the pectic part of the vegetable are the main culprits behind soft rot, one of the main diseases that cause vegetables to decay. The pectin creates the intermediate lamella, a mechanical barrier that stiffens the cells and acts as a glue between them[9]. A bacteria called Pectobacterium carotovorum has been linked to stem and soft rot in a number of crops, including tomatoes, onions, and potatoes[10]. Nigerian canteen operators occasionally purchase vegetables that are spoiled or damaged, such as tomatoes and peppers. This is because they are cheaper and help to reduce the overhead cost of production[9]. The presence of *Pectobacterium carotovarum* in waste water can therefore be as a result of the usage of tomatoes, onions and peppers affected by soft rot for cooking.

Acinetobacter baumanii was also isolated. This organism has been implicated as a life - threatening pathogen that is associated with community acquired and nosocomial infections mainly pneumonia[11]. This organism have been found on fruits and vegetables and are believed to have gotten on them while growing in soil, during harvesting, from organic fertilizers, contaminated irrigation water as well as during transportation and handling[12]. This could also be the source of this organism found in the waste water and sludge of the canteens.

Bacillus cereus was one of the recovered Bacillus species in the microbiological analysis of various ready-to-eat foods[13]. According to the study, the canteens' hygienic standards had no bearing on the occurrence of Bacillus spp. This could be as a result of the ease with which Bacillus cereus can enter foods due to their natural occurrence in soil. Bacillus safensis is a rod-shaped, mobile bacterium that has been used in biotechnological applications and has a strong resistance for salt, heavy metals, UV, and gamma radiation. It can be found in many different settings, including soil and oil-polluted areas where it performs biodegradative functions[14]. The environment around canteens is typically polluted by cooking oil, which mixes with waste water to form a sludge, making it an ideal environment for the growth of this bacteria[14].

Proteus mirabilis was isolated from the foods. It is commonly considered to be an opportunistic pathogen causing urinary tract infections (UTIs) in humans. However, some strains of *P. mirabilis* were found to be association with food poisoning outbreaks, with the pathogenic mechanism still unclear[15]. It is widely found in soil and water, and its presence may be from contaminated water used in cleaning of utensils and from minute quantities of soil contaminating the foods.

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Chapter Five

Conclusion

5.1 Conclusion

Foods that we eat are hardly sterile, but the most important thing is our foods do not contain pathogens or potential pathogens. It is also important that the environment where our foods are prepared should be clean and free from pathogens likely to cause health hazards. A single food canteen has the potential to start an epidemic. According to the research's findings, restaurants' sludge and waste water can be dangerous to people's health since they include pathogenic bacteria that grow in the presence of organic matter and other bio-nutrients. Clearly, some of hese germs come from the ingredients used in cooking, while others are the result of unclean procedures. As a result, the food that is now supplied and the environment in tertiary institutions' canteens put the people who use them in risk.

5.2 Recommendation

A thorough profiling of the genes associated with antibiotic resistance for pathogenic bacteria found in restaurant sludge and waste water should be provided by medical specialists.

Governments must establish rules for managing restaurant sludge and waste water in a way that ensures waste water is detoxified and sterilised, preventing health problems for people, aquatic species, and terrestrial plant life.

Restaurants should be encouraged to recycle their used water because their constant, excessive water use in Oyo State can reduce the amount of water available.

Restaurants owned by public and private institutions are required to have suitable facilities for disposing of their waste water and sludge.

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Appendix I

Source: Lab Work, 2022.

Appendix II

Source: Lab Work, 2022

Bio-data

A. Personal Data:

1. Full Name:	Adeyemi-EKEOLU Bukola Marufat Nô. 8 Kajola Estate, Apapa Moniya, Ibadan. adewunmibukola66@gmail.com 08066851684
2. Date and Place of Birth:	19th July, 1986; Ibadan
3. Nationality:	Nigerian
4. Name and Address of Next of Kin:	Adeyemi Odunayo Adams. Nô. 8 Kajola Estate, Apapa Moniya, Ibadan.

B. Educational Background:

School Attended	Date	Qualifications
❖ Methodist Primary School, Ibadan.	1992-1998	First Leaving Sch. Cert.
❖ Isbatudeen Girls School,Ibadan.	1998-2004	S.S.C.E
❖ Federal Polytechnic, Offa.	2006-2008	OND
❖ Federal Polytechnic, Offa.	2009-2011	HND
❖ Lead City University, Ibadan.	2020-2022	M.Sc in view

C. Working Experience with Dates

Laboratory Technologist 2014-Till Date

D. Awards and Fellowship Nill

E. Membrship of Academi Professional Bodies Nill

F. Publication: 3rd FASCON Conference 2022 Poster Presentation

Date

Signature

University Compliance Certification

This is to certify that the Thesis by Adeyemi-Ekeolu Marurat Bukola with Matric No LCU/PG/001815 in the department of **Biological Sciences**, Faculty of Natural and Applied Sciences, Lead City University, is in full compliance with the approved university format and style.

Name and Signature **Date**