ENVIRONMENTAL HYGIENE AND MICROBIOLOGICAL ASSESSMENT OF FOOD SERVICE ESTABLISHMENTS IN SELECTED BOARDING HIGH SCHOOLS IN IBADAN, NIGERIA

BY

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A Thesis in the Department of Microbiology submitted to the Faculty of Science in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

JULY, 2023

CERTIFICATION

This is to certify that the research work reported in this thesis was carried out by KAFAYAT ADENRELE ADEBAYO (Matric Number: 173918), under our supervision in the Department of Microbiology, University of Ibadan, Nigeria.

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DEDICATION

To school children

Who are gifted and talented

Whose complete well-being must be nurtured

In a safe and healthy environment

For they are the future and hope of our great nation

ACKNOWLEDGEMENTS

All glories belong to the Almighty God for granting me the ability to pursue this research study at the University of Ibadan.

I want to express my profound appreciation to my supervisor Prof. O.E. Fagade, for his continuous mentoring and advice. He gave me the vigour to strive hard until the completion of this study. He believed in me and pushed me to succeed. May God grant you good health, long life and happiness always.

I am indebted to my co-supervisor Dr. Elizabeth O. Oloruntoba of the Department of Environmental Health Sciences, Faculty of Public Health for her attention, patience, scrutiny and persistence in seeing this research to a logical conclusion. May the goodness you bring to others always be upon you and your family.

I am grateful to the Head of the Department of Microbiology, Prof. A.A. Ogunjobi for his prompt attention to issues and commitment to ensuring proper administrative work. I also appreciate the members of the Environmental Unit, Dr. O.O. Adelowo, Dr. A.I. Falodun, Dr. Blessing Nwadike, Dr. A.O. Adekambi, and Mrs Bola Okpeku for their moral support, guidance and technical inputs in this work. A very special appreciation goes to Dr. Bolade Oyelade of the New Jersey Department of Environmental Protection, Leeds Point Office, Leeds Point, NJ, USA for taking the time to peruse this write-up and his useful contributions to making this work a reality.

The molecular unit team of the Nigerian Institute of Medical Research (NIMR) led by Dr. Muinah Fowora gave me the most exciting research experience. Special thanks to Mrs Nike Aiyedogbon for doing a thorough analytical work. They gave me an enabling research environment and put me through very important biotechnology techniques. May God always meet your need where you least expected them. I am highly indebted to Dr. Agre Paterni of the yam breeding unit at the International Institute of Tropical Agriculture (IITA) for putting me through the DNA fingerprint analysis.

I am thankful to Prof. A. A. Onilude, and Prof. Sherifat M. Wakil for their kindness and words of encouragement during this study. I appreciate the PG coordinator, Dr. Olubusola O. Odeniyi for her technical input and moral support. I am indeed grateful to every member of staff of the Department of Microbiology, University of Ibadan for their

support and encouragement to make this work a success. I want to thank all my colleagues and other graduate students in the department for making the study environment calm and accommodating.

I hereby express my warmest appreciation to the LISA team, Department of Statistics, University of Ibadan for accepting to analyse my questionnaires. Special thanks to Dr. A.T. Adeniran and Mr. Olajide Akinpeloye for their prompt responses whenever needed. Thank you for teaching me the rudiments of biostatistics.

I wish to sincerely thank my employer (Federal Ministry of Health, Port Health Services, Ibadan) for allowing me to pursue this challenging but quite fulfilling degree course. I thank all my colleagues at Ibadan for standing in for me whenever I needed to take time off during this research. I appreciate the Oyo State Ministry of Education for permitting me to conduct this study. I am grateful to all schools' head teachers for their support and cooperation. My gratitude goes to the entire staff of Nigerian Stored Product Research Institute (NSPRI), Ibadan for making available their Microbiology Laboratory for the preliminary stage of my research work.

My heartfelt gratitude goes to my darling husband, Mr. Shakir Oladipo Adebayo. He was very supportive both financially and morally. He believed in me and accepted to relocate my whole family to Ibadan without a doubt in pursuit of the degree. This work was made possible with the cooperation and sacrifice made by my loving children: Mr. Abdulmalik Adebayo, Miss Aliyyah Adebayo, Master Abduljabbar Adebayo, Miss Aneesah Adebayo, and Master Abdulhakeem Adebayo. I am grateful to my in-laws for their love, prayers and moral support. May God protect you all for good.

I am highly indebted to my immediate family members: My parents, Alhaji Gani and late Alhaja Kudirat Oladimeji, you set the foundation of honesty, knowledge, and dedication to work; my siblings (Mrs. Yemisi Dawodu, Dr. Mutiat Obileye, Dr. Fatimat Akinlusi, Prof. Morufat Balogun, Mrs. Aminat Idowu, and Mrs. Zaynab Ashiru-Mobolaji, without you all, I could not have been here today.

I appreciate my good friends from my undergraduate studies (Abdulkabir Aliu, Tajudeen Ogunmola, Sulaiman, Yahaya, Ismail Leasu and Nurain Lawal) for their support, advice and goodwill when it seemed all was unachievable. Many thanks to my neighbours, (they made the home peaceful and even cared for my family when I had to travel out of

town) and ever-punctual driver, Mr Kamor Salawu for assisting with daily family responsibilities. I will always admire your simplicity and kindness.

I am indeed grateful to my research assistant, Mr Lekan Olanrewaju, for paying attention to details while the work was in progress and to all my study participants for making my data collection a possibility.

Special thanks to everyone who has contributed in one way or the other to the success of this research study. May God bless you all.

Kafayat Adenrele Adebayo

July 2023

ABSTRACT

Schools' Food Service Establishments (FSEs) have been incriminated in numerous foodborne diseases outbreaks globally and have been linked to the environment and food handling procedures in the establishments. Despite this, FSEs in Nigerian boarding schools have been poorly investigated. In order to provide baseline data for infection control, this study was designed to assess environmental hygiene and food handlers' Knowledge, Attitude and Practices (KAP) and investigate food-related microbial contamination from selected boarding schools' FSEs in Ibadan, Nigeria.

Observational checklist and interviewer-administered questionnaire were used to evaluate environmental hygiene parameters, food handlers' KAP in four schools' FSEs out of forty-three schools by inclusion criteria and balloting. Swabs from Food Contact Surfaces (FCS): utensils and surfaces; 20 food handlers' hands and samples of Ready-to-Eat (RTE) foods were examined for Aerobic Plate Count (APC), Total Coliform (TC), Faecal Coliform (FC) and selected important foodborne pathogens counts using standard methods. Isolated bacteria were characterised phenotypically and subjected to 16S rRNA sequencing. Antibiotic susceptibility testing was determined using disc diffusion and E-strip techniques based on CLSI and EUCAST standards, respectively. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Schools FSEs' compliance mean scores for environmental hygiene parameters were 82.2, 56.8, 52.7 and 65.6% for toilets, dining areas, kitchens and observed food handlers at work, respectively. The food handlers had good knowledge (61.9%), positive attitude (81.4%) to ensure food safety, but poor hygiene practices (52.6%) which differed significantly among schools ($p=0.012$, $\chi^2=10.15$). Major unsanitary practices observed were: use of basins and buckets for dish washing, uncovered solid waste receptacles, non-availability of sanitising agents and inadequate handwashing. Mean logCFU/cm^2 of APC for counter tops, chopping boards, grinders, trays and knives were 5.59±1.56, 4.38±2.62, 4.01±0.77, 2.47±2.23 and 2.38±1.75, respectively. Food handlers' hands' mean logCFU/cm^2 of APC, TC, FC, *Staphylococcus* and *Bacillus* species were 3.10±1.78, 2.62±1.23, 2.80±1.74, 1.94±1.04 and 1.97±1.39, respectively. Seventy-eight percent of RTE foods conformed to acceptable limit of < 4logCFU/g for APC. The distribution of bacteria from schools FSEs were 62.0% (FCS), 19.0% (food handlers' hands) and 19.0% (RTE foods). The identified food-related bacteria were *Alcaligenes faecalis*, *Achromobacter xylosoxidans*, *Bacillus cereus*, *Ochrobactrum anthropi*,

Proteus mirabilis, *Serratia marcescens*, *Staphylococcus saprophyticus* and *Bordetella* species. *Alcaligenes faecalis* resistance (%) to cefixime, cefuroxime, ceftazidime, gentamicin, augmentin, nitrofurantoin, ofloxacin and ciprofloxacin were 76.2, 71.4, 66.7, 61.9, 57.1, 42.9, 4.8 and 4.8, while for *Bacillus cereus*, they were 85.7,100.0, 57.1, 85.7, 28.6, 57.1, 0.0 and 0.0, respectively. The minimum inhibitory concentration of colistin for *Alcaligenes faecalis* ranged from 1.5 µg/mL to >256 µg/mL which was highly significant ($F=9.194$, $p<0.05$) compared to other antibiotics. Two *Bacillus cereus* were resistant to imipenem, 81.0% were multi-antibiotic resistant, while none of the identified bacteria showed resistance to piperacillin/tazobactam.

Food contact surfaces and food handlers' hands were grossly contaminated. The presence of colistin-resistant *Alcaligenes faecalis* and resistance of *Bacillus cereus* to imipenem in boarding schools' food service establishments is a serious public health concern. These findings will be useful in policy formulation and the development of food safety guidelines in boarding schools.

Keywords: Environmental hygiene practice, Food-related microbial contamination, Food service establishments, Antibiotic-resistant bacteria

Word Count: 497

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
APC	Aerobic Plate Count
BCC	Bacillus Cereus Count
CAGR	Compound Annual Growth Rate
CDC	Centre for Disease Control
CFU	Colony Forming Unit
DALY	Disability Adjusted Life Years
EDTA	ethylenediaminetetraacetic acid
FC	Faecal Coliform
FAO	Food and Agricultural Administration
FBD	Foodborne Disease
FCS	Food Contact Surface
FDA	Food and Drug Administration
FSEs	Food Service Establishments
GAP	Good Agricultural Practices
GMP	Good Manufacturing Practices
GPS	Global Positioning System
HACCP	Hazard Analysis Critical Control Points
HPA	Health Protection Agency
IDSR	Integrated Disease Surveillance and Response

LMICs	Low and Middle-Income Countries
MGE	Mobile Genetic Elements
NBS	National Bureau of Statistics
NCBI	National Centre for Biotechnology Information
NSFP	National School Feeding Program
NCDC	Nigeria Centre for Disease Control
NPC	National Population Commission
PCR	Polymerase Chain Reaction
RTE	Ready-to-Eat
rRNA	ribosomal Ribonucleic acid
SAC	Staphylococcus Aureus Count
SDG	Sustainable Development Goals
SHP	School Health Programme
SSC	Salmonella Shigella Count
TC	Total Coliform
UNICEF	United Nations Children Funds
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background

More than 50% of children deaths worldwide are attributed to malnutrition, with the highest occurrence observed in underdeveloped nations, particularly in Sub-Saharan Africa and South Asia (Walson and Berkley, 2018). Access to nutritious and safe foods, healthy environments, basic water supply, hygiene, health, and sanitation are crucial steps toward alleviating these problems. Malnutrition has been linked to death in diarrhoeal infections, however, it is seldom stated as a causative agent (WHO, 2011a).

United Nations Sustainable Development Goals 2, 3, and 6 which are zero hunger; good health and well-being; clean water and sanitation are all directly tied to food safety and public health (UN, 2020). Guaranteeing food safety and security in today's globalised world is becoming increasingly difficult and underestimated by governments, businesses and individuals alike (Fukuda, 2015). Consumption of contaminated food can result in illnesses and their long-term consequences, including decreased life expectancy and disruption of maternal/child health. Diarrhoeal diseases kill around 2.2 million people globally each year, with contaminated water, inadequate sanitation, and poor hygiene being responsible for 56% of those deaths. The majority of these deaths are due to diarrhoea and jeopardize international development efforts (WHO, 2015).

Globalisation of the food supply chain has created new obstacles for food safety, aggravating worldwide public health problems associated with foodborne infections. Rapid urbanisation, increased food intake away from home, and the introduction of novel or antibiotic-resistant bacteria and food vehicles have all been cited as contributing factors (Fukuda, 2015). The variety of meals available in various sectors of Food Service Establishments (FSEs) such as restaurants, schools, street-vended foods, neighbourhood supermarkets, hospitals and assisted living facilities has a significant impact on customer choices and healthy eating. It becomes vital to monitor and assess operations along the

food supply chain from field to table on a constant basis (Omojokun, 2013). While food producers and distributors take necessary procedures to ensure food safety until it reaches the client, all food handlers and consumers are responsible for maintaining these protections until the food is consumed (Odeyemi *et al.,* 2019). Periodic visual inspections concentrating on sanitary procedures and microbiological monitoring of surfaces prone to cross-contamination could give critical insights for enhancing food workers' knowledge, attitudes and safe food handling practices (Garayoa *et al*., 2017). While outbreaks associated with commercially processed foods attract much public attention, many cases of foodborne illnesses go unreported in schools, restaurants, informal catering services, and households. Foodborne diseases (FBDs) continue to cause significant illnesses and death among the populace particularly in at-risk populations including, children, adolescents, aged elderly and those with weakened immune systems (Marzano and Balzaretti, 2013).

According to UNICEF (2016), adolescents aged 10 to 19 years, account for 16% of the world's population of 7.2 billion with adolescents accounting for 23% of the population in Sub-Saharan Africa. Diarrhoeal diseases are the most common illnesses resulting from consumption of contaminated food, causing approximately 600 million people to fall ill and 240,000 deaths annually (WHO, 2015). Also, the National Bureau of Statistics (NBS) reported in 2009 that Nigeria had over one million (1,069,133) cases of diarrhoea.

School feeding service is defined as an aspect of School Health Programme (SHP) and includes design of a food service facility, cleaning and sanitation, waste disposal and pest control (Moronkola, 2012). It is aimed at providing at least a balanced meal a day to school children. The confined nature of the school environment could increase the risk of spread of infectious microorganisms if food hygiene and proper monitoring are not implemented. Persistent FBDs will result in malnutrition, interrupt education, can lead to chronic diseases and affects growth and development in the community. Therefore, reducing exposure to bacterial contaminants in young people/students is probably more beneficial and effective than trying to improve their health later in life (Machado *et al.,* 2014).

Poor hygiene contributes significantly to the spread of respiratory and gastrointestinal tract infections among school children. Some of these infections have developed

antibiotic resistance and are extremely difficult to treat (Maillard *et al.,* 2020). Antibiotic-resistant bacteria and antibiotic-resistant genes can rapidly spread across the food supply chain and cause human illnesses (FAO, 2015). Multiple studies have reported antibiotic-resistant strains spreading, infecting and contaminating food animals and products, making the spread of antibiotic resistanct organisms along the food chain a major worldwide public health problem. Because of the problem of antibiotic resistance, which hinders the effective treatment of infectious bacterial diseases, it is increasingly important to employ improved hygiene practices in order to limit the spread of antibiotic resistant strains (Maillard *et al.,* 2020).

1.2 Statement of Problem

Global food consumption patterns have shifted away from traditional home cooking and moved toward modern outdoor and mass caterings. Ready-to-Eat (RTE) foods are the results of human actions such as economics, education, a healthier lifestyle, a desire for nutritious foods and a variety of food options. Daily life situations, including schools, where no hygiene regulation is normally enforced, create various opportunities for infection transmission and its implications. The majority of infections are caused by resistant pathogenic bacteria, making serious bacterial infections difficult to treat. This could increase the risk of more deadly infections and also the cost of healthcare, leading to a greater prevalence of morbidity and even mortality, exerting a considerable strain on the country's economy.

In 2016, around 10.3 million Nigerian pupils were enrolled in junior and senior secondary schools (FME, 2017), accounting for 20% of the country's teenage population. These schools serve at least one meal per day, either through food vendors or in a school cafeteria. Although underreported, there is growing evidence of contamination in school foods. There is a scarcity of data on the microbiological state and occurrence of antibiotic resistant bacteria in boarding FSEs, which could result in challenging foodborne diseases.

1.3 Justification

Boarding school system is an important context for health index surveillance and subsequently health promotions. An assessment of food safety concerns in school food facilities is a good starting point since it will provide baseline information on food

hygiene awareness and the microbiological status of school food service establishments. This research effort will improve the understanding of pathogen spread in food, the environment, and antibiotic resistance in schools' FSEs, generate data that may be beneficial in evaluating health risk factors and controlling infection, and aid in the development of policies and the provision of food safety guidelines for school health programmes. Although extensive microbiological studies have been documented on different segments of schools' FSEs, there is limited data on boarding high schools' FSEs in Nigeria, hence the need for this study. These results could proffer desirable changes among food handlers, school management and students behaviour and practices. The findings will lead to overall provision of healthy, safe and nourishing foods and better behavioural attitudes to choosing safe foods.

1.4 Research Questions

1. What factors influence environmental hygiene and food safety practices among food handlers in boarding high schools' FSEs?
2. How prevalent and diverse are the bacteria associated with these schools' FSEs?
3. What are the antibiotic resistance pattern of identified bacteria from boarding schools' FSEs?

1.5 Aim

This aim of this study was to assess the environmental hygiene, food handlers' practices and microbiological status of food service establishments in selected boarding high schools in Ibadan, Nigeria

1.6 Specific Objectives

The objectives of the study were to:

1. Assess boarding high school FSEs' environmental conditions and food handlers' knowledge, attitudes and practices.
2. Identify by conventional and molecular methods prevalent bacteria on food contact surfaces, food handlers' hands and Ready-to-Eat foods in selected boarding high schools' FSEs.
3. Determine the antibiotic susceptibility profiles of identified bacteria.

CHAPTER TWO

LITERATURE REVIEW

2.1 Food Service Establishments

Food Service Establishments (FSEs) are defined as all establishments where food is regularly served outside the home. It is also defined as the physical access to food, an important element of food environment. Food environment can be thought of as where food is available in a community, how customers feel inside food stores, how services and infrastructures work in institutions, or how much information can be assessed about food (Engler-Stringer *et al.*, 2014). They are classified as commercial and non-commercial FSEs. Commercial FSEs include restaurant, hotel, fast food, supermarket, vending machines, and some industrial catering services (public houses, travel caterers). Non-commercial FSEs include institutional/educational settings in hospitals, schools, social services, armed forces (army, navy, air force), elderly homes, child-care centres and nursing homes (Payne-Palacio and Theis, 2005).

As civilisation progresses, changing lifestyles, healthier eating habits, urbanisation, increasing public interest in nutrition and freshness influenced the development of food service establishments. In recent years, the industry has grown significantly and seen profound changes. The industry's primary drivers of growth are socioeconomic conditions such as high disposable income, a busy and increasingly mobile lifestyle, the changing status of women, increasing number of single person households, special needs, presence of a more affluent society, families having time out together, an increase in corporations outsourcing non-core services, and market expansion into new sectors such as transportation and retail. Additionally, tourism, immigration, and culinary variety demands for healthier, nourishing and safer foods are of major impact (Payne-Palacio and Theis, 2005; Jaffee *et al*., 2019).

The global market of food service industry was estimated at US$3 Trillion in the year 2020 and with a prospect of US$4.1 Trillion in 2026, growing at a CAGR of 5.4%. The

USA, Canada, China and Europe will drive the 3.4% CAGR (Research and Market, 2022). In Africa, FSEs are growing fast with an estimated CAGR of 7.9% between 2020 and 2025. Nigeria is expected to take the lead with 6.4% CAGR being the largest African economy (Research and Market, 2021). Also, most countries have a large share of their human labour working in the food service sector. It has contributed to socio-economic improvements and expansion of tourism industry (Signe *et al*., 2018). These FSEs often vary in sizes depending on the menu size and consumer number. The consequence of this is increased challenges in provision of safe foods in the food service establishments. It has been reported that schools, hospitals, childcare centres and elderly homes should be given special attention with regard to food safety due to vulnerable populations often served (Elson, 2007; Lund, 2015).

2.2 Food Safety and Sustainable Development Goals

Food safety is concerned with maintaining minimal food contaminants such as microbial, chemical, physical hazards and even allergens that may occur along the food supply and cause injury or illness to the consumers (WHO, 2015). Food and nutritional security become a reality only when the basic components of a healthy diet are safe to ingest as perceived by the consumers (Grace, 2015a). Food safety encompasses all steps along the food value chain of growing, harvesting, transporting, cooking, distribution, preparing, storing, serving and consuming, with the goal of preventing infection and contamination throughout the food production chain and assisting in the maintenance of food quality and wholesomeness to stay healthy.

According to WHO (2015) estimates, 31 global hazards sickened 600 million people and killed 420,000 people annually. Also, World Bank estimated that disease, disability, and early deaths caused by unsafe food resulted in productivity losses of approximately US$95.2 billion per year in Low and Middle-Income Countries (LMICs) (Jaffee *et al*., 2019). Unsafe food endangers food and nutritional security, human growth, the food industry as a whole, and international trade. Food safety hazards that have been addressed include microbial pathogens, parasites, zoonotic diseases, aflatoxins and other chemical contaminants (WHO, 2015). According to Jaffee *et al.* (2019) and Grace (2015b), food safety will be critical to attaining many of the United Nations Sustainable Development Goals (SDGs), including the following specific goals:

SDG 1 - End Poverty: Foodborne disease (FBD) is the major cause of illness among the poor, and it is associated with a number of costs affecting them, including lost workdays, out-of-pocket spending, and lower value of farm animals and other properties.

SDG 2 - End hunger: FBD has an effect on both food security and nutrition. Toxins, for example, may directly contribute to malnutrition; some of the healthiest foods seem to be the most frequently linked in FBD and consumer concerns about food safety may lead them to buy less healthy food.

SDG 3 - Good health and well-being: FBD has a comparable consequence to malaria, HIV/AIDS, and tuberculosis, with infants, pregnant women, the aged, and those with compromised immune systems being particularly vulnerable.

SDG 6 - Clean water and sanitation. Inadequate access to safe drinking water increases the risk of food contamination during processing, excessive chemical use in food production can contaminate water sources, and infectious FBDs can be spread via water.

2.3 Hazards associated with Food Service Establishments

Hazards are defined as anything found in food that can contaminate it and cause harm to the consumers, whether it is added intentionally or not. According to Ababio *et al*. (2016), Valero *et al.* (2016), Sridhar and Oloruntoba (2018) and WHO (2019), food safety hazards are classified into four groups: physical chemical, biological hazards and allergens:

1. Physical hazards: These are things that could be harmful that aren't normally in food such as stones, glass, nails, metal, hair, bones, dirt, plastic, wood, and animal faeces. They are the first to be identified as contaminants since they can be seen by naked eyes.
2. Chemical hazards: A chemical hazard occurs when there is presence of unwanted chemical in food/water. The most common chemicals are sanitizers, pesticides, detergents, polishes, glass cleaners, cleaning and drying agents, food additives, agro-chemicals, dioxins, polychlorinated biphenyls, heavy metals and antimicrobial residues.
3. Biological hazards: These are found in nature among humans, animals and/or the environment (soil, water and air). They may be bacteria, viruses, fungi, parasitic

protozoa, their toxins and metabolites. Common examples include *Escherichia coli, Campylobacter* spp. *Salmonella* spp., *Noroviruses* and *Aspergillus* spp.

4. Allergens: Most food allergens are proteins such as egg, nuts, seafood and milk. Cereals and fruits have also been implicated.

2.4 Foodborne pathogens

Foodborne pathogens are microorganisms that are implicated in most foodborne diseases, which mostly resulted from consumption of contaminated food and water. These pathogens normally colonize the human gastrointestinal tract when proper hygiene and sanitation of food and water have been compromised along food supply chain. The most commonly isolated pathogens from contaminated food and water are bacterial organisms such as enterotoxigenic *E. coli, Campylobacter jejuni, Salmonella* spp., *Shigella* spp., *Listeria monocytogenes, Vibrio* species, *Bacillus cereus, Staphylococcus aureus* and *Clostridim perfringens*. Others are viruses like Norwalk virus, rotavirus, Hepatitis A virus; and protozoan parasites which include *Entamoeba histolytica, Toxoplasma gondii, Trichinella,* and *Giardia lamblia* (Blackburn and McClure, 2002). These bacteria are responsible for over 200 diseases, which range from gastroenteritis to cancer (WHO, 2015). Diarrhoea is a symptom of foodborne diseases caused by a variety of microorganisms, most of which are transmitted by contaminated food and water. The majority of gastroenteritis cases are infectious and can be transmitted directly or indirectly through contact with infected people's faeces.

Bacteria are responsible for more than 60% of foodborne illnesses because they possess different virulence factors such as ability to adhere and adapt to host cells, toxin production and presence of antibiotic resistant genes. Bacterial foodborne diseases are classified into three types: infections, intoxications, and toxicoinfections. Foodborne infection is contracted through the consumption of food infected with live bacteria such as *Escherichia coli* or *Campylobacter* spp., which grow and establish themselves in the host, causing disease. Foodborne bacterial poisoning occurs when food contains pre-formed bacterial toxins, such as those produced by *Staphylococcus aureus* and *Clostridium botulinum*, as a result of bacterial growth in the food. Foodborne toxicoinfection occurs when bacteria (*Clostridium perfringens*) found in food, generate a toxin in the host (IFT, 2004; Hernández-Cortez *et al.*, 2017). The common bacterial food pathogens are described below.

2.4.1 *Escherichia coli*

Many domestic animals and humans are naturally infected with *Escherichia coli*. It is an Enterobacteriaceae Gram-negative rod. The phenotypic and pathogenicity characteristics of *E. coli* strains are used to create a classification system. The enteropathogenic *E. coli* (EPEC) strains causes infantile diarrhoea, the enterotoxigenic *E. coli* (ETEC) strains, which cause diarrhoea (travellers disease) due to their enterotoxin, the enteroinvasive *E. coli* (EIEC) strains, which is responsible for dysentery-like illnesses, the enteroaggregative *E. coli* (EAEC), the enteroheamorraghic *E. coli* (EHEC), a subset of the Shiga toxin-producing *E. coli* (STEC) and the diffusely adherent *E. coli* (DAEC) strains (FDA, 2012; Nyenje and Ndip, 2013).

Outbreaks of FBDs have been traced to the enteroheamorraghic (EHEC), the most prevalent *Escherichia coli* strain in terms of food safety and their toxins are very similar with that of *Shigella dysenteriae*. The most common EHEC causing human illness is *E. coli* O157:H7. When compared with *Salmonella* spp. and *Campylobacter* spp., EHEC infection cases are fewer, but the potentially fatal effects of this disease, particularly in children and the elderly, make it a major public health concern. EHEC infection is responsible for 70% of all cases of paediatric kidney failure (HPA, 2009). Consumption of raw or undercooked ground beef, as well as other ground beef-based foods such as burger patties and meatloaves, is a major source of infection. Cross-contamination, as well as poor personal and kitchen hygiene, are important transmission routes.

Plasmids and bacteriophages, which are mobile genetic elements that can be transferred horizontally, can encode *Escherichia coli* virulence genes. During slaughtering and carcass processing, both *E. coli* O157:H7 and non-O157 STEC can enter the intestines of cows and cause contamination (Hernández-Cortez *et al.*, 2017). It is common during outbreaks to have an incubation period of between three and four days, but it can be shorter or longer. People of all ages are at risk of contracting an infection, but it is more prevalent among the young. The O157:H7 strain of *E. coli* is considered an invasive pathogen because of its ability to rapidly grow and adhere to intestinal cells following an infection. It is easier for toxins to be absorbed into the tiny intestinal wall's capillaries because of this close contact. Within 24 hours, bloody diarrhoea, severe stomach cramps, and fever are all symptoms of colitis caused by *E. coli* O157:H7 (FDA, 2012).

The study of this organism and its qualities is crucial for minimising illness spread in LMICs, where alarming rates of unsanitary environments, malnutrition, and limited health care persist. According to an investigation in Nigeria, the presence of commercial fast food and roadside cafes, diarrhoea, the wet seasons of the year, and asymptomatic carriers might all contribute to *E. coli* O157:H7 infection. Good food handling techniques, personal and environmental hygiene are the ultimate line of protection against *E. coli* O157:H7 illness. Thoroughly cooking meat, consuming pasteurised milk, and washing fruits and vegetables before eating them raw are all examples of food hygiene practices that may help reduce the spread of disease (Isibor *et al*., 2013).

2.4.2 *Campylobacter* spp.

Campylobacter infection is a zoonotic disease that have been responsible for outbreaks and sporadic infections. The genus Campylobacter are comma-shaped, Gram-negative rods that do not produce spores and it consists of about 20 species, linked to human infections. *Campylobater jejuni* and *Campylobater coli* are the most often reported in human illnesses where it causes acute enteritis. Many more species have been isolated from animal (FDA, 2012).

It is found in faeces of warm-blooded animals like cow and sheep. Other species that thermo-tolerant in nature but not often reported are *Campylobater lari* and *Campylobater upsaliensis.* It is often isolated from poultry, contaminated water and food. Average annual incidence rate (IR) was 11.4 cases/100,000 persons in the USA (Geissler *et al*., 2017) . Campylobacter is found in 1.5 percent to 18 percent of young children in Sub-Saharan Africa (Mason *et al*., 2013). Campylobacter infections are self-limiting not requiring use of antibacterial drugs. The clinical symptoms of the infection are abdominal pain, fever, headache, and sometimes vomiting which mimic gastroenteritis from other enteric bacterial pathogens. The symptoms start to manifest when as few as 500 organisms have been consumed between two to five days of infection. Campylobacter species are easily destroyed by heat, and have been found to be reduced in numbers when contaminated foodstuffs are frozen (Blackburn and McClure, 2002).

This bacteria foodborne transmission is thought to be more common with inadequately cooked meat products, poultry, and even contaminated dairy products, and infections

can also be spread via contaminated water or ice (Gillespie, 2007). A significant number of cases occur as a result of contaminated vegetables and shellfish, and swimming activities involving polluted water. It is critical for public health to deploy comprehensive biocontrol strategies to minimise the transmission of this group of pathogens. There are facilities in the developed world to test for these pathogens using improved technology such as Polymerase Chain Reaction (PCR) based typing, pulsed-field gel electrophoresis and ribotyping. Cross-contamination of RTE foods during food preparation is a significant mode of transmission (FDA, 2012).

In order to prevent Campylobacter infections, it is important to cook or pasteurise food properly, and to minimise cross-contamination of cooked or RTE meals with dirty utensils, equipment, or food contact surfaces. Pasteurisation will kill viable campylobacters in milk, but safe food handling techniques and safe irrigation must be used throughout harvesting to assure food safety (CDC, 2011a). Campylobacteriosis has been linked to autoimmune diseases such as Guillain-Barre Syndrome, Haemolytic Uremic Syndrome, and recurrent colitis (FDA, 2012).

2.4.3 *Salmonella* spp.

Bacteria of the genus Salmonella are aerobic, Gram-negative rods that do not produce spores with more than 2000 serotypes. The ingestion of roughly a million living cells causes the illness with an incubation period which varies from 12 to 36 hours. It has been found in the intestines of humans and a wide range of other animals (cattle, pigs, poultry, dogs and rodents). Poultry offal, such as eggshell, is a frequent source of Salmonella. Insect pests, domestic pets, and food workers could all transfer it to foods. Salmonella can cause enteric fever, a life-threatening infection, and salmonellosis, a more frequent foodborne condition (Grace, 2015a). Salmonella is a genus comprised of two species that can cause sickness in humans: *Salmonella enterica* and *Salmonella bongori.* Serotype designations are frequently used to refer to *Salmonella* species. Serotypes are defined by the antigenic characteristics of the surface and flagella. Salmonella enterica, the most serious public health threat, is divided into six subspecies. For instance, *Salmonella enterica* subsp. enterica is further classified into several serotypes, including *Salmonella enterica* serotype Enteritidis and *Salmonella enterica* serotype Typhimurium, the most common causes of human salmonellosis (FDA, 2012).

Diseases like typhoid and paratyphoid (also known as enteric fever) caused by *Salmonella typhi* A, B and C are prevalent in underdeveloped countries. Non-typhoidal species cause salmonellosis which is often under reported because it is a self- limiting acute gastroenteritis (Horn *et al.*, 2016). In an Ethiopian study, 6.9% of food handlers excreted salmonella species in their stool specimen, and most did no medical screening before working (Mama and Alemu, 2016). Inadequate washing of fruits and vegetables before consuming them, and lack of thorough cleaning of work surfaces in food preparation can be a source of salmonella. Non-typhoidal Salmonella serovar Typhimurium and Salmonella serovar Enteritidis are the most common causes of human salmonellosis.

Some *Salmonella* spp. have evolved to adhere and infect vegetables (Nyenje and Ndip, 2013). Salmonella species were found in 10.9 percent of the broiler/layer poultry farms in Jos, Nigeria (Agada *et al*., 2014). The study also found environmental risk factors like untreated water supply, other farm animals as well as vermin, wild bird, farm workers and equipment.

2.4.4 *Shigella* spp.

Shigella spp. are Gram-negative, facultative anaerobic rods that do not produce spores. They have a strong resemblance to Salmonella species. *Shigella* spp. are highly adaptable pathogen that typically infects just humans and a few other animals. Contaminated water and food are vectors for the bacterium (FDA, 2012). Shigella infection is often acquired through ingestion (faecal-oral route), depending on the host's age and health status. A very low number of cells (100 cells) can be sufficient to initiate an infection. Certain strains produce enterotoxin shiga toxin, which is identical to *E. coli* O157:H7 verotoxin. *S. dysenteriae*, *S. flexneri*, *S. sonnei*, and *S. boydii* are the most important species (Girma, 2015).

Shigellosis is a severe invasive enteric infection that causes diarrhoea ranging from watery stool to severe life-threatening dysentery. The disease is widespread in a number of impoverished nations and outbreaks can result in severe morbidity and mortality. Controlling shigellosis is tough due to its ease of transmission and quick development of antibiotic resistance (WHO, 2005). Shigellosis is prevalent in nations with poor hygiene standards. Incubation might last between two and seven days. Shigella is mainly

an invasive pathogen, with the colon being the primary site of infection. Ulceration is produced by the build-up of metabolic products and the release of endotoxin. Infected individuals' stool will contain blood, mucous, or pus. Personal and food handling hygiene are necessary components of preventing disease transmission from food handlers to customers. Handwashing before food handling and properly cooking all meals prior to consumption help lower the chance of developing shigellosis (Jay *et al.*, 2005; Mama and Alemu, 2016). The majority of instances of shigellosis resulted from consuming food or drink contaminated with faeces. The bacteria can spread from infected carriers by a variety of channels, including food, fingers, faeces, flies, and fomites (FDA, 2012).

2.4.5 *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive, facultative anaerobic rod that causes listeriosis. Unlike many other pathogens, it is hardy, salt-tolerant, and grows at low temperatures (below 1 °C). Its existence often persists in food processing environments (FDA, 2012). There are invasive and non-invasive forms of infection with *Listeria monocytogenes*. The non-invasive form is characterised by gastroenteritis in the absence of more serious symptoms like septicemia, meningitis, and abortion following foodborne infection with *Listeria monocytogenes.* It has been suggested that the occurrence of non-invasive listeriosis may be underestimated as *Listeria monocytogenes* is not among the pathogens routinely investigated in outbreaks of gastrointestinal diseases. It is often associated with soil, water, vegetation and sewage (Akano *et al.*, 2013).

Most human infection follows consumption of contaminated food. Foods normally associated with listeria outbreaks include soft cheeses, fermented sausages, coleslaw and other assorted salads. Because of the pathogen high mortality rates in pregnant women, unborn children, neonates, the elderly, and immunocompromised individuals, it should be extremely low in food products (Valero *et al.*, 2016). Moist surfaces in food processing plants are ideal for this bacterium. In an appropriate growth medium, *L. monocytogenes* may thrive in a pH range of 4.4 - 9.4. (Arunava *et al.*, 2019). Flu-like symptoms can be caused by ingesting as few as 1000 bacterium cells that invade macrophages, thereby causing malaise, diarrhoea, and mild fever. It is possible that Listeria virulent strains multiply after macrophage invasion, disrupting these cells and resulting in sepsis. It is at this point that microbes can spread throughout the body and can impair the central nervous system, the eyes, the heart, as well as the foetus of

pregnant women. It is common to find *L. monocytogenes* as a post-contamination pathogen in meals such as cooked slices of meat or smoked salmon or chopped vegetables and ready-to-eat foods (Valero *et al.*, 2016).

Processed Ready-to-Eat (RTE) foods are a major source of *L. monocytogenes* infection because of their long shelf life (weeks) and low-temperature storage until consumption. Many RTE food-borne listeriosis cases have been recorded globally due to the fact that RTE meals are not further cooked, allowing *L. monocytogenes* to survive. In 2013, the European Food Safety Authority (EFSA) reported 1,763 confirmed listeriosis cases from 27 member nations, with 191 deaths. Despite the implementation of food safety criteria, human listeriosis has increased dramatically. It is difficult to attribute human instances to specific meals, but Whole Genome Sequencing (WGS) paired with epidemiological data offer the ability to do so (Ricci *et al*., 2018).

This pathogen was found to be prevalent in poultry and poultry products from Oyo State, Nigeria with an overall prevalence of 91.8 percent. Higher incidence in meat suggests post-slaughter contamination and could indicate public health risks from contact between raw meat and other processed foods (Ishola *et al*., 2016). In another study in Enugu, Nigeria, Listeria organisms were found in a wide range of Nigerian environments, including soil and foods of both animal and plant origin, which may pose a health risk for immunocompromised individuals (Ikeh, 2010). *L. monocytogenes* was found in 25% of smoked fish collected from a variety of retail stores and markets in Sokoto, Nigeria (Salihu *et al*., 2008).

2.4.6 *Vibrio* spp.

The genus Vibrio consists of Gram-negative, comma-shaped oxidase-positive, facultative anaerobic rods with polar flagellum. Members of the Vibrio genus are endemic to aquatic habitats and perform critical functions in maintaining the aquatic environment. The genus has over 100 species, the majority of which are marine or freshwater in origin, and its taxonomy is periodically changed as new species are discovered. The primary mode of transmission of Vibrio infections to humans is via intake of polluted water and undercooked seafood products. *Vibrio parahaemolyticus, Vibrio cholerae, Vibrio vulnificus, Vibrio tubiashi,* and *Vibrio fluvialis* are all pathogenic Vibrio species of public health significance that are typically spread by water and shellfish. (Osunla and Okoh, 2017).

In underdeveloped nations, the prevalence of certain vibrios is correlated with inadequate hygiene and sanitation, while in developed ones, the prevalence of other species is linked with regular foodborne infections. The most serious pathogen is *Vibrio cholerae*, which causes cholera, a severe disease with worldwide impact. *Vibrio parahaemolyticus* and *Vibrio vulnificus* are significant pathogens identified from recreational water and discovered to exhibit virulent antibiotic resistance genes (Oyelade *et al.*, 2018). *V. cholerae* is a non-invasive organism that affects solely the small intestine by the production of enterotoxin and is the causative agent of cholera, whereas *V. parahaemolyticus* and *V. vulnificus* are regarded to be invasive germs that mostly impact the colon.

While cooking kills the bacteria, infection is usually caused by raw seafood, inadequate processing, or cross-contamination with a raw product. Serogroups O1 and O139 can proliferate in contaminated seafood following cooking, and rapid chilling of food leftovers is vital to avoid this infection. In locations where *Vibrio cholerae* serogroups O1 and/or O139 are endemic, illnesses can arise through consuming polluted water, as well as contaminated food and seafood. Cholera is a highly contagious disease that can affect both young and old. It is accompanied with an onset of acute diarrhoea that occurs rapidly. Without rehydration therapy, this condition has a mortality rate of 30% to 50%; however, with proper care, the fatality rate drops to less than 1% (FDA, 2012). *V. cholera* has been implicated in numerous foodborne diseases epidemics in schools, hospitals, nursing homes, restaurants, and street vendors. Food-borne infections can be avoided and decreased via the combined efforts of all stakeholders engaged in the production, transportation, processing, distribution, regulation, and preparation of food products. Where cholera is endemic, it is critical to interrupt the infection cycle by safeguarding food against sewage contamination. This disease is prevalent year-round in regions with poor sanitation in Nigeria, where it is endemic and occurs most frequently during the rainy season. In 2018, Nigeria recorded 42,466 suspected cases including 830 deaths, for a case fatality rate of 1.95 percent in 20 of 36 states (NCDC, 2019).

Pseudomonas aeruginosa and other Non-Fermentative Gram-Negative Bacilli (NF-GNB)

Pseudomonas aeruginosa is a Gram-negative, motile, rod-shaped, aerobic, and non-fermentative organism. It is ubiquitous, found in soil, water, plants, and animals and can survive for a long time in a variety of environments and situations. *P. aeruginosa's* environmental resilience and its many virulence factors enable it a human opportunistic pathogen, primarily affecting immunocompromised people. It is a nosocomial and community acquired pathogen (Gupte *et al*., 2015). *P. aeruginosa*, which causes 10.1% of all hospital-acquired infections, is the fourth most isolated causative agent (CDC, 2011b). Reservoirs include disinfectants, respiratory equipment, food, sinks, taps, and mops. This bacterium was found in 20.3 percent of post-operative wound swabs from four hospitals in Benue, Nigeria. (Iduh *et al.,* 2015).

This organism is often brought into hospitals on fruits, plants, and vegetables, as well as by visitors and patients from other hospitals. Transmission happens via hospital personnel's hands, direct patient contact with contaminated reservoirs, and contaminated food and drink. Hand hygiene and environmental cleanliness can significantly reduce infection risk in hospitals. Antimicrobial resistance genes are among the pathogen's virulence factors. Multi-antibiotic resistant infections are difficult to treat and can be fatal, especially in immunocompromised patients (Al Dawodeyah *et al*., 2018).

Other NF-GN bacilli include *Acinetobacter baumanii, Achromobacter xylosoxidans, Alcaligenes faecalis, Ochrobactrum anthropi, Burkholderia species, Sphingomonas paucimobilis, Moraxella species, Rastonia picketti, Shewenella putrefaciens, Stenotrophomonas maltophilia* among others. These organisms are found in soil, water and the environment (Gales, *et al*., 2005; Chawla *et al*., 2013). Infections due to NF-GNB other than *Pseudomonas aeruginosa* are uncommon but their incidence is increasing in recent years. They cause infections in immunocompromised hosts and are important in clinical diagnosis. Their identification to species level is difficult as a result of their phenotypic variation and slow growth rate. They are being increasingly isolated from diverse ecological niches including food service and hospital settings. The availability of improved microbiology techniques such as molecular bio-typing could have influenced their earlier misidentification by conventional methods. These organisms have been found to exhibit resistance to commonly used antimicrobial agents

and some last-resort antibiotics like colistin and carbapenems. They have been shown to produce a wide range of beta-lactamases and metallo-lactamases (Chawla *et al*., 2013; Grewal *et al.*, 2017).

2.4.7 *Bacillus* spp.

The genus comprises of four main species namely *Bacillus anthracis, Bacillus cereus, Bacillus mycoides,* and *Bacillus thuringiensis*. *Bacillus anthracis* causes anthrax, *Bacillus cereus* produces emetic toxin and enterotoxin, causing foodborne sickness, and *Bacillus thuringiensis* is a pathogen that infects insects. Several scientists believe that these closely related species should all be classified together as members of the *B. cereus* (Rosenquist, *et al*., 2005). *Bacillus cereus* is an aerobic, Gram-positive rod that forms spores and releases exotoxin into food. Fresh and marine waters, decomposing organic debris, dirt, vegetables, and invertebrates' digestive tracts are all part of this organism's natural environment (Horn *et al.,* 2016). It's found in a lot of cereal meals, especially rice and corn flour (HPA, 2009). The spore is resistant to heat and acid, and it is not killed by typical pasteurisation or sanitation methods. Temperature abuse is said to be the cause of the majority of outbreaks. Vomiting, stomach cramps, and some diarrhoea occur during the incubation period, which lasts between 1 and 16 hours. Because *Bacillus cereus* poisoning is not a notifiable disease, its prevalence is frequently underestimated (Christison *et al*., 2008).

Bacillus cereus produces two distinct types of food poisoning: diarrhoea (infection) caused by enterotoxins and emetic (intoxication) caused by a preformed short-cyclic peptide called cereulide. The first, and most well-known enterotoxin, causes abdominal pain and diarrhoea, with 4-16-hour incubation period and symptoms lasting between 12 and 24 hours. A haemolysin and a non-haemolytic enterotoxin are the most common examples. When enterotoxin is released in the gut, it can cause an intermediate sort of food poisoning, although the toxin can also be pre-formed in foods. Its symptoms are similar to those of an *S. aureus* infection (*Jay et al.,* 2005). In previous outbreaks, cooked meat, rice dishes, vanilla sauce, custards, soups, and raw vegetable sprouts were implicated. The second, emetic toxin is characterised by an acute episode of nausea and vomiting that occurs 1–5 hours after a meal; diarrhoea is not a common symptom. *B. cereus* emetic food poisoning is mainly caused by starchy foods, including boiled or fried rice, potatoes, pasta, and noodles. Since *B. cereus* can form spores (a heat-resistant

survival mode) at high temperatures, cooked food should be refrigerated at 4°C or lower temperatures (FDA, 2012).

2.4.8 *Staphylococcus aureus*

Staphylococcus aureus are facultative, aerobic, Gram-positive cocci. Most warm-blooded animals and humans have *S. aureus* on their skin and in their nasal cavities. They do not produce spores, but rather a heat-resistant toxin (survives 100°C for 30 minutes). *S. aureus* is the third leading cause of FBD globally and grows when cooked foods are kept at a temperature between 20°C and 40°C for an extended period of time (Zare *et al*., 2019).

There are at least 14 distinct Staphylococcal Enterotoxins (SE) described (designated A-O). Although type A is the most frequently implicated toxin in food poisoning, types C, B, D, and E have also been implicated. The majority of outbreaks involve SE-A and SE-D, which are caused by a broader range of environmental conditions. Numerous environmental factors, including pH, water activity, temperature, food type, and processing conditions, have been implicated in the production of SEs (Valero *et al*., 2016). Staphylococcal enterotoxins are a common cause of food poisoning. They are contracted after consuming contaminated foods, particularly processed meat and dairy products, that have been exposed to *S. aureus* because of poor handling and inappropriate time and temperature control during processing. Outbreaks have also been linked to pasta, sandwiches and sausages. Nausea and severe vomiting (sometimes with diarrhoea), can occur suddenly. The illness is typically self-limiting, with only severe cases necessitating hospitalisation (Argudin *et al*., 2010; Rossi *et al.*, 2018).

The enterotoxin is excreted when bacteria grow in incorrectly treated foods. The toxin-contaminated food causes fever, chills, headache, chest pain, and coughing. Food intoxication is distinct from bacterial foodborne infection, which occurs when pathogens proliferate after ingestion of food. Cooked food contamination must be prevented with extreme caution. In the case of staphylococcal entero-intoxication, food handlers must take specific care because indirect source of contaminated food could be asymptomatic *S. aureus* infected persons (Argudín *et al*., 2010).

2.4.9 *Clostridium* spp.

Clostridium species are Gram-positive, rod-shaped bacteria that may generate endospores, allowing them to survive in adverse environments (Montso and Ateba, 2014). Clostridia can infect both humans and animals. The most common species are *Clostridium botulinum, Clostridium difficile, Clostridium perfringens and Clostridium tetani.* They cause illness when large numbers of living cells which subsequently release an enterotoxin in the alimentary canal is ingested. The pathogen is carried in human and animal intestines, soil and dust. *C. perfringens* is the second most common foodborne pathogen after Campylobacter with cases underreported (FSN, 2015). The vehicle for spread of *C. perfringes* include cooked meat, poultry, fish and vegetable dishes stored at ambient temperature with long cooling periods with optimum growth temperature 43-45°C.

According to Gillespie (2007), the symptoms of *C. perfringes* gastroenteritis is an intestinal disorder, and symptoms are diarrhoea, nausea, but no vomiting or fever (incubation period is between 8-22 hours). In healthy persons, it usually causes a mild condition that lasts one day or less. It can spread from raw to cooked food, or from unhygienic food handlers. Vulnerable persons are more prone to serious sickness and eating food contaminated with soil or faeces and storing it in an environment that enhances organism growth (HPA, 2009). Poorly cooked or reheated foods, such as stews, meat pies and gravies containing beef, turkey or chicken, are linked with almost every epidemic. Spores germinate and proliferate when cooked foods are cooled slowly, held at room temperature, or reheating is insufficient.

C. perfringens (Type A) causes food poisoning (and gas gangrene); type C causes necrotizing enteritis. Institutional settings (including school cafeterias, hospitals, nursing homes, and prisons) are the most common source of *C. perfringens* poisoning and it often causes food poisoning among children and the elderly. As a preventive measure, food handlers should be educated about the hazards of large-scale cooking, especially meat dishes (FDA, 2012).

2.5 Risk Factors associated with foodborne diseases

Risk is described as an individual's attribute, characteristics, or exposure that increases the likelihood of getting an illness or harm. It is the likelihood of a hazard occurring in

food handling operations. The risk is proportional to the number of consumers who may be harmed, particularly in large-scale operations (Melngaile and Karklina, 2013). Since food is transmitted along the food chain from primary production to the consumers, failure in activities along the food supply are responsible for the risks often involved in foodborne diseases (Hernández-Cortez *et al.*, 2017).

Despite the implementation of Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP) and much structured science-based risk analysis Hazard Analysis Critical Control Points (HACCP) by formal food service establishments, food safety risks are on the increase. Food safety inspection violations are often high in most informal mass food service establishments like school catering, street foods in developing countries because they do not have the required food safety standards for implementation nor evaluation (Melngaile and Karklina, 2013).

It is apparent that environmental mishandling and misappropriation are not done on purpose to hurt or jeopardize the health of consumers. Errors frequently develop as a result of lack of understanding of the likely implications (Olumakaiye and Bakare, 2013). The occurrence of FBDs is caused by a number of key risk factors: foods from unhealthy sources, lack of drinkable water, insufficient cooking, unsuitable storage temperatures, contaminated FCSs, inadequate sanitation, and poor personnel hygiene (Grace, 2015a; FDA, 2018).

Unsafe raw material: According to Hernández-Cortez *et al.* (2017), most materials used for food processing are agricultural products from plants, animal and aquaculture. The risk of contamination is often due to lack of GAP such as unsafe irrigation water and contamination with faecal matters. Also, RTE foods like vegetable salads, fresh fruits, and others that will not be further processed before consumption must be sourced from safe and approved sources. The majority of foodborne infections are linked to animal products. Slaughtering animals could introduce huge concentrations of pathogens including antibiotic resistant bacteria into food industries (Lund, 2015). When receiving food, food handlers should ensure that it is at the proper temperature, free of pests, and has not been tampered with in any way. (FDA, 2018). Water is used in food processing, cleaning, and handwashing. The availability of potable water during food processing to minimise the risk of microbial and chemical contaminations is very essential (Valero *et al.*, 2016). Implementing correct water treatment and control

techniques, as well as evaluating their performance, is critical for preventing waterborne illness.

Inappropriate time and temperature control during food processing: Proper control of time/temperature during food production is the most important single process that can kill bacteria and prevent them from multiplying. The temperature range (danger zone) that must be avoided during holding potentially hazardous foods is 5°C and 65°C. Food plant managers must take every care to keep potentially hazardous foods out of the danger zone as long as possible (Valero *et al*., 2016). Food handlers lack information regarding appropriate cooking and refrigeration temperatures for preventing the growth and survival of germs, insufficient cooling and reheating of food and preparation of food in advance prior to consumption. Many studies in developing countries have reported poor temperature control and non-use of thermometer by food handlers during food processing (Oranusi *et al*., 2007; Rossi *et al*., 2018; Elsherbiny *et al*., 2019).

Contaminated Food Contact Surfaces (FCSs): The FCSs encompass all processing and storage equipment, utensils, facilities, food handler hands and PPE, as well as packaging materials. Microbial contamination can occur through, utensils (cutting board, knives, crockery), food processing equipment and countertops. If surfaces and utensils are not properly maintained, food remnants might accumulate and create biofilms, which have been linked to cross-contamination between food contact surfaces and other food products (Sibanyoni and Tabit, 2019). To avoid this, utensils, food preparation equipment, and any other contact surfaces should be cleansed, rinsed, and sanitised on a regular basis. RTE foods must be kept away from raw and unprocessed foods to avoid cross-contamination (Hernández-Cortez *et al*., 2017).

Poor food handlers' hygiene and sanitation: The term "food handler" refers to anyone who comes into direct contact with food when preparing, producing, processing, packaging, storing, transporting, distributing, selling, supplying, or serving it (Valero *et al.,* 2016). Food contamination is mostly determined by the health status of food handlers, their personal hygiene, their understanding and practice of food hygiene and safety (Mama and Alemu, 2016). Food handlers can easily transfer pathogens such as bacteria to the food through cross-contamination, as a result of poor food safety principles. Food workers must be adequately trained (regularly and continuously) in what they do to minimise the spread of bacteria in the food preparation area. Proper

handwashing, medical examination, basic knowledge about food safety are very important in order to minimise incidences of foodborne illness. It was reported in a South African school nutrition study that, while handling food, less than 50 percent of respondents wash their hands multiple times (Nomakhushe and Wilkenson, 2018). Whereas, another study reported that the prevalence of coliforms (*Enterobacter sp. E. coli, Klebsiella aerogenes, Proteus vulgaris* and *Shigella sonnei*) on the hands of fast-food handlers in Abeokuta, Nigeria (Bankole *et al.,* 2009).

2.6 Global estimate of foodborne diseases

Estimating the burden of FBDs is more difficult than estimating the burden of individual illnesses such as malaria or tuberculosis. These foodborne diseases continue to be a significant cause of illness and death in the global human population, affecting mostly vulnerable groups such as babies, the elderly, and the immunocompromised. The most frequent symptom is diarrhoea, however, the majority of cases go undiagnosed in the laboratory (Grace, 2015a). Dietary contamination is the leading cause of diarrhoea-related mortality which has hindered worldwide developmental projects. According to the WHO's global estimates of FBDs, almost 1 in 10 people get sick from contaminated food each year, resulting in 33 million lost healthy years. Foodborne diseases kill 125,000 children under five years of age every year (WHO, 2015). According to the World Bank, the overall productivity loss associated with FBD in LMICs is around $95.2 billion per year, while the annual cost of treating these diseases involved several billion dollars (Jaffee *et al*., 2019).

Every year, nearly one in six Americans (or 48 million individuals) become ill, 128,000 are hospitalised, and 3,000 die from FBDs (CDC, 2011a). Between 10,200 and 17,800 people are hospitalised annually in France because of these pathogens. An estimated one million children deaths occurred annually in South East Asia as a result of diarrheal infections caused by contaminated food and water. A study in Gansu predicted an annual occurrence of about 30 million episodes of acute intestinal disease in China, necessitating almost 20 million medical treatments and antibiotic prescriptions (Sang *et al*., 2014).

In Africa, poverty and malnutrition are the primary drivers of unhealthy food intake. Lack of access to safe drinking water, a dysfunctional government structure,

communicable diseases, trade pressure, and unfavourable environmental circumstances are all noteworthy reasons. The high prevalence of diarrheal infections among children is indicative of the African regions' food hygiene status. Microbial infections are commonly implicated as a result of unsafe food intake and an uncertified supply chain (Grace, 2015a). On the African continent, multiple severe foodborne outbreaks have been documented; Kenya witnessed an acute aflatoxicosis outbreak in 2004 due to maize, while Angola documented 400 cases of bromide poisoning in 2007 due to the usage of sodium bromide as cooking salt.(Nyenje and Ndip, 2013). Between 2017 and 2018, 674 cases and 183 deaths due to Listeriosis outbreak was reported in South Africa and the outbreak was traced to polony, a sausage-like product was implicated (WHO, 2020).

The World Health Organisation African Region was estimated to have the highest burden of foodborne diseases per population with an estimated 91 million illnesses and 137,000 deaths occurring every year (WHO, 2015). There is increase in rates of diarrhoea and deaths especially in Democratic Republic of Congo, Chad, Angola, Nigeria, Somalia and Ethiopia. Infectious diarrhoeal illnesses account for more than half of all foodborne illness worldwide. People living in the poorest area of the world suffer 53% of all foodborne illness and succumb to 75% of related deaths. FBDs in Africa are largely caused by diarrheal diseases, accounting for 70 percent of the total (Jaffee *et al*., 2019).

In Nigeria, diarrhoeal diseases are one of the priority diseases of public health significance by the Federal Ministry of Health (IDSR, 2005; NCDC, 2019). The Federal Ministry of Health, the Standards Organisation of Nigeria, the Federal Ministry of Agriculture and Rural Development, the National Agency for Food and Drug Administration and Control (NAFDAC), the Nigeria Plant Quarantine Services, the Consumer Protection Agency, the Federal Ministry of Environment, and the Federal Ministry of Education, are the most important institutions in Nigeria for monitoring food safety standards and regulations. However, the lack of harmonisation and enforcement of legislation by these various regulatory organisations, as well as public ignorance of food safety issues, have contributed to low food safety standards (Omojokun, 2013).

According to WHO (2009), more than 200,000 people die of food poisoning annually in Nigeria from foodborne pathogens especially *E. coli* and *Salmonella* species. It was

estimated that FBD cost Nigeria about three million US dollars which represented 17-25% of the total cost of all illnesses. Also, a total of 1,069,133 cases of diarrhoeal diseases were reported in 2007 with 2,368 deaths (NBS, 2009). In 2010, the overall expenditure on health care in Nigeria was estimated to be US$3 billion, or roughly 1.25 percent of gross domestic product (ILRI, 2011). Cholera is a major public health issue in WHO African Region. Between 1st January and 11th November 2013, a total of 39,898 cholera patients with 862 deaths were recorded from 21 countries with 10 % cases from Nigeria (4220 cases and 145 deaths, reported from 16 states (WHO, 2013).

Food safety interventions in underdeveloped nations are lacking in evidence-based practice. There are numerous benefits to training farmers GAPs and informal value chain participants. Food safety can also be improved by new technologies, better public awareness, and more emphasis on food system governance. (Grace, 2015).

2.7 School Health Programme

The School Health Programme (SHP) is an educational and health programme aimed at meeting the health needs of students and employees with the help of parents, community members, and the government. Raising children who are well-adjusted, physically fit, and who possess the behaviours, attitudes, and knowledge necessary to make informed decisions about their future is the main focus of school health programme (FME, 2006; Moronkola, 2012). It encompasses all school-based projects/activities aimed at promoting the healthy development of the school system. According to the Federal Ministry of Education, national school health policy, the SHP's scope includes the following: a healthy school environment; school feeding services; skilled-based health education; school health services; and links between the school, home, and community. School-based health services are an integral part of the public health system, as they contribute to children being effective learners and healthy citizens. The components must function together in order to enhance students' and families' lives (FME, 2006).

Research studies in Nigeria have shown that SHP faces numerous difficulties. It was reported that in Kogi State, neither public nor private senior secondary schools include regular food inspections, vaccinations, health evaluations, and the availability of safe drinking water (Sarkin-Kebbi and Kwashabawa, 2016). Also, in a related study on SHP implementation in selected public secondary schools in Ibadan, Nigeria, reported that

most school lunch services and sanitation should be improved. It also revealed a lack of implementation, particularly in school services and a healthy school environment. As a result, SHP must be revived nationwide to protect, preserve, and promote student health for better learning outcomes and educational objectives (Ademokun *et al.,* 2014).

2.7.1 Healthful school environment

The goal of a healthful school environment is to create secure learning, working, and living environments that promote students' emotional, physical, and social well-being. In boarding schools, students reside in the school except for vacations, therefore the school environment should be healthy. Healthful schools, according to Moronkola (2012), provide basic necessities like shelter, warmth, food, water, light and ventilation as well as emergency medical care and protection from biological hazards like pathogens, worms, rodents, dangerous insects, poisonous animals and insect pests. They also protect students from chemical hazards such as air and waterborne contaminants and cleaning agents.

School health services in most of the schools in Nigeria have been so poor and these however pose a significant threat to all sectors of the national economy. Akerele (2011) asserted that many children are being afflicted with communicable diseases such as measles, cholera, diarrhoea, malaria, typhoid fever, dysentery and pneumonia, which can be prevented or treated but could cause increased morbidity and sometimes death. This leaves most of the affected students with no other option than to drop out of school in the final analysis (Odongo *et al.,* 2015) determined the wide variation in the prevalence of communicable diseases among students in Kisumu county, Kenya. He reported malaria had the highest rate at 20.7%, diarrhoea was second highest at 15.1% and pneumonia was lowest at 5.2%. Many research studies have been conducted on health challenges facing children that are under five-year old, but there is paucity of information on the extent of prevalence rates of diarrhoeal diseases and the risk factors affecting their variability among adolescents in secondary schools. Improves environmental sanitation and students' hygiene practices in schools can significantly reduce diarrhoeal diseases among adolescents (Ramani *et al.,* 2017).

2.7.2 School feeding service

According to FAO (2019), children in school are an important target population for nutrition education, and schools are a great starting point for teaching students the basics of food, nutrition and health. Promoting nutrition in schools can have a wide-ranging impact on the health and nutrition of families and communities (Hachem, 2016). Students' academic success, physical and mental development, long-term health and well-being all hinge on their abilities to maintain regular, healthy eating habits throughout their lives (Moronkola, 2012). For effective school feeding services, there must be school feeding policy and a convenient food supply system. The service must provide at least one meal a day for all day students and three meals for boarding students. The services of trained food vendors (off-site system) or cafeteria system (on-site system) may be adopted.

School feeding programme can be classified as lunch service and boarding school service. The meals must be well planned to provide all the required nutrients in the right proportion. The lunch service is often practiced in day schools. The meal may be prepared on school site or outside the school and packaged for distribution. The programme ensures that pupils receive at least one adequate meal each day in form of lunch service. The 2016-2025 National School Feeding Program (NSFP), adopted by the United Nations which declared a decade of action on nutrition, in which systems that promote healthy diets and provide a safe and enabling environment for all ages were identified as essential tools (Hachem, 2016). It has been found to improve school attendance and wellbeing of students.

Numerous schools worldwide have implemented the provision of a balanced meal to school-aged children in order to improve their wellbeing as well as promote continuous education in developing countries. An estimated one million pupils receive lunch daily in Ghana (Ababio *et al.,* 2016), 37 million students in Brazil and an estimated 2,700,000 pupils benefit from public school dining services in Italy (Marzano and Balzaretti, 2013). Also, Nigeria's National Home-Grown School Feeding Programme (NHGSFP) provides daily meals to almost ten million students drawn from over 50,000 public primary schools in classes one to three (Vanguard, 2021). About 80,000 farmers and over 102, 097 cooks across 26 states are participating in the programme.

School health authorities in conjunction with local and state health regulatory bodies are in charge of food safety control checks on school FSEs (both day and boarding). The number of boarding secondary schools in Nigeria is increasing and it becomes important that the student's food be properly managed. Many adolescents attend these boarding schools and become exposed to various health challenges (foodborne infections, obesity, poor diets). The early detection of adolescents' state of health and appropriate intervention will help in forming right decisions and thus influencing the overall wellbeing of students before they become full adults (Machado *et al*., 2014). In a review, out of 26 published articles on school health programme in public and private schools, only seven schools carried out screening and inspection of school food vendors while all schools reported availability of midday meal (Dania and Adebayo, 2019).

The boarding school food service involves catering for students who are resident in the school. Many students are enrolled in boarding schools because of quality education, international exposure, busy schedule of parents, orphans, change of environment, improved learning environment. In Nigeria, the national school feeding programme for boarding school recommends that every school should have a kitchen with basic facilities. Trained workers should be employed to manage raw materials sourcing, storage, handling, preparation and services. But the food supply may be contracted out while still being prepared in the school premises (Moronkola, 2012). Kitchens in schools should be strategically located so that they have access to clean water, a well-ventilated store, a functional freezer and a refrigerator for perishables, as well as electricity, a safe waste disposal system, and even school farms or gardens. The meals should be provided in a dining area where teachers are available to supervise and instruct students on good eating habits. Food service in Nigerian schools is frequently unsatisfactory, and children's poor dietary habits can be traced back to a combination of factors, including poverty, a lack of knowledge about healthy eating habits, and the carelessness of food handlers and poor hygiene practices at home. Many studies have linked poor nutrition with low school attendance, retention, and achievement among students. Improper food handling and storage usually result in the growth of food/water borne pathogens such as bacteria, viruses, and intestinal parasites in school children, with serious implications for nutrient absorption and utilisation, resulting in poor growth and development. (Ramani *et al.,* 2017).

2.8 Outbreaks of foodborne diseases in schools

Foodborne diseases have a negative impact on children, accounting for approximately half of all reported cases, with the majority of these cases affecting children under the age of 15 (Hachem, 2016; FDA, 2018). These children's immune systems are still developing, limiting their ability to fight infection. They have a smaller body weight, which lowers the dose required for a pathogen to infect them. They have little control over their nutrition and the hazards associated with food safety. Their stomach acid production is lower, reducing their ability to destroy pathogenic microorganisms (Marzano and Balzaretti, 2013).

Several outbreaks of FBD in schools are inadequately reported mainly because of various factors which could include self-medication, self-limiting infections, poor health records keeping, poor surveillance, late notification, lack of capacity, focus on selected notifiable diseases, ignorance, and fear among students. There have been several reports of FBD epidemics in schools where school lunches are mostly implicated around the world, but the scope of these outbreaks has not been fully examined. The most often reported food preparation procedures that are responsible for these school-related outbreaks were improper food storage and holding temperatures, as well as poor food handling practices (Marzano and Balzaretti, 2013; De Oliveira *et al.,* 2014).

In November 2015, about 30 people were infected by *Campylobacter* at a school in New Jersey, USA (FSN, 2015). According to Brazilian Ministry of Health, estimate of 10.6% cases were associated with day-care and schools between 2008 and 2018 (Finger *et al.,* 2019). In Asia, numerous outbreaks were also recorded: 5.6% of a Malaysian boarding school were infected with Norovirus (Subahir *et al*., 2019); about 30% of religious school students in Islamabad were infected from a leftover meal that was not properly handled before service (Akram *et al.*, 2021); in India, more than 35 students were sent to hospital with gastroenteritis symptoms after eating lunch that consisted of rice, pulse and vegetables at a public school in Tikai village (Zeenews, 2012) and at least 60 pupils of a government primary school in Korba, Chhattisgarh fell ill after eating food provided by the government's mid-day meal programme (NDTV, 2016). About 200 students were affected in an outbreak at a boarding school in the Shunyi District of Beijing, China. It was traced to infected canteen food handlers that led to contaminated food consumed by students (Chen *et al*., 2019).

In Africa, in October 2014, 279 pupils at a local school in Koster, South Africa, were impacted by a suspected outbreak of foodborne sickness. The source of infection was suspected to be contaminated school food (NICD, 2014). An outbreak of gastro-enteritis occurred in a primary school in Ghana after eating the lunch served by school feeding programme. Although, source of infection could not be ascertained, poor food safety practices was suspected (Malm *et al*., 2015). Another foodborne outbreak caused by consumption of contaminated food served at a college dinner in Lusaka, Zambia showed that food workers and food samples were contaminated with *S. aureus* and faecal coliforms (Kapaya *et al*., 2017).

Since Nigeria's FME 2006 school health policy has not been adequately implemented, several incidences of food poisoning in schools have also been reported, resulting in hospitalisations and sometimes deaths. In March 2016, two students at Government Unity Secondary School in Gummi, Zamfara State, were reported dead after suffering from food poisoning, while nine others were hospitalised in critical condition (Maradun, 2016). Similarly, 71 girls of the Government Girls Secondary School in Kalgo, Kebbi State, were hospitalised after consuming tainted food purchased from a food seller on the school compound (Ogbeche, 2016). Bacterial infection in Queens College, Lagos, resulted in two students' death with more than 50 others hospitalised in March, 2017. The results of two independent laboratories indicated that the kitchen water and the school's water factory, contained a high concentration of pathogens. Coliforms, *Escherichia coli, Salmonella, Klebsiella ozoana,* and *Aeromonas hydrophilica* are among the microorganisms identified (Ezeamalu, 2018). Also, 42 girls were hospitalised and one death recorded in a Government Girls School Kawo, Kaduna. The infection was caused by faecal contamination of water because of poor toilet facility and environmental hygiene (Sabiu, 2018).

2.9 The burden of foodborne diseases

An estimated 1,300 Disability Adjusted Life Years (DALYs) are lost per 100,000 individuals in Africa due to foodborne illnesses. Each year of healthy life that is lost due to a disease, disability or death is called a Disability Adjusted Life Year (DALY). In contrast, the United States, Canada, Cuba, and the rest of the developed world lose only 35 DALYs per 100,000 people. Diarrheal diseases cause almost fifty percent of the global burden of FBDs, affecting 550 million people and killing 230,000 people each

year, primarily children, with 220 million falling unwell and 96,000 dying. Consuming tainted raw or undercooked meat, eggs, fresh fruit, or dairy products frequently results in diarrhoea. Microbial pathogens were responsible for the majority (79 percent) of the burden each year, resulting in 584 million cases of disease, approximately 450,000 deaths, and 26 million DALYs. *Salmonella* spp., toxigenic *Escherichia coli*, Norovirus, and *Campylobacter* spp. were the most prevalent pathogens in that order (WHO, 2015).

Foodborne diseases are leading causes of malnutrition in underdeveloped countries, impairing infants' and children's growth and disease resistance, making them more susceptible to a variety of diseases such as respiratory infections, which contribute to the downward spiral of additional malnutrition consequences (Marzano and Balzaretti, 2013; Rossi *et al*., 2018). Additionally, individuals may suffer retarded physical and mental development, which can prevent them from realising their full potential in society. Food also plays a critical role at the interface of human and animal diseases, as infections that evolve in animals can be transmitted to humans via food. Disease outbreaks due to *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus*, and haemorrhagic *E. coli* strains associated with both animal and plant food vehicles (FDA, 2012).

The burden of foodborne diseases can be social, economic, and multidimensional, particularly now that economic globalisation has raised the risk of epidemics spreading beyond national borders, highlighting the importance of a thorough worldwide assessment of the burden of food and waterborne infections. Bacterial foodborne illnesses in schools could result in a significant socio-economic burden such as absence from class, lack of academic excellence, loss of appetite for school meals due to fear, reduced productivity, cost of medical treatment, cost of outbreak investigation, loss of school reputation, poor growth due to underlying infections, lack of confidence in school meals, as well as the cost of disposing contaminated foods. During times of disease and food recalls, the impact on national production is considerable. As a result, there may be a decrease in consumer and investor confidence, as well as disruptions in trade flows, job losses, morbidity, and early death (Jaffee *et al*., 2019). The secondary consequences of foodborne diseases could include chronic ulcer, liver failure, septicaemia and other long-term health complications. Public health education and evaluation of food handlers regarding hygienic food storage and preparation in elementary and secondary schools

and other educational institutions mass catering is a critical public health policy that should be actually considered.

2.10 Antibiotic resistance: A food safety challenge

Antimicrobials (antibiotics, antifungals, antivirals, and antiparasites); heavy metals and biocides (disinfectants and surfactants) are three well-characterised types of resistance-generating compounds. Antibiotics are naturally occurring substances obtained from plants or microbes that have the ability to prevent the growth of pathogenic germs that cause human diseases (Singer *et al.,* 2016). Antibiotic resistance is a significant issue in the treatment of serious bacterial foodborne infections, as antibiotic usage in animals selectively produces resistant foodborne bacteria that can be transmitted to people via food contamination. (Nyenje and Ndip, 2013; FAO, 2015). Antibiotics are vital for human and animal health, and also food production (primary production, biocides, disinfectants, food and feed preservatives, or decontaminants), yet their abuse has increased resistance concerns to humans and agro-ecosystems (Capita and Alonso, 2013). As a result of its ability to selectively kill just certain types of bacteria, antibiotics can also leave behind or select unusual variants that can continue to thrive in the presence of the antibiotic. These resistant varieties subsequently grow to form the dominant bacterial population, passing on their resistance genes down through generations (Founou *et al.,* 2016).

According to their primary mode of action, antibiotics are categorised as interfering with cell wall synthesis, inhibiting protein synthesis, interfering with nucleic acid synthesis, and inhibiting a metabolic pathway (Todar, 2006). Resistance develops when an antibiotic is rendered ineffective due to a unique genetic mutation in its DNA (chromosomal resistance) or due to the acquisition of mobile genetic elements from another resistant bacterium (Horizontal Gene Transfer). Often, resistance to one type of antibiotic might result in resistance to others in the same class (cross-selection) or in another class (co-selection). MGEs like plasmids, transposons, and integrons are frequently responsible for co-selection, while cross-selection of various resistance genes is frequently achieved via efflux pumps (Singer *et al.,* 2016). Antibiotic resistance genes can potentially be transferred from commensal bacteria to human pathogens and spread internationally through global trade (Bengtsson-Palme *et al*., 2018).

Antibiotic resistance has increased morbidity and mortality in humans. The high cost for newer more expensive drugs and longer hospital stay is part of the economic impact of antibiotic resistance in the community. Among the practices that contribute to the proliferation of antibiotic-resistant bacteria in the environment include contamination of municipal water system when run-off from housing facilities and factories contaminate streams and underground water; non-therapeutic use of antibiotics in livestock to prevent infection and improve growth; and transferring them to human after consumption thereby increasing its resistance. Additionally, the use of antibiotics in Genetically Modified (GM) crops when antibiotic resistant genes are used as markers to detect specific genes and inappropriate use in medical environment such as incomplete empirical treatment of infections and general overuse have been linked to antibiotic resistance development (WHO, 2011b).

The burden of antibiotic resistance arising from various ecological sources throughout the entire food chain must be assessed in order to effectively manage the its threat to public health (farm, abattoir, market, school and food industry). This is more apparent with limited data available on the use of antibiotics, resistance pathogens, and foodborne illnesses in developing countries. Additional research on bacteria isolated from these diverse sources will help in the better understanding of resistance reservoirs in the environment. The antibiotic resistance surveillance will strengthen national capabilities for generating data on its prevalence and trends in order to control or limit the development of antibiotic resistance and to inform risk-based management decisions (FAO, 2015). One Health approach and Codex Alimentarius Commission guidelines must be adopted in order to complete the data gaps in food chain surveillance. Long-term studies (epidemic and molecular) from farm-to-fork must also be conducted (Founou *et al*., 2016).

According to FAO (2015), sectors must collaborate to reduce antibiotic overuse in food and animal production through measures like implementing GAPs, infection control, and targeted hygiene. Additionally, promoting awareness, monitoring school food services, and implementing food safety regulations can help reduce infections and the need for antibiotics (Maillard *et al.*, 2020).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Description of the study area

The research was carried out in selected boarding high schools in Ibadan, Nigeria. With a population of 2,338,659, Ibadan is the capital of Oyo State and the third largest metropolitan city in Nigeria after Lagos and Kano. The city population is projected to be 4,091,361 in 2023, with a population growth rate of 3.29 percent. Ibadan is located in South-Western Nigeria, 128 kilometres northeast of Lagos and 530 kilometres southwest of Abuja. It is at latitude 7.3775 North and longitude 3.9470 East. It is located on a hill with urban and rural surroundings encompassing a total land area of 3,123 km^2 (NPC, 2010). The Ibadan metropolitan region has a population density of 586 people per square kilometre. Eleven Local Government Areas (LGAs) make up the city of Ibadan. There are five urban and six rural LGAs, which include: Ibadan North, Ibadan North East, Ibadan North West, Ibadan South East, Ibadan South West, Akinyele, Egbeda, Ido Lagelu, Oluyole, and Ona Ara LGAs (Figure 3.1).

3.2 Study design and population

The study employed a descriptive cross-sectional design with comparative and microbiological components, and selected participating schools using a multistage sampling technique (Imam, 2013). It involved selected private and public boarding high schools located within both rural and urban communities in Ibadan. The study population was total sample size of thirty food handlers. It consisted of everyone that were involved in raw food storage, food preparation and service in each of the selected school. Samples were collected from selected schools' FSEs between April and December 2017.

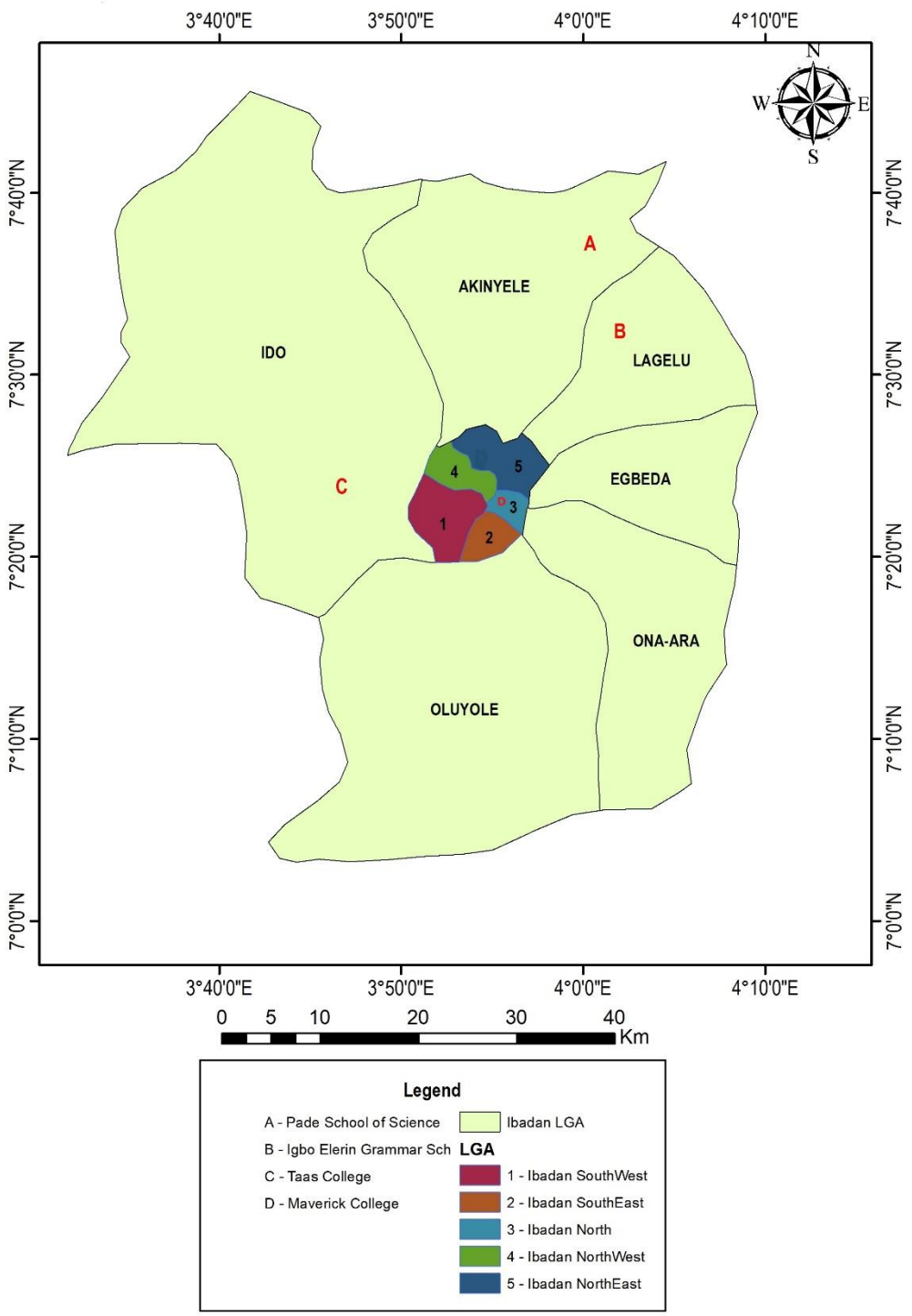


Figure 3.1: Map of Ibadan city showing study locations

Source: Adapted from Balogun (2011)

3.3 Selection of study locations

The Oyo State Ministry of Education provided a full list of all private and public high schools in Ibadan as at the time of this study (MoE, 2015). Seventy-two schools had boarding facilities in eight out of eleven local government areas in Ibadan. This list was then stratified by series of inclusion and exclusion criteria. Stratification was aimed at avoiding over or under-representation of certain types of establishments (Imam, 2013). A map showing study spatial distribution and features of selected boarding secondary schools in Ibadan is shown in Figure 3.2. A 4-stage sampling was used to select LGAs, schools and food handlers working in boarding school kitchens, and the stages are described below:

Stage 1: Classification into urban and rural LGAs.
Stage 2: Selection of LGAs with both public and private boarding schools.
Stage 3: Selection of schools using the inclusion and exclusion criteria.
Stage 4: Selection of four schools (including all food handlers) by balloting.

Inclusion criteria: The selection criteria included all boarding private and public secondary school in the selected LGAs in Ibadan with boarder's population of at least seventy students and at least five food handlers.

Exclusion criteria No part time food handlers were involved in the study.

Four schools (two public and two private) were randomly selected by balloting and the schools were in four different LGAs comprising of Akinyele, Ibadan North, Ido and Lagelu (See Appendix 1).

3.4 Description of food service establishments of selected schools

3.4.1 School A

This is a public school located in Pade village, a rural area in Akinyele LGA. The school population was 622 with 297 girls and 325 boys. Food was prepared in the open space behind the dining hall. There was an extension of the kitchen made with zinc roof and low wall. Firewood and bottled cooking gas were used as fuel for cooking. The floor was cemented but had many cracks and was littered with paper. Goats roamed freely in the food preparation area and even in the dining hall. The dining area was dirty with uncleaned tables, dusty windows, damaged furniture with crevices and cobwebs on the wall.

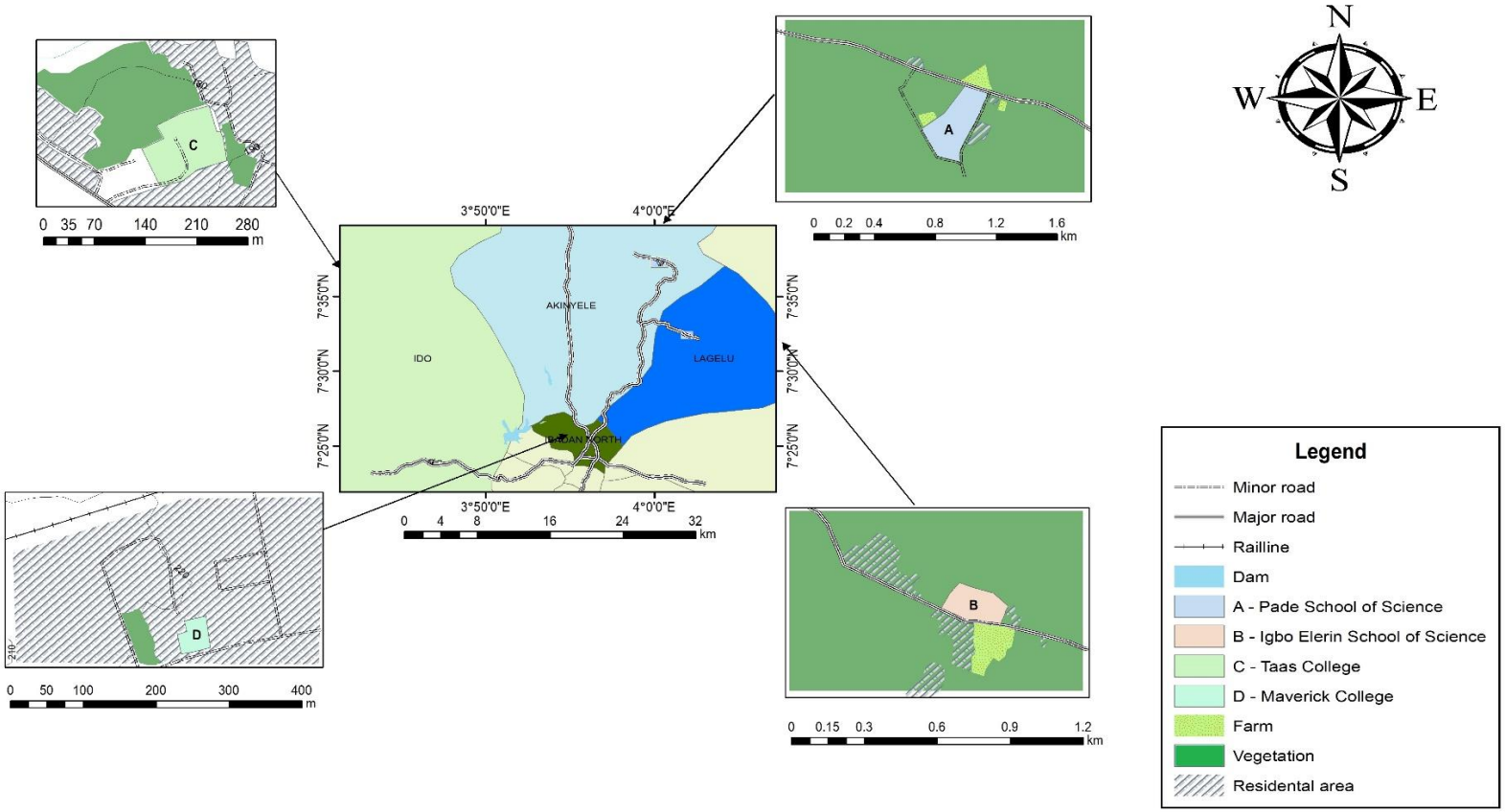


Figure 3.2: Spatial distribution and features of locations of selected boarding high schools in Ibadan

Description of the dining area is shown in Plate 3.1. There were two borehole water sources (for drinking and dish washing). Prepared food was served in bowls and insulated food boxes but the bowls were covered with used rice sacks. Students provided their plates and cutleries.

3.4.2 School B

The school is a public school located in Igbo Elerin, a rural area in Lagelu LGA. It had both day and boarding facilities with boarders' population of 650 students. There was an enclosed kitchen and an unenclosed extension covered with zinc roof and low walls located behind the dining hall. Two water sources were available (borehole), distributed through a standing tap and stored in drums and a nearby deep well used as alternative when tap was not running. Cooking fuel was only firewood. Food was served in insulated food boxes and distributed to students in the dining hall. Students provided their cutleries and plates. Goats roamed freely in the kitchen environment. Basins and buckets were used to wash utensils and plates. The food handlers put on Personal Protective Equipment (PPE) such as head scarves, footwear and aprons.

3.4.3 School C

The school is a private school located at Omi Adio town, a rural area of Ido LGA. The scholl population was two hundred students with full boarding facilities. There was a small kitchen enclosed within the dining area. The main food preparation area was an open extension beside the dining area and roofed with zinc sheets. Bottled cooking gas and charcoal were sources of fuel. Water was distributed through the kitchen taps for cooking and drinking by a motorised borehole. There were filters (water guard) attached to the distribution taps that served as drinking water.

The open drainage behind the kitchen was clean and without odour. Utensils were adequately washed and stacked for adequate drying. Meals were served from insulated food boxes in the kitchen through a service window to students. The storage freezer was in good working conditions with food items frozen. The food handlers were dressed in specially sewn uniform with head scarves, they wore apron and footwear and no jewelries. The food handlers when at work were neatly dressed (Plate 3.2).

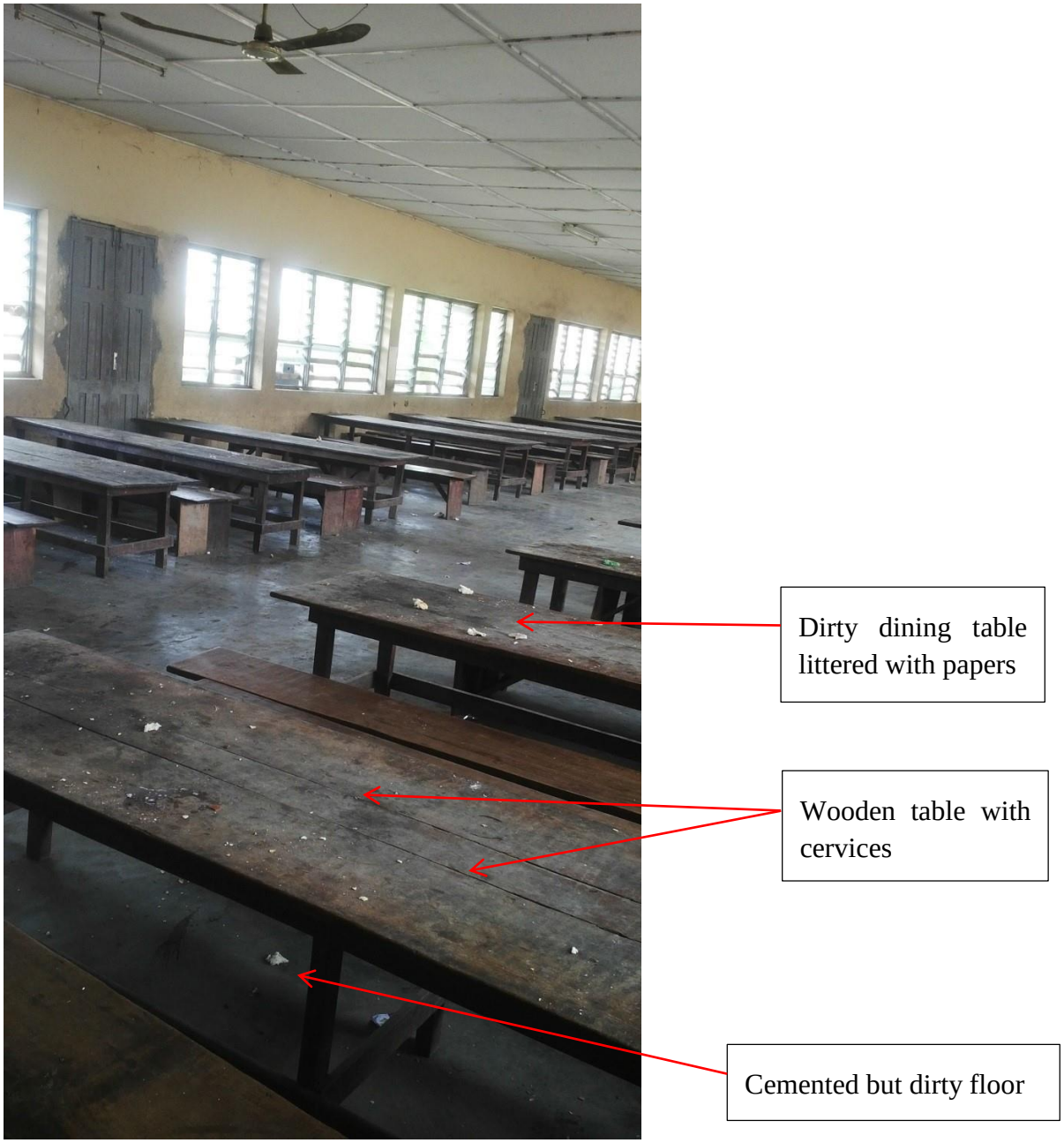


Plate 3.1: Dining hall after a meal at School A

Plate 3.2: Food handlers with head coverings at School C

3.4.4 School D

The school is a private school located in urban area in Ibadan North LGA. The school had both day and boarding facilities with seventy boarders. There was a small separately built kitchen with a large roofed and partly enclosed extension. Bottled cooking gas and charcoal were used as fuel. Meals were served in insulated food boxes and arranged on the dining tables for students. Cooking water was obtained from motorised deep well and stored in water tanks. Drinking water was borehole water supplied by third party, and distributed through water dispensers. Most food handlers wore PPE like apron, head scarves and footwear and others did not. There was a big tree that serves as a shade in front of the kitchen. The only male handler did not wear any head cover. The characteristics and description of the four selected schools are summarised and shown in Table 3.1.

3.5 Ethical Approval

Before field work began, this study was approved by the Oyo State Ministry of Health Ethical Review Committee under Reference No. AD13479/339 (See Appendix 2). Additionally, authorisation was secured in writing from the Oyo State Ministry of Education and the Local Inspector for Education in each LGA of the selected schools. Before the interview began, the participants were informed of the study's objectives and completed individual consent forms were acquired. Participation was entirely voluntary, and no coercive measures were used. Participants were assured of the confidentiality of all information acquired, and respondents' names were omitted from the questionnaire to maintain anonymity.

3.6 Data collection instruments

Data from the selected boarding schools' FSEs were obtained through three instruments. These were Key Informant Interview (KII) guide, an observational checklist, and a semi-structured interviewer-administered questionnaire

3.6.1 Key informant interview guide

Key Informant Interview (KII) guide was developed based on research study by Ademokun *et al*. (2012) to collect in-depth qualitative information (See Appendix 3).

Table 3.1: Characteristics and description of the selected boarding high schools

School Code	A	B	C	D
Location	Pade	Igbo Elerin	Omi Adio	Bodija
LGA	Akinyele	Lagelu	Ido	Ibadan North
School status	Public, Rural	Public, Rural	Private, Rural	Private, Urban
GPS	N7.622, E4.007	N7.539, E4.034	N7.398, E3.779	N7.423, E3.907
No of schools in the LGA	76	52	70	81
No of private schools	41	23	52	45
No of public schools	35	29	18	36
Boarding schools	4	6	2	10
School type	Full boarding	Day/Boarding	Full Boarding	Day/Boarding
No of boarders	622	650	200	70
No of food handlers	9	6	7	8
Source of water	Borehole & Deep well	Borehole & Deep well	Borehole	Borehole

The KII guide was used to collect information from staff in charge of school food service on knowledge about school health programme and available facilities for school feeding services; nutrition and food safety; procurement and storage of food items; food handlers' welfare; safe water supply plan and waste disposal. Six key informants (two housemasters, one housemistress, one kitchen head, one vice-principal and one director of welfare) were interviewed. Jottings of responses were made and interviews entirely conducted in English language. Texts were described using thematic approach and analysed. The selected key informants are listed below:

Respondent 1: Matron A

Respondent 2: Vice principal B

Respondent 3: Housemaster B

Respondent 4: Director of Welfare C

Respondent 5: Housemaster D

Respondent 6: Matron D

3.6.2 Observational checklist

The observational checklist (see Appendix 4) was used to assess the schools' FSEs physically while food preparation was taking place. The checklist was based on Imam (2013) and consisted of four parts: kitchen sanitation (P1), toilet hygiene (P2), dining area assessment (P3) and observed food handlers at work (P4). The food handlers were observed while performing their chores to assess their food handling practices. The sanitary conditions of the facilities and observations during food preparation and handling were also recorded. The checklists were scored as yes/no (1/0). Specific attributes were selected from the checklist and the total percentage average scores of the four school were obtained. The grading was as follows: very Good (≥70%), Good (50-69%) and poor (<50%).

3.6.3 Semi-structured interviewer-administered questionnaire

The use of a semi-structured questionnaire (See Appendix 5) was employed to obtain information from food handlers concerning their environmental sanitation, food safety

knowledge, attitude and practices (KAP). It was adapted and modified from previously published work of Imam (2013). The questionnaire was structured into six distinctive parts: socio-demographic data; food safety training and medical examination; environment and food safety; knowledge about risk factors associated with food handling; attitude towards food hygiene and practices including handwashing by food handlers.

Socio-demographic data: Age, gender, marital status, level of education, job designation, years of experience and method of skill acquisition were all covered in this section.

Food safety training and medical examination: This section covered type of training, duration and number, pre-employment and type of medical tests.

Environmental hygiene and food safety: It comprised of sources of water, pest control method of sewage and refuse disposal.

Food safety and hygiene knowledge: This section comprised of 42 questions that were graded using four categories: foodborne diseases; symptoms of foodborne diseases; food handling practices and health conditions that could threaten food safety. To minimise biased results, respondents were instructed to select one of three options: correct, wrong, or don't know, with scores ranging from 0 to 42 points. Each correct answer scored two point and incorrect scored no point (See Appendix 5).

Food handlers' attitude: It covered 12 questions that were statements about food hygiene and safety. It was assessed on three-point rating scale of (agree, disagree, uncertain) and scored between 0 and 24 points. Each correct answer scored two point and incorrect scored no point.

Food hygiene practices including handwashing: This section consisted of a total of 37 questions. Twelve questions were included in the handwashing practices section. The responses were graded on a five-point Likert scale (never=0, rarely=1, sometimes=2, most times=3, and always=4) and ranged from 0 to 48 points. Seven questions were included in the materials for handwashing, with a maximum Likert score of 28 points. The remaining 18 questions had a yes or no option. Each correct response scored one point, while incorrect responses received none. The KAP scores were then adjusted to

100 points and scores less than 60% were considered poor while 60% and above were rated good. Negative statements were used with positive ones to counterbalance any potential bias in answer selection and the scoring was done in the opposite direction for positive statements.

3.7 Laboratory used for analyses

The laboratory analyses were carried out at the Biotechnology Laboratory, Department of Microbiology, University of Ibadan; Nigerian Institute of Stored Product Research Institute (NSPRI), Ibadan, and Molecular Biology and Biotechnology Laboratory, Nigerian Institute of Medical Research (NIMR) Yaba, Lagos.

3.8 Sample collection and handling

From the four participating schools' FSEs, eighty-seven (87) samples were randomly collected: food contact surfaces (n=42), food handlers' hand swabs (n=20), RTE foods (n=18) and water samples (n=7). The distribution of different types of samples collected in each FSE is shown in Table 3.2. The type of food samples taken from the FSEs varied according to their menu and availability on the given sampling day. All samples were kept at 4^{o}C on ice packs and transported to the laboratory for further analysis.

3.8.1 Food contact surfaces

A total of 42 FCS samples from the four selected school FSEs were collected. Samples included counter tops (3), chopping boards (8), cutting knives (9), serving trays (9), dining table (5), drinking water tap (2) and domestic grinding machines (6). Each surface (100 cm^2) was swabbed with pre-moistened commercial sterile swab-stick dipped in 10 mL of sterile 0.1% peptone water (Oxoid, UK) and the solution was considered as 0.1 dilution (Rossi *et al.*, 2018).

3.8.2 Food handlers' hand swabs

Food handlers' hand were swabbed after handwashing procedure. Each sterile swab stick was pre-moistened in a test tube containing 10 mL of 0.1% sterile peptone water (Oxoid, UK). A fresh sterile stick was swabbed over the right hand palm of each food handler while lunch was being served (Christison *et al*., 2008, Lee *et al.*, 2017).

Table 3.2: Samples collected from selected boarding schools' FSEs in Ibadan

Category	Type	Number of samples per school				Total Samples
		A	B	C	D	
Food Contact Surfaces	Chopping board	2	2	2	2	8
	Countertop	0	0	1	2	3
	Dining Table	2	0	2	1	5
	Drinking water tap	1	0	0	1	2
	Grinder	2	1	2	1	6
	Knife	1	4	2	2	9
	Tray	2	2	3	2	9
Food Handlers	Hand swabs	5	4	4	7	20
RTE foods	Bread	1	1	1	1	4
	Beans	2	0	0	0	2
	Rice	1	1	1	1	4
	Fish Stew	0	0	1	1	2
	Meat Stew	0	0	1	1	2
	Egg	1	0	0	1	2
	Semolina	0	0	0	1	1
	Amala	1	0	0	0	1
Water	Cooking & Drinking	2	1	2	2	7
Total		**23**	**16**	**22**	**27**	**87**

3.8.3 Ready-to-Eat foods

Samples of RTE foods were randomly collected from the selected schools based on the meals on the school menu. The samples were collected into sterile Ziploc bags immediately after preparation from the storage warmers with serving spoons (Marzano and Balzaretti, 2013). One hundred grams of each food type was collected during each sampling period.

3.8.4 Water samples

Drinking and cooking water were collected from each school. The tap was allowed to run for one minute and sterile 0.75ml bottles were completely filled with water samples excluding air bubbles (Edema *et al*., 2011). Altogether, seven water samples were aseptically collected and labelled appropriately. Location and description of water samples are shown in Table 3.3.

3.9 Bacteriological analyses of samples

3.9.1 Sterilisation

All non-disposable glassware used for the experiments were properly washed in liquid soap and rinsed with tap water All glasswares were air dried and later sterilised in the oven at 160°C for six hours. All culture media and reagents used in this study were appropriately prepared according to manufacturers' instruction and sterilised inside the autoclave at 120°C and pressure of 15psi for 15 minutes (See Appendix 6). All microbiological procedures were according to the methods described by (AOAC, 2005).

3.9.2 Sample preparation

For FCSs and food handlers' hands, one millilitre of the 0.1 dilution was serially diluted to 10^{-2} through 10^{-4} dilutions and 10g of each RTE food samples was homogenised with 90 ml of sterile 0.1% peptone water and decimal dilutions up to 10^{-6} were prepared. Ten millilitres of each water sample for bacteriological evaluation was aseptically transferred into 90 mL of 0.1% sterile peptone water (Oxoid Ltd, Basingstoke, UK), vortexed and six-fold serial dilutions of 10^{-1} to 10^{-6} prepared. Selected dilutions were used for plate counts. Media used were MacConkey Agar, Eosin-Methylene Blue (EMB) Agar and Salmonella-Shigella Agar (Hi Media, India); Baird Parker agar and Mannitol Egg Yolk polymyxin (MYP) Agar (Oxoid, UK).

Table 3.3: Water sample collected from the selected schools

Number	Sample code	Sample description
1	WA1	School A drinking water (collected from a storage tank supplied by a borehole in the dining area)
2	WA2	School A cooking water (collected from a storage tank supplied by another borehole)
3	WB	School B drinking and cooking water (collected from kitchen tap supplied by a borehole)
4	WC1	School C drinking water (collected from a kitchen tap with filters attached)
5	WC2	School C cooking water (collected from kitchen tap without filters)
6	WD1	School D drinking water (collected from water dispenser in the dining hall)
7	WD2	School D cooking water (collected from kitchen tap supplied by a borehole)

3.9.3 Bacteria isolation, subculturing and preservation

One millilitre aliquot each of the selected dilutions was plated out on Nutrient agar (Hi Media, India) and appropriate selective media using the standard pour plate technique (AOAC, 2005). Plates were incubated aerobically at 37°C for 24-48 hours except *B. cereus* that was cultured at 30°C. The plates were observed for growth after the incubation period. Colonies were first identified using cell morphology and the Gram reaction, while repeated streaking on selective media yielded pure cultures. Pure culture of the bacteria obtained were stored on Nutrient Agar at 4°C; and Brain Heart Infusion broth (containing 15% glycerol) kept at -80°C for further studies.

3.9.4 Aerobic plate count

The Nutrient agar cultured plates from selected dilutions (10^{-2}, 10^{-4} and 10^{-6}) were enumerated in duplicate to obtain aerobic plate count. All discrete colonies between 10-300 colonies were counted and results calculated according to the standard formula in Equation 3.1.

$$N = \frac{\sum C}{[V(n1 + n2)d]} \tag{3.1}$$

N= Aerobic Plate Count

∑C = total number of colonies counted of successive dilutions

V = volume of inoculum applied to each plate in millilitres

n1= number of plates retained in the first dilution

n2= number of plates retained in the second dilution

d= dilution ratio corresponding to the first dilution retained

Results were written as logCFU/g for food samples, logCFU/mL for water samples and logCFU/cm^2 for hand swabs and FCSs.

3.9.5 Total coliform

MacConkey agar was used to isolate Gram-negative, lactose-fermenting Enterobacteria species. Presumptive pink colonies were enumerated as total coliform (TC) indicating the presence of indicator organisms.

3.9.6 Faecal coliform

Faecal coliforms (FC) are subgroup of total coliform mainly *E. coli* and are mostly found in the intestine of warm blooded animals. Samples were inoculated on Eosin Methylene Blue (EMB) agar and incubated at 44.5°C. The plate showing growth of blue/black with green metallic sheen colonies were confirmed as *E. coli.*

3.9.7 Salmonella-Shigella count

Salmonella-Shigella agar was used to culture non-lactose fermenting bacteria. Colourless colonies with black centres were presumptively selected as *Salmonella* spp., while clear colonies were S*higella* spp.

3.9.8 *Staphylococcus aureus* count

Black/grey colonies with or without clear zones were counted on Baird Parker agar. The gram-positive, catalase positive and coagulase positive were preliminarily identified as *S. aureus.*

3.9.9 *Bacillus cereus* count

From Mannitol egg yolk polymyxin (MYP) agar plate, eosin-pink colonies surrounded by a zone of inhibition were selected, enumerated and presumptively identified as *B. cereus.* The isolates were transferred to nutrient agar slants for confirmation.

3.10 Biochemical characterisation of bacteria isolates

The isolates' identities were confirmed by an array of biochemical tests as follows: oxidase, catalase, methyl red, Voges Proskauer, Sulphur Indole Motility test (SIM), Triple Sugar Iron (TSI), urease, citrate utilisation, coagulase activity and sugar fermentation tests (AOAC, 2005).

3.10.1 Oxidase test

Dried filter paper was soaked with oxidase reagent solution (tetramethylparaphenylenediammine-2-HCL) and the test microorganism was smeared onto the impregnated surface using platinum inoculating loop. A purplish-blue colouration within 30-60 seconds indicated a positive reaction.

3.10.2 Catalase test

A young culture (12-18 hours) was placed on a clean microscope slide and then 3% hydrogen peroxide was added using a wire loop. The production of white effervescence froth indicated that the organism was catalase positive.

3.10.3 Methyl Red test

Five drops of methyl red indicator was added to bacteria culture grown on MR-VP broth and incubated at 37°C for 48 hours. The development of stable red colour in the surface of the medium indicated a positive result.

3.10.4 Voges Proskauer

One millilitre of 0.5 mL of 6% alpha naphthol was added to the test bacteria culture broth from MR test and followed by 0.5 mL of 40% KOH. The tube was agitated to expose the medium to atmospheric oxygen and remained undisturbed. A red colour after two hours indicated a positive result.

3.10.5 Sulphur Indole Motility

This is a multiple test that simultaneously test for motility, indole production and hydrogen sulphite production. A sterile straight inoculating wire was used to pick a colony of young culture and stabbed into prepared Sulphur Indole Motility (SIM) medium in tubes to a depth of 5 mm. Also, a sterile swab stick was used to create a lawn on the surface of SIM medium (Hi Media, India). The tubes were incubated at 37°C for 24-48 hours. Motility was observed if medium was cloudy. Development of black precipitate indicated presence of Hydrogen sulphite. Two drops of Kovac's reagent was added to the culture and the appearance of a dark red colour indicated a positive indole test.

3.10.6 Triple Sugar Iron

Triple sugar iron (TSI) agar butt was stabbed with the test organism and also streaked on the surface of the slant. The tubes were incubated at 37°C for 48 hours and observed for change in colour and production of gas. The results were compared with standards.

3.10.7 Urease

The urea medium was heavily inoculated with the culture being tested and incubated for 24 hours at 37°C. Positive results were determined by changes in colour from yellow to pink.

3.10.8 Citrate

A test-tube of Koser citrate medium was inoculated with the organism and incubated at 35°C for 96 hours. Turbidity was recorded as positive due to growth.

3.10.9 Coagulase

Free coagulase was determined by transferring presumptive *Staphylococcus aureus* colonies into tubes containing 5 mL of Brain Heart infusion broth. The tubes were incubated for 18-24 hours at 37°C. A volume of 0.5 mL of the coagulase plasma with ethylenediaminetetraacetic acid (EDTA) was added to 0.2 mL of broth culture. The tubes were incubated at 35-37°C and examined periodically during a 6-hour interval for clot formation. A clot formation is considered a positive reaction for *Staphylococcus aureus*.

3.10.10 Sugar fermentation

Sugar fermentation tests were done using phenol red broth basal medium. One gram of each sugar was dissolved into 10 mL of distilled water. A volume of 10mL the basal medium was measured into each McCartney bottles (28 mL) and 0.1mL of different sugar solution (lactose, glucose, fructose, maltose, raffinose and sucrose) was added, inverted Durham tube was placed in each bottle and sterilised at 121°C for 10 minutes in an autoclave. Each bottle was inoculated with two drops of 18-hour broth culture of suspected organism and then incubated at 37°C for 18-24 hours. The production of acid was determined by change in colour from yellow to red while the production of gas was determined by the accumulation of gas in the inverted Durham tube.

3.11 Physico-chemical analysis of water

Physico-chemical parameters of the water samples were analysed according to the standard methods given in American Public Health Association (APHA, 2012).

3.11.1 Determination of temperature

A handheld digital thermometer was used to record the temperature (Checktemp1, Hanna Instruments, Italy). As soon as the reading remained stable, the value obtained was documented.

3.11.2 Determination of pH

The calibration of the pH meter was done using standard buffer solutions of pH 4 and 7 respectively (Hanna Instruments, pH 211, Italy). The measurement was recorded after obtaining a stable reading upon inserting the probe into the water samples in a beaker.

3.11.3 Determination of total dissolved solid

A volume of 10 mL of filtered water sample (2 µm whatman filter paper) was transferred to a pre-weighed evaporating dish and allowed to completely evaporate at 98°C in a conventional oven. After drying the dish to a constant weight at 105°C, it was then cooled in a desiccator and reweighed. The filterable residue was quantified as total dissolved solid (TDS) using Equation 3.2.

$$\text{TDS (mg/L)} = \frac{(W_2 - W_1) \times 1000}{Vol\ of\ filtrate\ used} \qquad (3.2)$$

W_1 = initial weight of evaporating dish

W_2 = Final weight of the dish (evaporating dish + residue)

3.11.4 Determination of conductivity

A Hannah conductivity meter (4510 model) was used to measure the conductivity at room temperature. For calibration purposes, Potassium Chloride (KCl) 0.01 M solution with a conductivity of 1408 µS/cm was used as the standard solution. A steady reading was recorded after the probe was dipped into the sample container.

3.11.5 Determination of turbidity

Two grams of Barium Chloride ($BaCl_2$) was measured into a 100 ml volumetric flask, marked up with distilled water and allowed to stand for 2 hours at room temperature. This was a standard of 100 ppm and lower standards of 10, 20, 40, 60 and 80 ppm were also prepared from the stock. Absorbance of $BaCl_2$ standard was measured on the spectrophotometer at 420 nm while distilled water was used as blank. A graph of absorbance against standard concentration values was plotted. Absorbance of water samples was also taken and turbidity was calculated from the gradient of the curve.

3.11.6 Determination of calcium hardness

Water samples (25 mL each) were poured in separate 250 mL conical flasks, 5 mL of ammonium chloride in concentrated ammonia buffer (N8.5 M NH_3-NH_4Cl) and two drops of Eriochrome black T indicator was added. This was titrated against 0.01M ethylenediaminetetraacetic acid (EDTA) solution until a colour change from violet to blue developed and hardness calculated according to Equation 3.3.

$$Hardness\ (\text{mg/L}\ CaCO_3) = \frac{V \times M \times 1000}{Vol\ of\ sample} \qquad (3.3)$$

M= Molarity of EDTA

V= Volume of EDTA

3.11.7 Determination of phosphate

A volume of 5 mL of water sample was placed in 50 mL volumetric flask, also the same volume (5 mL) of phosphate standard solutions (0, 1, 2, 3, 4, 5 ppm) were equally placed in 50 mL volumetric flasks. Then, 10 mL of Vanadate-molybdate reagent was added to each flask, mixed and allowed to stand for 15-30 min (for the blue colour to develop). The readings were taken in a Red Halliday colorimeter at 630 nm. Absorbance was plotted against concentration for the phosphate solution to obtain a standard curve. Phosphate content in water samples were calculated from the obtained graph.

3.11.8 Determination of sulphate

A volume of 10mL of water sample was measured into 25 mL volumetric flask and 1mL of gelatin $BaCl_2$ reagent was added and made up with distilled water. For the standard solution, a set of sulphate solution containing 0, 1, 2, 3, 4, 5 ppm was prepared from the

working standard solution (100ppm). The absorbance reading was taken with a spectrophotometer (Model Spectronic 20) at 420 nm after 30 min. The absorbance was plotted against concentration of the standard solution to obtain a standard curve. The water sample sulphate content was calculated in mg/L from the curve.

3.11.9 Determination of nitrate

A 3.25 mL volume of water was added to 0.5 g of activated carbon and 25 mL of extracting solution was added. It was mixed and filtered, then 10ml of the aliquot was evaporated to dryness and 1 mL of phenolsulphonic acid was added. Another 10 mL of distilled water was added after 10 minutes and the aliquot made alkaline by adding 4 mL of concentrated NH_4OH. Standard calibration curve was prepared with 2.5, 10, 15, 20, 25 mL of Nitrate-Nitrogen (NO_3-N). The absorbance of the sample was taken at 410 nm with a spectrophotometer (Model Spectronic 20). Nitrate content of water was determined from the curve.

3.11.10 Determination of chloride

A 50ml volume of water sample was adjusted to pH 8.2 with NaOH, mixed with 1ml of potassium chromate indicator and then the solution was titrated with standard silver nitrate ($AgNO_3$) solution until it reached a persistent orange-red end point. A blank solution was also titrated with the standard solution and chloride content computed as in Equation 3.4:

$$Chloride\ (mg/L) = \frac{\{(A - B) \times N \times 35450\}}{Vol\ of\ sample\ used\ for\ titration} \qquad (3.4)$$

A = volume of $AgNO_3$ used in titration

B = volume of $AgNO_3$ for blank

N = Normality of $AgNO_3$

3.11.11 Determination of iron and lead content

A set of standard iron and lead solutions (0.2, 0.4, 0.6, 0.8, and 1.0 mg/L) were prepared by diluting their stock solution with distilled water to the correct concentrations. Iron and lead contents were assessed by wet oxidation of samples in a digestion process and extracts from the above digestion were aspirated using an Atomic Absorption Spectrophotometer (ASS: Model 210 VGP) with air-acetylene gas mixture as oxidant at

280 nm. Standard curves were obtained for each element. A volume of 20 mL of water sample was measured into the digestion flask with 5 mL of nitric perchloric acid, digested, cooled and distilled water added. The solution was then filtered into a 50 mL volumetric flask, diluted to volume and extract from the above digestion was aspirated. The readings were recorded from ASS as mg/L of solution (digested sample). The results were calculated by multiplying reading by the dilution factor and sample metal content obtained from the standard curve.

3.12 Most Probable Number for water bacteriological examination

A five-tube fermentation most probable number (MPN) method was used to determine total coliform and faecal coliform during bacteriological examination of water samples. Each set of tube A, B and C containing sterile 10mL lactose broth with bromocresol purple as an indicator were inoculated with specified volumes of water A (five 10 mL), B (five 1 mL) and C (five 0.1 mL). Set A was double strength while sets B and C were single strength. An inverted Durham tube was placed in each test tube for gas collection. The tubes were incubated at 37°C for 24 hours and examined for growth (positive tubes). All negative tubes were further incubated up to 48 hours. Gas and acid production were evidenced by a colour change from purple to yellow and presence of gas in the Durham tubes. Positive tubes were counted and results compared with McCradys probability table at 95% Confidence Limit. The results obtained were expressed as MPN/100 mL. A loopful from positive tubes were streaked on EMB plates and incubated at 44.5°C for 24 hours to confirm presence of *Escherichia coli* (APHA, 2012).

3.13 Antibiotic susceptibility testing

3.13.1 Kirby-Bauer disc diffusion assay

The Kirby-Bauer disc diffusion method was used for antibiotic susceptibility testing as described by Clinical and Laboratory Standard Institute (CLSI, 2018). The isolates were sub-cultured on Mueller-Hinton agar (Oxoid, UK) to obtain discrete colonies and incubated at 37°C overnight (16-18 hours.). A sterile wire loop was used to pick discrete colonies of the isolates, emulsified in pre-labelled bottle of sterile 3-5 mL of physiological saline, and turbidity was compared with 0.5 McFarland's opacity standard. Sterile Mueller-Hinton agar plates were then inoculated with aid of sterile swab sticks. The swabbed plates were allowed to dry for about 3-5 min.

The bacteria were tested against eight commercial antibiotics. The commercially prepared antibiotic discs and their concentrations in microgram for the Gram-negative bacteria were Cefixime (5 µg), Ofloxacin (5 µg), Augumentin (30 µg), Nitrofurantoin (300 µg), Ciprofloxacin (5 µg), Ceftazidime (30 µg), Cefuroxime (3 µg), and Gentamicin (10 µg). Also, the Gram-positive bacteria were equally tested against eight commercial antibiotics: Ceftriaxone (30 µg), Erythromycin (5 µg), Cloxacillin (5 µg), Ofloxacin (5 µg), Augmentin (30 µg), Ceftazidime (30 µg), Cefuroxime (30 µg) and Gentamicin (10 µg) (Abtek Biological Ltd, England). Sterile forceps were used to place discs onto the plates. The antibiotics were then pressed down to ensure it had contact with the agar. The plates were then covered and incubated aerobically at 37°C for 18 hours (Grewal *et al.,* 2017). After overnight incubation, the growth was examined, the diameters of the zones of inhibition were measured in millimetres with a ruler (See Appendix 8). They were then categorised as sensitive and resistant according to CLSI (2018) standard tables. Multi-antibiotic resistance (MAR) was evaluated by grouping the organisms that are resistant to three or more classes of antibiotics as multi-antibiotic resistant bacteria.

3.13.2 Minimum Inhibitory Concentration by E-test

The minimum inhibitory concentration (MIC) E-test strips (Liofilchem srl, Roseto degli Abruzzi, Italy), with concentration gradients ranging from 0.016 µg/mL to 256 µg/mL each for ceftriaxone (3rd Generation cephalosporin); imipenem (carbapenem); colistin (polymyxin) and piperacillin/tazobactam (penicillin/monobactam) were used to determine the minimum inhibitory concentration according to the manufacturer's instructions on Muller-Hinton agar plates. Bacteria cultures (18-24 hours) were harvested into sterile saline (0.85% NaCl) in tubes and compared with 0.5 Mcfarland's standard. A sterile swab stick was placed into each saline tube and drained along the tube edge to remove excess saline. The swab stick was then used to streak evenly on a Muller-Hilton Agar (MHA) plates (Hi Media, India). The streak covered the whole of the plates and streaking was done at angle 60^{o} three times. The plates were left in the incubator for 20 minutes, so that the agar could absorb the inoculum. The E strips were placed directly in the middle of the plates. The plates were incubated at 37°C for 24 hours and observed afterwards. The MIC values were taken where the edge of the inhibition zone intersected the strips (Rana-Khara *et al.,* 2016). The MIC values were

interpreted using European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2020) breakpoint guidelines.

3.14 Molecular methods for bacteria identification

3.14.1 Bacteria Deoxyribonucleaic acid (DNA) harvesting and extraction

Total genomic DNA of bacteria isolated from the boarding schools' food service establishments were extracted from freshly prepared overnight cultures on nutrient agar medium. DNA extraction was carried out on the samples using Bacteria DNA extraction kits (Jena Bioscience, Germany) according to the manufacturers' instructions.

Gram-negative bacteria

Harvesting and cell lysis: A loopful of single bacterium colony was taken from pure culture plate and suspended in 1 mL sterile deionised water in 2 mL Eppendorf tube, vortexed and centrifuged at 10,000 rpm for 1 min. The supernatant was discarded. According to manufacturer's guidelines, 300 µL lysis Buffer and 2 µL RNase A was added to cell pellet, vortexed vigorously for 30-60 seconds, later 8 µL of Proteinase K was added and mixed by pipetting. The product was incubated at 60°C for 10 min. Then, 300 µL of Binding Buffer was added, pulse-vortexed and centrifuged for 5 min at 10,000 rpm.

Lysate washing and DNA elution: The lysate was pippeted into a spin column in a 2 mL collection tube and centrifuged for 1 min at 10,000 rpm. Washing Buffer (500 µL) was added and further centrifuged for 30 seconds at 10,000 rpm in a two-repetition step. Lastly, 40 µL Elution Buffer was added into the centre of the column, incubated at room temperature for 1 min and centrifuged at 10,000 rpm for 2 min. The DNA product was stored at - 20°C.

Gram-positive bacteria

Cell harvesting: A loopful of bacterial growth was taken from the first streaking area of the pure plate and suspended in 1 mL sterile deionised water in 2 mL Eppendorf tube, vortexed, centrifuged at 10,000 rpm for 1 min and the supernatant was discarded.

Cell resuspension: Due to the nature of Gram-positive bacteria cell wall, a procedure called cell resuspension was carried out before cell lysis. Cell pellet above was

resuspended in 300 µL of Resuspension Buffer and 2 µL of lysozyme solution was added. The suspension was mixed by inverting several times and incubated at 37°C for one hour and centrifuged at 10,000 rpm for I min.

Cell lysis, washing and DNA elution: This was as described for Gram-negative bacteria above.

The purity and concentration of the extracted Gram-negative and Gram-positive DNA was evaluated using a NANODROP (ND 1000) Spectrophotometer (Thermo Scientific, USA). All the samples showed a DNA yield between 60 ng and 120 ng, and the extracted DNA was optimally pure with purity ratio of 1.60 nm to 1.90 nm.

3.14.2 Randomly Amplified Polymorphic DNA-PCR

The molecular variability among the bacteria isolates were analysed by means of Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) using two random primers (OPA 06: 5'-GGT CCC TGA C-3'and OPA 11: 5'- CAA TCG CCG T-3') (Operon Biotechnologies, Cologne, Germany). Amplification was performed in a 10 µL reaction volume containing 2.0 µL of Ready-to-load Master Mix (Solis Biodyne, Estonia), 2.0 µL DNA template and nuclease-free sterile water was used to make up the reaction volume. The PCR was performed using Eppendorf Master Cycler. The initial denaturation step was for 5 min at 95°C, followed by 40 cycles of I min at 95°C (denaturation), 1 min at 30°C (primer annealing) and 2 min at 72°C (elongation), with a final extension for 10 min at 72°C. Following amplification, 10 µL of each PCR product was separated by electrophoresis in 1% (w/v) agarose gel in Tris-acetate-EDTA (TAE) buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 6.8). A 100- base-pair (bp) DNA ladder (Solis Biodyne, Estonia) was used as a size standard. The DNA fragments were visualised after staining the gels with Ethidium bromide of concentration 0.1 µg/mL and then photographed under transmitted ultraviolet light. Binary matrix was constructed pair-wise and presence and absence of RAPD bands were rated 1 or 0, accordingly, in each row. The genetic distance dendrogram was built using ape 5.0: R-package (Paradis and Schliep, 2019). The diversity and distribution of RAPD-PCR fingerprints of bacteria cultures were analysed.

3.14.3 Partial 16S rRNA gene sequencing

From similarity index at 75% obtained after the RAPD-PCR dendrogram analysis, seventy bacterial isolates representative of the clustered groups were selected for 16S rRNA sequencing. The genomic DNA was initially checked for the conservative structure using 16S rRNA gene fragments with the universal primers pair 27F and 149R (5-'AGAGTTTGATCCTGGCTCAG-3') and (3'-GGTTACCTTGTTACGACTT-5') respectively). Amplification was performed in a 25 µL reaction mixture containing 5 µL of 1X Hot FirePol Blend Master Mix Ready-to-Load Buffer (Solis Biodyne, Estonia), 1.5 mM $MgCl_2$, 200 µM of each deoxynucleotide triphosphates (dNTPs), 2 units of Hot FIREPol DNA polymerase enzyme; 0.2 µL of each primer, 5 µL of the extracted DNA, and double-distilled sterile water was used to make up the reaction mixture. Thermal cycling was conducted in a Peltier Thermal Cycler (PTC 100) for an initial denaturation of 95°C for 5 min followed by 35 amplification cycles of 30 seconds at 95°C; 30 seconds at 61°C, and 1 min at 72°C. This was followed by a final extension step of 10 min at 72°C. The amplification product was separated on a 1.0% agarose gel (pH 6.8) and electrophoresis was carried out at 100V for 1 hour in a horizontal electrophoresis Chamber (Cleaver Scientific, Rugby, UK). After electrophoresis, DNA bands were visualised using a UV illuminator, the size of the amplicons were verified using a 1Kb DNA ladder (Solis Biodyne, Estonia). The amplicons were purified to obtain pure PCR products prior to sequencing the PCR products using forward primer 27F (5-'AGAGTTTGATCCTGGCTCAG-3'). The DNA was loaded on the ABI 3130xl genetic analyser (Applied Biosystems) to generate the sequences.

The 16S rRNA gene sequences were compared to sequences in the Gene Bank database using the National Centre for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLAST) to determine sequence similarity with previously published sequences. The NCBI gene bank assigned an accession number to each related sequence. The 16S rRNA gene sequences of isolates with a high degree of similarity (>75%) to Gen Bank sequences were examined for relatedness (Alsanie *et al.*, 2018).

To reconstruct the phylogeny, sample sequences were chosen and aligned using MEGA X software (Appendix 10). After alignments, a neighbour-joining phylogenetic tree was constructed in MEGA X using substitution model and a thousand bootstrap replications (Kumar *et al*., 2018). The evolutionary distances were calculated according to the

Maximum Composite Likelihood method (Tamura *et al*., 2004) and were expressed in terms of base substitutions per site.

3.15 Data Management

The Key Informant Interview (KII) data were transcribed, edited, and saved correctly in a rich text format, and the data were analysed using the content analysis technique (Ademokun *et al*., 2014). All other data were analysed using SPSS version 25 and illustrated using charts, tables, and figures. All data were summarised using the mean, frequency, and standard deviations. Student T-test, One-way ANOVA and chi-square tests were utilised for data analysis at $\alpha_{0.05}$.

3.16 Limitations of this study

There were few public boarding schools in Ibadan and most private boarding schools refused to grant access for this research, even with the letter of permission from government authorities. Therefore, very few selected boarding schools were involved in this study.

CHAPTER FOUR

RESULTS

4.1 Implementation of school health programme and school feeding service

Selected key informants were school representatives in charge of school feeding services. Some were management or teaching staff, while other were non-management staff. Their responses varied based on their assigned responsibilities. All the respondents (100%) stated that they had basic understanding about school health programme and its implementation is according to their capacity. They highlighted its main components as: healthful environment, school feeding services, school health services and school skilled based services. They mentioned that the government did not officially provide guidelines on SHP implementation and that there was no specified requirement needed to achieve the objectives of SHP, but appealed to the government to be more committed in its implementation. Respondent 2 (a vice-principal) stated:

"I heard about school health programme when a circular was sent to the school long time ago on it. I think it also involves setting up a school health club where students, teachers and parents will discuss students' healthy living".

Majority of respondents said students' health issues were referred to private hospital if beyond the school sick bay capacity. Facilities available for school feeding services were assessed based on five main themes and key informants' responses summarised in Figure 4.1.

4.1.1 Nutrition and food safety

Majority of the respondents agreed that understanding children nutritional needs and provision of safe nutritious meals are most important in any school feeding service. All respondents (100%) schools' menu plans showed moderately balanced meals, but fruits were not regularly served except as reported by few informants (33.3%). The respondents stated that they were well informed about the importance of food safety in preventing the spread of foodborne infections.

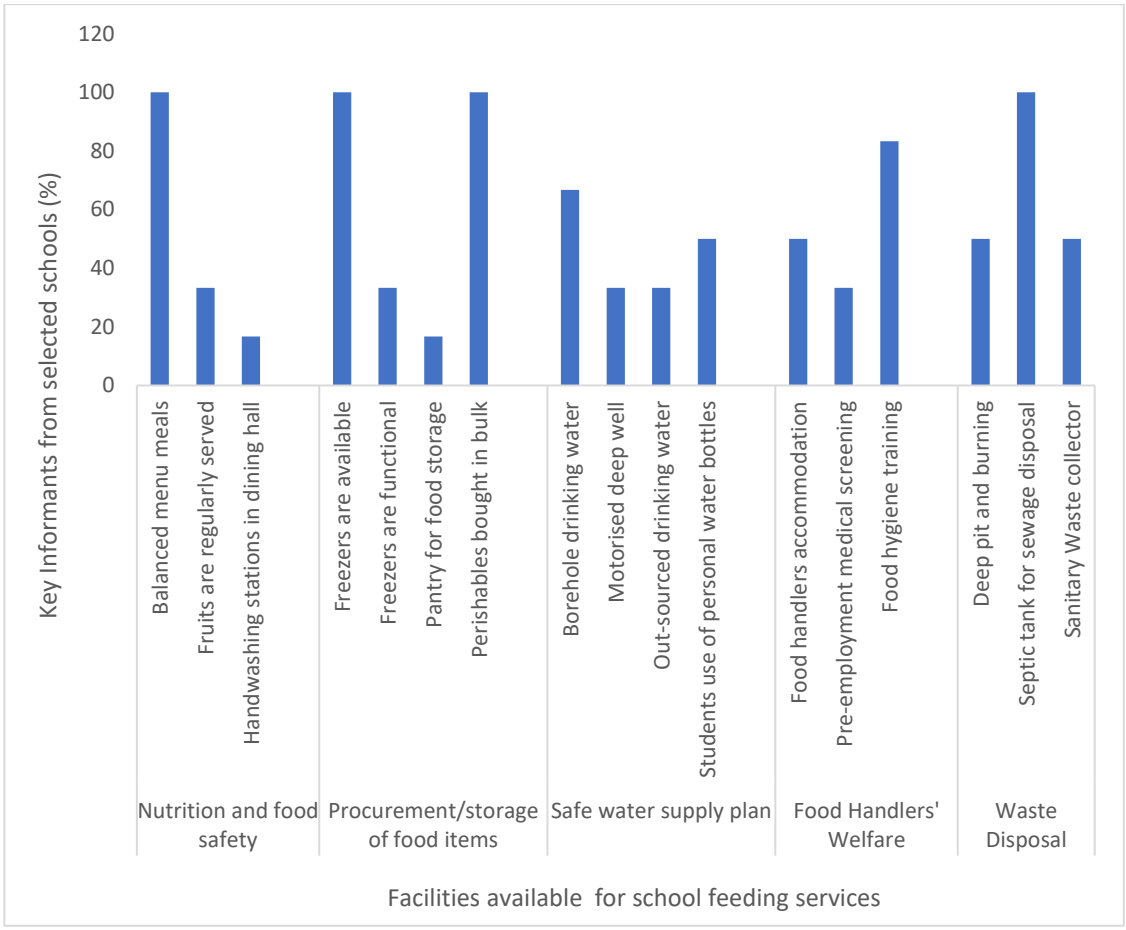


Figure 4.1: Summary of key informants' responses on facilities available for school feeding services

However, they emphasised that most food contamination occurred due to inadequate basic facilities like well-maintained food contact surfaces and handwashing stations. For instance, in this study, only school C informant (16.7%) reported availability of handwashing stations with soap in the dining hall.

4.1.2 Procurement and storage of food items

Almost all of the respondents reported buying non-perishable food items fortnightly which were kept in the kitchen stores. Although, all respondents (100%) stated that freezers were available, but only 33.3% reported freezers that were in good working conditions. Just one (16.7%) respondent mentioned the use of pantry for food storage. All respondents (100%) said perishables items needed for food service were bought as needed but most vegetables were freshly bought. Items such as beef, tomatoes and pepper were often bought in bulk fortnightly, cooked, fried or boiled to extend their shelf lives. The lack of functional freezer was attributed to poor power supply. Respondent 3 (a housemaster) said that:

"the school agricultural farm could be a source of fresh and cheap food while teaching students on food production".

4.1.3 Safe water supply plan

Most respondents (67%) reported the use of on-site borehole water for drinking. Some respondents confirmed the availability of motorised deep wells which are usually used for cooking and washing. Every respondent agreed that drinking water must be potable and stored in clean containers with lids, all participants obtained their drinking water from borehole while two respondents (33.3%) said their water was out-sourced from a water distribution company. Half (50%) respondents stated that students were encouraged to have personal drinking water bottles in order to reduce cross-contamination that can result from sharing.

4.1.4 Food handlers' welfare

Majority of respondents (83%) had in place a good food handlers' welfare plan. They recruit food handlers with experience in catering through old staff and by referral. Some respondents (50%) indicated the availability of accommodation for the food handlers in school premises, while other respondents said no accommodation was provided.

Sometimes, no running taps were available in the toilets. Most schools run two working shifts and each food handler is given one day off work in every week. This helped retain food handlers in the schools for a longer period. All accommodation facilities in place were adequate in terms of comfortable rooms and toilets. Most food handlers attended food hygiene training at government approved centres once in a year, while many took part in more regular in-house trainings according to 83.3% of the respondents. Most food handlers were not pre-screened for medical fitness.

4.1.5 Waste disposal

Exactly half (50%) of the respondents stated that their school had deep pit for refuse collection, while the others had waste receptacles collected by waste collectors. Those using deep pits were located in the rural area due to availability of verse expanse of un-used land for refuse collection, and waste burning was done on regular basis. The others were located in the urban areas where organised collectors were available. All respondents (100%) reported that sewage disposal was by septic tank system. However, most key informants agreed that inappropriate waste management could be source of infection due to transmission of pathogens by pests such as cockroaches, rats and houseflies. In those schools where waste collectors were engaged, it is important that school waste should be collected more regularly to prevent cross-contamination as a result of over-filled receptacles that could attract insects and other pests.

4.2 Environmental hygiene of boarding schools' FSEs

The environmental hygiene parameters of boarding schools' FSEs from the observational checklist is highlighted in Table 4.1. The scores for selected sanitary parameters were 21.4%, 35.7%, 71.4% and 28.6% for schools A, B, C and D, respectively. Only school C had a very good grade (>70%), while the remaining three schools were rated poor. The main area of unsanitary practices in the kitchen include; unavailability of dish washing sinks, the use of basins and buckets for dish washing, presence of domestic animal in the kitchen, uncovered waste receptacles and non-availability of sanitising agents. The dining area do not sit all students at a meal in all the schools, the dining floors were littered and there was no soap for handwashing except in School C. Most food handlers did not wash their hands frequently during food preparation. Also, visitors traffic and dressing were not adequately controlled.

Schools' FSEs' compliance mean scores for environmental hygiene parameters were 82.2%, 56.8%. 52.7% and 65.6% for toilets, dining areas, kitchens and observed food handlers at work, respectively (Table 4.2). For toilet assessment, the highest score was obtained in School C and D (100%), while 85.7% and 42.9% were recorded for school's B and A, respectively. In terms of dining area facilities and hygiene, three-quarter of the schools scored below 50% with School C scoring 90.9%, while Schools A, B and D scored 45.5% each. Concerning kitchen, the scores were 77.8%, 61.1%, 44.4% and 27.8% for Schools C, D, B and A, respectively. The two public schools (A and B) had poor score, while the private schools' (C and D) performance was good. The scores for observed food handlers at work were 100%. 62.5%, 62.5% and 37.5% for schools C, B, D and A, respectively. Overall scores for Schools A, B, C and D were 38.4%, 59.5%, 92.2% and 67.3% respectively with 75% of the schools scoring above 50% pass mark.

Table 4.1: Environmental hygiene status of selected boarding schools' FSEs

Environmental hygiene parameters	School scores			
	A	**B**	**C**	**D**
Kitchen interior is clean and tidy	No	Yes	Yes	Yes
Absence of domestic animals in kitchen premises	No	No	Yes	Yes
Piped water is available	Yes	Yes	Yes	Yes
Sinks are used for dishing washing rather than basins/buckets	No	No	No	No
Cobweb not seen on the kitchen walls	No	No	Yes	No
Solid waste receptacle is covered	No	No	No	No
Sanitising agents are available	No	No	No	No
Running taps are functional in the toilet	No	No	Yes	Yes
The dining area sit all students at a meal	Yes	Yes	No	No
No dirty plates/food remnants on dining table	No	Yes	Yes	No
Soap for handwashing is available	No	No	Yes	No
Employees wear proper hair covering	Yes	Yes	Yes	No
Employees wash their hands frequently	No	No	Yes	No
Visitors traffic is controlled	No	No	Yes	No
Total correct scores	3	5	10	4
Conformity for selected parameters (%)	21.4	35.7	71.4	28.6

Table 4.2: Mean environmental hygiene compliance scores of boarding schools' FSEs

	Observational checklist score of schools' FSEs (%)					
School	**Kitchen**	**Toilet**	**Dining Area**	**Food Handlers at work**	**Total (%)**	**Rating**
A	27.8	42.9	45.5	37.5	38.4	Poor
B	44.4	85.7	45.5	62.5	59.5	Good
C	77.8	100	90.9	100	92.2	Very Good
D	61.1	100	45.5	62.5	67.3	Good
Mean Compliance Score (%)	52.8	82.2	54.6	65.6		

Key: Very Good (≥70%), Good (50-69%), Poor (<50%).

4.3 Food handlers Knowledge, Attitude and Practice

4.3.1 Socio-demographic characteristics of food handlers

The social-demographic characteristics of the food handlers is presented in Table 4.3. The mean age of food handlers was 40.7 ± 11.2 years, 22 (73.3%) were 30 years and above. Forty percent of respondents had completed primary, 43.3% had completed secondary education, while 10% had no formal education. Majority (80%) of the food handlers were designated as cooks and 90% were female. Almost all of the food handlers 26 (86.7%), were ever married while only 4 (13.3%) were single. More than half 17 (56.7%) of the respondents acquired knowledge of food preparation through mentoring, nine (30.3%) from home, while only 13.3% had formal training. About two-third (56.7%) had personal interest in choice of the profession. The average length of employment in food handling was 7.6 years, with majority having more than 5 years of experience. About half (53.3%) of the food handlers did not go for pre-employment medical examination screening.

4.3.2 Food handlers report on water supply and sanitation facilities

The water supply, sanitation facilities and food safety within the school food preparation areas are represented in Table 4.4. Food handlers stated that water supply sources available were motorised borehole (93.3%), protected deep well (30.0%) and hand pumped borehole (3.3%). In addition, all the key informants earlier established the availability of treated motorised borehole while others reported using motorised deep wells. All schools had on-site water supplies, except for School D, which opted to outsource its drinking water commercially. Similarly, on-site observation showed that all (100%) of the schools' kitchen have protected water sources including protected well, hand pump borehole and motorised borehole. Moreover, there was no pest infestation in any of the school's buildings. Almost all 27 (90 percent) food handlers reported that the distance between the water source and the food preparation location was less than 250 meters. Majority 24 (80.0%) of the respondents reported that pour flush was the method of excreta disposal in the school, 50% stated organised waste collector as their way of refuse disposal. The major cooking fuel mentioned by the handlers were bottled gas 27 (90.0%), firewood 14 (46.7%) and charcoal 12 (40.0%).

Table 4.3: Socio-demographic characteristics of food handlers from selected boarding Schools' FSEs

Characteristics	Frequency (N)	Percentage (%)	Mean ± S.D	Range
Age in years				
< 30	8	26.7	40.7± 11.2	22-62
≥ 30	22	73.3		
Level of Education				
No formal education	3	10.0		
Primary	12	40.0		
Secondary	13	43.3		
Tertiary	2	6.7		
Job Designation				
Cook	24	80.0		
Others*	6	20.0		
Gender				
Male	3	10.0		
Female	27	90.0		
Marital status				
Ever Married (Married, Divorced & Widowed)	26	86.7		
Single	4	13.3		
Why did you choose this profession				
Personal interest	17	56.7		
No other alternative	13	43.3		
How did you learn this profession				
Catering school	4	13.3		
Home	9	30.0		
Mentoring	17	56.7		
Year of experience as food handler				
< 5 years	19	63.3	7.6 ± 8.8	
≥ 5 years	11	36.7		
Training on food safety and hygiene				
Yes	17	56.7		
No	13	43.3		

Others*= Kitchen assistants, matron

Table 4.4: Water supply and sanitation facilities at boarding schools' FSEs

Water supply and sanitation facility	Frequency (N)	Percentage (%)
Water Supply		
Protected well	9	30.0
Hand pumped borehole	1	3.3
Motorised borehole	28	93.3
Distance between water source and food preparation location		
Less than 250m	27	90.0
Between 250m and 500m	2	6.7
No response	1	3.3
Method of sewage disposal in the school		
Septic tank	2	6.7
Pour flush	24	80.0
Bush disposal	4	13.3
Method of refuse disposal		
Throwing on empty land	5	16.7
Organised waste collector	15	50.0
Throwing in a dug ground	10	33.3
Major cooking fuel for school food preparation		
Bottled gas	27	90.0
Firewood	14	46.7
Charcoal	12	40.0
Kerosene stove	2	6.7

4.3.3 Knowledge about risk factors associated with food handling

The mean knowledge score was 26.0±4.58 (range=18-32), with most respondents having good knowledge (61.9%) about risk factors associated with food handling. Food handlers' knowledge scores about types of foodborne diseases, its symptoms, food handling techniques and diseases to be reported by food handlers were 63.64%, 62.86%, 70.42% and 64.58%, respectively (Table 4.5). Food handler's overall performance on knowledge about risk factor associated with food handling was good with the least being 62.86% and the highest 70.42%. Also, the results of respondents' knowledge about risk factors associated with food handling is given in Table 4.6. Most respondents 24 (80%) and about half 16 (53.3%) agreed that typhoid and cholera are FBDs respectively but majority (86.7%) did not know that Hepatitis A infection is a foodborne disease. About two-third (60%) responded that food contamination is unlikely to occur if food is prepared in advance before serving. Majority of the food respondents 28 (93.3%) did not know that cooked food should not be kept between 5°C and 65°C. Approximately half (46.7 percent) were aware that food hygiene can be impaired by food handlers with long fingernails and exposed hair. Most participants (83.3%), on the other hand, were aware of symptoms associated with foodborne infections, such as stomach cramps and vomiting. Majority 21 (70.0%) stated that prolong service period can contribute to food contamination while 10 (33.3) % said improper cleaning of equipment, such as a grinding machine, does not increase the risk of foodborne disease. About one-fifth (23.3%) revealed that hands must be washed after cooking food, not prior to it. Most (90.0%) stated that cooked and uncooked foods should be preserved separately, 83.3% revealed that food workers can possibly cause food contamination, while 25 (83.3) % said that meats should be placed on the lower shelves of the refrigerator, while vegetables should be kept at the top.

Knowledge score was compared with the food handlers' socio-demographic characteristics as presented in Table 4.7. Food handlers in private schools were more knowledgeable compared to their public-school counterparts, but the difference was not significant. Also, there were no significant differences between food handlers' age category, job designation, choice of profession, years of experience and knowledge score. In contrast, there was a significant association between method of skill acquisition ($p= 0.017$) and knowledge score.

Table 4.5: Knowledge scores of food handlers about risk factors associated with food handling

Variables	Food handlers knowledge scores			
	Good Knowledge (%)	Poor Knowledge N(%)	Overall score	Rating
Foodborne diseases (11 marks)	23 (76.7)	7 (23.3)	63.64	Good
Symptoms of foodborne diseases (7 marks)	21(70)	9(30)	62.86	Good
Food handling techniques (12 marks)	28(93.3)	2(6.7)	70.42	Very Good
Disease that must be reported by food handlers (12 marks)	24(80)	6 (20)	64.58	Good

Key: Very Good: > 70%, Good: 60-100%, Poor: < 60%.

Table 4.6: Knowledge about risk factors associated with food handling

Knowledge Statements	Have Knowledge N(%)	No knowledge N (%)
Hepatitis A is foodborne disease	4(13.3)	26(86.7)
Tuberculosis is foodborne disease	7(23.33)	23(76.7)
Typhoid fever is foodborne disease	24(80)	6(20)
Cholera is foodborne in nature	16(53.3)	14(46.6)
Stomach aches and cramps are symptoms of foodborne disease	17(56.7)	13(43.3)
Vomiting is a symptom of foodborne disease	25(83.3)	5(16.7)
Food contamination is unlikely to occur if food is prepared in advance before serving.	12 (40)	18 (60)
Prolonged service period can contribute to food contamination	21 (70.0)	9 (30.0)
The improper cleaning of equipment, such as grinding machine, does not enhance the risk of foodborne disease.	20(66.7)	10(33.3)
Handwashing is necessary only after food preparation process, not prior to food preparation	23 (76.7)	7 (23.3)
Cooked food should not be kept between 5°C and 65°C to avoid growth of food pathogens	2 (6.7)	28 (93.3)
Insects like houseflies, ants and cockroaches can spread foodborne pathogens	25(83.3)	5(16.7)
Food hygiene can be impaired by food handlers with long fingernails and exposed hair	14 (46.7)	16 (53.3)
Cooked and uncooked foods should be preserved separately	27(90)	3(10)
Food workers can possibly cause food contamination	25 (83.3)	5 (16.7)
Meats should be placed on the lower shelves of the refrigerator, while vegetables should be kept at the top.	25 (83.3)	5 (16.7)
Handwashing before touching food helps to keep the food safe to eat.	29(96.7)	1(3.3)

Table 4.7: Association between selected food handlers' socio-demographic characteristics and knowledge scores

Characteristics	N	Mean±SD	P-value
School status			
Public	15	25.9±4.7	0.877*
Private	15	26.1±4.6	
Age category in years			
< 30	7	26.6±4.4	0.113*
≥ 30	23	25.8±4.7	
Job Designation			
Cook	24	25.5±4.7	0.280*
Others+	3	27.8±3.9	
Why do you choose this profession?			
Personal interest	17	24.8±4.9	0.839*
No option	13	26.8±4.2	
How do you learn this profession?			
Catering school	4	25.5±5.3	0.017**
Home	9	25.4±5.3	
Mentoring	17	26.4±4.2	
Year of practice as food handler			
< 5 years	19	26.5±4.5	0.466*
≥ 5 years	11	25.1±4.8	

+Matron, kitchen assistant

* T-test, One-way ANOVA **

4.3.4 Attitude of food handlers about risk factors associated with food handling

Table 4.8 shows the attitude of food handlers towards risk factors associated with food handling. The mean attitude score was 19.53±2.86 (range=14-24), with most respondents having positive attitude (81.4%) about risk factors associated with food handling. The majority (93.3 %) of the respondents agreed that to prevent cross-contamination, cooked and uncooked foods should be preserved separately, and roughly half (53.3 %) agreed refreezing already-thawed food can result in food contamination. Furthermore, 93.3 percent agreed that food safety knowledge and training are important to them. Two-thirds (63.3 percent) disagreed that personal protective equipment such as head scarves, face masks, and hand gloves cannot reduce the risk of food contamination. Several food handlers (46.7%) agreed that hands can be wiped with apron after handwashing. Slightly more than half (53.3%) agreed that foodborne outbreaks are natural life events while 53.3% agreed that food handlers should not put on clean overall regularly during food preparation. A fifth (20%) were undecided on the importance of throwing away expired food. Most (90.0%) of the respondents agreed that pest control is part of requirements to achieve food safety.

4.3.5 Food safety training and medical examination of food handlers

The results for food safety training and medical examination is shown in Table 4.9. About half (53.3%) received food hygiene training. Government organised the training of half (50.0%) of the trained food handlers. Five (31.3%) food workers received the training once, while 11 (68.7%) received the training more than two times which lasted mostly for a day (56.3%). Majority (53.3%) of food handlers did pre-employment medical screening, while less than half (46.7%) were not examined prior to food handling job. About half (53.3%) of respondents had their urine examined, 40% for tuberculosis, 26.7 percent had a stool culture test, while only 10% did HIV screening. None were tested for Hepatitis A. Selected characteristics revealed significant differences in the schools' status of being private or public. At $p \leq 0.05$, level of food handler's education, pre-employment medical examination and on-job food hygiene training were all highly significant (Table 4.10).

Table 4.8: Attitude of food handlers towards risk factors associated with food handling

Attitude statement	Agree (%)	Disagree (%)	Undecided (%)
In order to prevent cross-contamination, cooked food should be preserved separately from uncooked food.	28 (93.3)	1 (3.3)	1 (3.3)
Refreezing already-thawed food can result in food contamination.	16 (53.3)	13 (43.3)	1 (3.3)
Personal protective equipment (PPE) such as a head scarves, face masks, and hand gloves cannot reduce the risk of food contamination.	11 (36.7)	19 (63.3)	0 (0.0)
Expired food must be thrown away.	19 (63.3)	5 (16.7)	6 (20.0)
Knowledge and training are crucial in order to assure food safety	28 (93.3)	2 (6.7)	0 (0.0)
Foodborne outbreaks are natural life events	16 (53.3)	14 (46.7)	0 (0.0)
Food handlers should put on clean overall occasionally during food preparation.	16 (53.3)	14 (46.7)	0 (0.0)
Same cutting board and knife should not be used for raw vegetables and meat.	24 (80.0)	6 (20.0)	0 (0.0)
Pest control is part of requirements to achieve food safety.	27 (90.0)	2 (6.7)	1 (3.3
Unwrapped foods should not be touched by food handlers with open wounds.	26 (86.7)	4 (13.3)	0 (0.0)
Good personal hygiene is important to prevent cross-contamination of food.	29 (96.7)	1 (3.3)	0 (0.0)
Hands can be wiped with apron after handwashing.	14 (46.7)	16 (53.3)	0 (0.0)

Table 4.9: Food safety training and medical examination of Food Handlers

Characteristics	Frequency (N)	Percentage (%)
Food Hygiene Training		
Yes	16	53.3
No	13	43.4
No response	1	3.3
Type of Food Hygiene Training received		
Formal	7	43.7
Informal	3	18.8
In-house Training	6	37.5
Organiser of the Training		
Your Institution	6	37.5
Government	8	50.0
Non-Government	2	12.5
Duration of Training		
1 day	9	56.3
≥ 2 days	7	43.7
Number of Time Trained		
Once	5	31.3
≥ 2 times	11	68.7
Pre-employment medical examination		
Yes	16	53.3
No	14	46.7
Type of medical examination (Multiple response)		
Tuberculosis (Sputum test)	12	40.0
HIV	3	10.0
Hepatitis A	0	0
Chest X-ray	6	20.0
Eye Test	3	10.0
Stool culture	8	26.7
Urine culture	16	53.3

Table 4.10: Association between socio-demographic characteristics of food handlers, food hygiene training and school status

Characteristics of Food handlers		School Status Respondents (N)		Chi-square
		Public	**Private**	**P-value**
Education	No formal	3	0	
	Primary	4	8	0.039*
	Secondary	6	7	
	Tertiary	2	0	
Pre-employment medical examination	Yes	2	13	0.000*
	No	12	3	
Food hygiene training on job	With certificate	5	2	
	Without certificate	1	2	0.007*
	In house	0	7	

* Statistically significant at $\alpha_{0.05}$

4.3.6 Handwashing and reported food hygiene practices of food handlers

Exactly half (50.0%) reported that they usually wash their hand when handling food, 43.4% and 36.7% respondents said they always wash their hands before and after cooking, respectively as presented in Table 4.11. Just below half (46.73%) of the handlers stated that they always wash their hands after touching skin, ear and face, only a few 2 (6.7%) did so after sneezing or coughing, while 40.0% did that after cleaning dirt and handling refuse. Majority of the food handlers were not practicing the critical role of general handwashing except 20 (66.7%) who reported that they always wash their hand after using the toilet.

According to Table 4.12, the results obtained on the materials used for handwashing by food handlers showed that 30% of the handlers affirmed that they always use water, 26.7% said they used water most of the times while 10% said they never used water. Almost half (43.3%) of the participants used water and soap to wash their hands and half of the handlers affirmed that they never used alcohol based sanitizer for cleaning their hands. More than half (60%) of the respondents said they never dried their hands with disposable paper towel, 23.3% said they use cloth towel most of the times while 13.3% said they used automatic hand dryer. Eight (26.7%) of the food handlers reported that they always use detergent to wash their hands while one respondent (3.3%) stated the use of liquid soap for handwashing.

Table 4.11: Pattern of handwashing practices among food handlers

STATEMENTS	**Responses**	
Reported handwashing practices	**Correct (%)**	**Incorrect(%)**
Wash hand when handling food	15 (50.0)	15 (50.0)
Wash hand before cooking starts	13 (43.3)	17 (56.7)
Wash hands after cooking ends	11 (36.7)	19 (63.3)
Wash hands before touching cooked or ready to eat foods	4 (13.3)	26 (86.7)
Wash hands after touching skin, ear and face	14 (46.7)	16 (53.3)
Wash hands after sneezing or coughing	2 (6.7)	28 (93.3)
Wash hands after cleaning dirts or handling refuse	12 (40.0)	18 (60.0)
Wash hands after using the toilet	20 (66.7)	10 (33.3)

Table 4.12: Materials for handwashing practices among food handlers

S/No	STATEMENTS	Always	Most times	Sometimes	Rarely	Never
		n (%)	n (%)	n (%)	n (%)	n (%)
1	Water	9 (30.0)	8 (26.7)	6 (20.0)	2 (6.7)	3 (10.0)
2	Water and soap (detergent)	8 (26.7)	13 (43.3)	7 (23.3)	-	2 (6.7)
3	Water and liquid soap	1 (3.3)	13 (43.3)	9 (30.0)	2 (6.7)	3 (10.0)
4	Use of alcohol hand sanitizer	1 (3.3)	1 (3.3)	-	12 (40.0)	15 (50.0)
5	Disposable paper towel	1 (3.3)	1 (3.3)	1 (3.3)	9 (30.0)	18 (60.0)
6	Cloth tower or cloth	1 (3.3)	7 (23.3)	5 (16.7)	6 (20.0)	11 (36.7)
7	Hand dryer (automatic)	-	4 (13.3)	2 (6.7)	1 (3.3)	22 (73.3)

The reported food hygiene practices of food handlers are given in Table 4.13. Almost all (83.3%) reported that they regularly checked the date of expiry on packaged food when being bought, two-third (66.7%) said they usually separated uncooked food from the cooked foods during storage. Majority of the respondents (73.3%) stated that there were separate sections for preparing raw and cooked foods. Only 1 (3.3%) regularly checked the temperature of raw food items during storage while 66.7% said that they regularly cleaned the sections and surfaces where food is prepared. About two-third (60%) stated that they had pest control procedures in place. Most respondents (60%) stated that they regularly used personal protective equipment during food handling, while about half (56.7%) of the respondents regularly took time off work when they were ill. The mean hygiene practices score was 9.45 ±1.87 (range =5-11). Food handlers had poor hygiene practices (52.6%) which differed significantly among schools (p=0.012; χ^2 =10.15).

There was a positive correlation between knowledge and attitude (r = 0.22), and knowledge and practices (r = 0.321), while there was a negative correlation between attitude and practices (r = - 0.113). None of the association shows significant correlation at 5% level of significance. This implies that knowledge level of food handlers had positive influence on their attitude and practices in handling food. The more knowledgeable a food handler is, the better is his attitude and practices towards food handling to minimise foodborne diseases. The negative attitude and practice correlation showed that food handlers' positive attitude did not translate into good practice though it was not significant (Table 4.14).

Association between school status and reported food safety practices is presented in Table 4.15. The findings showed that no significant association existed between school status (public or private schools) and whether they regularly check the date of expiry of packaged food when being bought, prepare food well in advance and take time off work when ill, and whether food handlers check refrigerator and freezer temperature. In contrast, a significant association exist between school status and whether food handlers reheat the food before serving. Significantly, more (26.7%) private schools' food handlers reheat already cooked food before serving compared to none (0.0%) of their public-school counterpart. However, the association between school status and the use of personal protective equipment was not significant.

Table 4.13: Reported food hygiene practices of food handlers

Food hygiene and safety practice	Frequency (N)	Percentage (%)
Check date of expiry of packaged food when being bought	25	83.3
Method of storing uncooked and cooked food		
Together	8	26.7
Separated	20	66.7
No particular form of storage	2	6.6
Regular check of temperature of raw food on storage	1	3.3
Separate sections for preparing raw and cooked food	22	73.3
Regular cleaning of sections and surfaces where food is prepared	20	66.7
Regularly take time off work when ill	17	56.7
Regular pest control in the kitchen and dining area	18	60.0
Regular use of personal protective equipment	18	60.0

Table 4.14: Correlation among KAP levels of food handlers in boarding schools' FSEs

Variables	R	P - value
Knowledge -Attitude	0.22	0.242
Knowledge - Practices	0.321	0.084
Attitude - Practices	- 0.113	0.551

Table 4.15: Association between school status and reported food safety practices

Food safety practices	School status		Chi-square	P-value
	Public (%)	Private (%)		
Check date of expiry				
Yes	12 (80.0)	13 (86.7)	0.240	0.500
No	3 (20.0)	2 (13.3)		
Check refrigerator and freezer temperature				
Yes	1 (6.7)	0(0.0)	1.034	0.500
No	14 (93.7)	15(100.0)		
Prepare meals ahead of time before serving				
Yes	12 (85.7)	9 (60.0)	1.283	0.129
No	3 (14.3)	6 (40.0)		
Reheat food before serving				
Yes	0 (0.0)	4 (26.7)	4.615	0.032 *
, No	15 (100.0)	11 (73.3)		
Take time off when ill				
Yes	9 (60.0)	8 (53.3)	0.136	0.712
No	6 (40.0)	7 (46.7)		
Availability of pest control programme				
Yes	7 (46.7)	12 (80.0)	3.220	0.073
No	8 (53.3)	3 (20.0)		
Use PPE when cooking				
Yes	7 (46.7)	11 (73.3)	2.222	0.136
No	8 (53.3)	4 (26.7)		

Statistically significant at $\alpha_{0.05}$; PPE- personal protective equipment

4.4 Food contact surfaces

The bacterial counts on food contact surfaces (FCSs) from selected boarding high schools' FSEs are given in Table 4.16. The general indication of the microbiological quality (APC) of FCS exceeded the acceptable limits (<2 logCFU/cm^2): drinking water tap (6.27±0.25 logCFU/cm^2), counter top (5.60±1.54 logCFU/cm^2), chopping board (4.38±2.62 logCFU/cm^2) grinder (4.0±0.77 logCFU/cm^2), knife (2.09±1.64 logCFU/cm^2), tray (1.77±2.3 logCFU/cm^2) and dining table (1.75±1.19 logCFU/cm^2). Also, SSC were 6.3±0 logCFU/cm^2, 6.26±0 logCFU/cm^2, 5.5± 1.57 logCFU/cm^2, 4.6±0 logCFU/cm^2, 4.5±0 logCFU/cm^2 and 0.77±0 logCFU/cm^2 for drinking water tap, tray, chopping board, grinder, counter top and dining table, respectively. For SAC, most surfaces exceeded the acceptable limit of <1.0 logCFU/cm^2 except chopping boards and dining tables. Grinder, countertop and drinking water tap recorded 2.47±1.66 logCFU/cm^2, 2.29±2.97 logCFU/cm^2 and 1.9±0 logCFU/cm^2, respectively. The bacterial count for BCC was the lowest among the measured variables, ranging from 0.39±0.74 logCFU/cm2 on the chopping board.to 2.8±0 logCFU/cm2 in the drinking water tap. On the other hand, TC and FC exhibited high levels of bacterial counts. Bacterial detection rate is shown in Table 4.17. The percentage incidences of bacterial contamination for different categories were as follows: SSC (19%), SAC (40%), BCC (24%), TC (55%), and FC (17%). Staphylococcus aureus was detected on all knives, grinders, and countertops, accounting for a 100% detection rate. Bacillus cereus was found on knives and dining tables, with a detection rate of less than 50%. Among these, the dining table exhibited the lowest incidence of bacterial contamination.

4.5 Food handlers hand swabs

Food handlers' hands were examined for bacterial counts (Table 4.18). The APC ranged between 1.16 logCFU/cm^2 and 6.33 logCFU/cm^2. Overall mean logCFU/cm^2 counts of food handlers for APC, SSC, SAC, BAC, TC and FC in were 3.10±1.78, 3.72±1.52, 1.94±1.04, 1.97±1.39, 2.62±1.23 and 2.80±1.74, respectively. Schools A and C food handlers hand count were satisfactory (<1.3 logCFU/cm^2), while highest counts were recorded in School D (>1.3 logCFU/cm^2). Bacterial percentage occurrence was 50% for APC, SAC and BCC, while 40% each was recorded for SSC, TC and FC implying high levels of contamination.

Table 4.16: Mean Bacterial counts from food contact surfaces in selected schools FSEs (LogCFU/cm^2)

Bacteria Count	Chopping Board	Dining Table	Drinking Water tap	Grinder	Knife	Tray	Counter Top
APC	4.38 ± 2.62	1.75 ± 1.19	6.27 ± 0.25	4.0 ± 0.77	2.09 ± 1.64	1.77 ± 2.3	5.6 ± 1.54
SSC	5.5 ± 1.57	0.77 ± 0	6.3 ± 0	4.6 ± 0	1.25 ± 0.35	6.26 ± 0	4.5 ± 0
SAC	0.82 ± 0.72	0.01 ± 0.44	1.9 ± 0	2.47 ± 1.66	1.02 ± 0.99	1.21 ± 1.2	2.29 ± 2.97
BCC	0.39 ± 0.74	0.46 ± 0.68	2.8 ± 0	1.93 ± 1.02	0.65 ± 0.36	0.89 ± 1.0	1.3 ± 1.03
TC	3.08 ± 2.0	0.09 ± 1.07	NA	2.82 ± 1.32	1.69 ± 1.04	1.46 ± 1.75	3.09 ± 1.96
FC	1.47 ± 0	NA	4.5 ± 0	NA	1.19 ± 0.35	2.1 ± 2.97	4.28 ± 0

Key: Aerobic Plate Count (APC), Salmonella-Shigella Count (SSC), *Staphylococcus aureus* count (SAC), *Bacillus cereus* Count (BCC), Total Coliforms (TC) and Faecal Coliform (FC); NA: Not Available, SD: Standard Deviation

APC: Acceptable limit is < 2 logCFU/cm^2 (Sibanyoni and Tabit, 2019)

Others: Acceptable limit is <1.0 logCFU/cm^2, SSC: Absent/Present (Balzaretti and Marazano. 2013)

Table 4.17: Bacteria counts on food contact surfaces from boarding schools' FSEs

Food Contact Surfaces	Total sample (n=42)	Microbial Detection Rate N(%)				
		SSC	SAC	BCC	TC	FC
Chopping Board	8	2 (25)	3 (38)	1 (13)	5 (63)	1 (13)
Countertop	3	1 (33)	1 (33)	1 (33)	2 (67)	1 (33)
Dining Table	5	0	0	0	1(20)	NA
Drinking water tap	2	1 (50)	1 (50)	1 (50)	NA	1 (50)
Grinder	6	1 (17)	5 (83)	5 (83)	5 (83)	NA
Knife	9	2 (22)	4 (44)	0	6 (66)	3 (33)
Tray	9	1 (11)	3 (33)	2 (22)	4 (44)	1 (11)
Total	42	8 (19)	17 (40)	10 (24)	23 (55)	7 (17)

Key: Salmonella-Shigella Count (SSC), *Staphylococcus aureus* count (SAC), *Bacillus cereus* Count (BCC), Total Coliforms (TC) and Faecal Coliform (FC); NA: Not Available

Table 4.18: Bacterial counts on food handlers' hands from boarding schools' FSEs

Sample	APC	SSC	SAC	BCC	TC	FC
	Log (CFU/cm^2)					
FHA1	<1	ND	2.48	2.85	ND	ND
FHA2	<1	ND	ND	2.9	ND	ND
FHA3	<1	ND	4.48	ND	2.3	ND
FHA4	<1	ND	ND	ND	ND	ND
FHA5	3.9	4.11	2.7	ND	3.4	2.9
FHB1	1.16	ND	0.72	0.92	ND	ND
FHB2	1.28	1.02	ND	1.01	0.71	0.4
FHB3	<1	ND	ND	0.91	ND	ND
FHB4	<1	ND	ND	ND	ND	ND
FHC1	<1	4.6	ND	ND	ND	ND
FHC2	<1	ND	ND	0.04	ND	ND
FHC3	<1	ND	ND	ND	ND	ND
FHC4	1.51	ND	1.44	0.56	ND	ND
FHD1	6.33	4.42	2.41	ND	4.17	2.1
FHD2	4.16	4.21	ND	ND	1.98	4.08
FHD3	4.29	4.13	1.71	3.77	2.24	4.01
FHD4	4.58	5.51	1.42	2	4.27	5.7
FHD5	1.49	ND	1.53	1.83	ND	1.04
FHD6	<1	ND	1.1	4.73	ND	ND
FHD7	2.34	1.76	1.36	2.06	1.89	2.14
Mean	3.10	3.72	1.94	1.97	2.62	2.80
SD	1.78	1.52	1.04	1.39	1.23	1.74
Maximum	6.33	5.51	4.48	4.73	4.27	5.7
Minimum	1.16	1.02	0.72	0.04	0.71	0.4
Positive (N)	10	8	10	10	8	8
Occurrence (%)	50	40	50	50	40	40

Key: Aerobic Plate Count (APC), Salmonella-Shigella Count (SSC), *Staphylococcus aureus* count (SAC). *Bacillus cereus* Count (BCC), Total Coliforms (TC) and Faecal Coliform (FC); ND: Not detected (below detection limit); FH, Food Handler, SD: Standard Deviation <1: 1.0 logCFU/cm^2 is the detection limit

Table 4.19 shows the percentage of food handlers that conformed with the microbiological standards of washed hands. High levels of non-conformity were recorded for all counts and ranged between 35% and 50% for all bacterial counts. Exactly half (50%) of food handlers' hands swabs were not within the acceptable limit for SAC. Salmonella/Shigella were detected in 40% of all samples. Forty percent were non-conformity for APC, BCC and FC, while thirty-five percent was recorded for TC. The hygiene levels of food handlers' hands were inadequate and could be due to poor cleaning and sanitation. *S. aureus* is normal flora of human and found on skin and nasal cavity.

4.6 Ready-to-Eat foods

The average counts (APC, SSC, SAC, BCC TC, FC) obtained from samples of RTE foods analysed are presented in Table 4.20 and Table 4.21. APC ranged between 2.60 logCFU/g and 6.43 logCFU/g with the mean count as 3.98 logCFU/g for RTE foods. The maximum count 6.43 logCFU/g was found in School D fish stew. The mean value of total coliform count was 3.23 logCFU/g with the highest count of 5.04 logCFU/g in egg sample from School A. Total coliforms were positive in 7 out of 18 samples such as fish stew, fried egg, bread, and yam flour (Amala) from Schools A, D and C.

Only five samples were positive for Salmonella-Shigella count, they were bread, beans, fish stew and egg dishes. The SSC ranged from 2.60 logCFU/g and 5.42 logCFU/g. Faecal coliforms were detected in only four samples bread, fish stew, egg and amala dishes. The faecal coliform count ranged between 2.75 logCFU/g and 4.75 logCFU/g. The mean FC count was 3.58 logCFU/g. The SAC ranged between 1.0 logCFU/g and 5.34 logCFU/g with mean of 3.04 logCFU/g. *Bacillus cereus* was recorded in 67% of the samples obtained from all samples with mean of 3.83 logCFU/g.

According to Table 4.21, levels of conformity for were 78%, 72%, 56%, 39%, 61% and 78% for APC, SSC SAC BCC, TC, and FC respectively. Fourteen (78%) RTE foods conformed with the acceptable limits of 4 logCFU/g for APC.

Table 4.19: Conformity of food handlers' hand swabs to microbiological standards

Sample	Assessment	Percentage of conformity											
		APC		SSC		SAC		BCC		TC		FC	
		N	%	N	%	N	%	N	%	N	%	N	%
Food Handlers (n=20)	Conformity	12	60	12	60	10	50	12	60	13	65	12	60
	Non-Conformity	8	40	8	40	10	50	8	40	7	35	8	40

Key: Aerobic Plate Count (APC), Salmonella-Shigella Count (SSC), *Staphylococcus aureus* Count, *Bacillus cereus* Count (BCC), Total Coliforms (TC) and Faecal Coliform (FC); APC: Acceptable limit is < 1.3 logCFU/cm^2 (Balzaretti and Marazano. 2013)
Others: Acceptable limit is <1.0 logCFU/cm^2, SSC: Absent/Present

Table 4.20: Bacterial counts of RTE foods isolated from boarding schools' FSEs

Bacterial Counts	Positive Sample (N)	Incidence (%)	Mean ± SD (LogCFU/g)	Min- Max (Log FU/g)
APC	13	72	3.98 ± 1.18	2.6-6.43
SSC	5	28	3.64 ± 1.06	2.6-5.42
SAC	9	50	3.04 ± 1.27	1.0-5.34
BCC	12	67	3.83 ± 1.14	2.48-5.45
TC	7	39	3.23 ± 1.12	2.0-5.04
FC	4	22	3.58 ± 0.98	2.75-4.75

Key: APC: Aerobic Plate Count, SSC: Salmonella-Shigella Count, SAC: Staphylococcus aureus Count, BCC: Bacillus Count, TC: Total Coliform, FC: Faecal Coliform

Table 4.21: Bacterial contamination of RTE foods from boarding schools' FSEs (Log/CFU/g)

Sample	**APC**	**SSC**	**SAC**	**BCC**	**TC**	**FC**
Bread A	3.53	5.42	ND	2.83	2.74	2.75
Bread B	3.1	ND	2.77	2.92	ND	ND
Bread C	2.6	ND	2.66	3	ND	ND
Bread D	<1	ND	ND	ND	2	ND
Beans A	<1	3.36	4.7	4.8	ND	ND
BeansA2	<1	ND	1	3.55	ND	ND
Rice A	<1	ND	5.34	5.38	ND	ND
Rice B	2.95	ND	ND	2.59	ND	ND
Rice C	3.23	ND	ND	ND	ND	ND
Rice D	5.35	ND	ND	ND	ND	ND
Fish Stew C	3.71	2.6	2.8	3.4	4.05	4.03
Fish Stew D	6.43	3.55	ND	5.28	3.78	ND
Meat C	3.44	ND	2.61	2.48	ND	ND
Meat D	3.44	ND	ND	ND	ND	ND
Fried Egg A	5.43	3.27	2.82	5.45	5.04	4.75
Fried Egg D	<1	ND	ND	ND	2	ND
Semolina D	5.13	ND	ND	4.26	ND	ND
Amala A	3.4	ND	2.7	ND	3.02	2.78
Non-Conformity	4	5	8	11	7	4
Conformity	14	13	10	7	11	14
Conformity (%)	78	72	56	39	61	78
Acceptable Limits*	<4	ND	<1.3	<3	<2	<1.3

Key: APC: Aerobic Plate Count, SSC: Salmonella-Shigella Count, SAC: *Staphylococcus aureus* Count, BCC: *Bacillus cereus* Count, TC: Total Coliform, FC: Faecal Coliform. ND: Not detected, <1: 1.0 logCFU/g is the detection limit (AOAC, 2005)

4.7 Physico-chemical parameters of water

The results of physico-chemical parameters for the water samples are given in Table 4.22. Only school B (WB) had same water for drinking and cooking. The temperature ranged from 26.6°C to 28.6°C, while the atmospheric temperature during the sample collection was between 24°C and 29°C. The pH values of the water samples tested ranged between 5.8 and 7.8 with sample WD1 having lowest value and WA1 sample with highest value. The pH of water samples from private schools are more acidic than those from public schools. Turbidity levels ranged from 0.1 to 13.12 NTU. Two samples WA2 and WB had turbidity values of 13.12 NTU and 11.93 NTU which exceeded the standard limit of 5 NTU. The majority of water samples were within the allowable limits.

The Total Dissolved Solids (TDS) ranged from 120 mg/L for school A cooking water (WA2) to 340 mg/L for school A drinking water (WA1), the values were all below the standard value of 500 mg/L. The conductivity also ranged between 180.7 µS/cm for WA2 and 503 µS/cm for WA1 respectively, which is lower than the allowable limit of 1000 µS/cm. The total hardness ranged between 8.51 mg/L and 56.481 and mg/L with school C cooking water (WC2) having the lowest value and school B drinking/cooking water (WB) with the highest value respectively.

Phosphate ion concentration ranged from 0.1mg/L (same in WA1, WA2 and WB) and 4.82 mg/L (WC1). The sulphate ion ranged between 0.74 mg/L and 5.84 mg/L with rural-public school cooking water WA2 having the least value and urban-private drinking water (WD1) having the highest value. Chloride ion ranged from 0.12 mg/L and 6.84 mg/L, lowest value was rural-public school cooking water WA2 and highest value was rural-private school drinking water (WC2). Nitrate and Lead were not detected in any of the water samples. Iron was detected in all samples and ranged from 0.66 mg/L to 2.03 mg/L, the values exceeded the NIS limits of 0.3 mg/L.

Table 4.22: Physico-chemical analyses of water from selected schools' FSEs

Sample Code	Public			Private				Min - Max	WHO	NIS (NSDWQ)
	WA1	WA2	WB	WC1	WC2	WD1	WD2			
Temperature (°C)	27.4	27.2	26.6	28.6	28.6	27.3	28.2	26.6 - 28.6	Ambient	Ambient
pH	7.8	6.6	6.7	6.3	6.3	5.8	6.8	5.8 - 7.8	6.5-8.5	6.5-8.5
Turbidity (NTU)	0.1	13.12	11.93	0.1	0.1	0.1	0.1	0.1 - 13.12	5	5
Total Dissolved Solids(mg/L)	340	120	150	175	176	196	173	120 - 340	1000	500
Conductivity (µScm-1)	503	180.7	225	262	264	291	259	180.7 - 503	250	1000
Calcium Hardness (mg/L)	2.59	6.68	56.48	21.23	8.40	10.17	45.52	0.84 - 56.48	NA	0.2
Magnesium Hardness (mg/L)	0.002	0.033	0.001	0.013	0.011	0.053	0.012	0.001 - 0.053	125	75
Total Hardness (mg/L)	2.592	6.713	56.481	21.243	8.51	10.223	45.532	0.851 - 56.481	500	150
Phosphate (mg/L)	0.1	0.1	0.1	4.82	2.05	1.98	2.72	0.1 - 4.82	NA	NA
Sulphate (mg/L)	1.19	0.74	2.45	5.03	2.03	4.63	5.84	0.74 - 5.84	500	100
Chloride (mg/L)	3.42	1.05	0.12	0.84	6.84	0.96	0.36	0.12 - 6.84	250	250
Iron (mg/L)	0.66	2.23	1.05	2.03	1.46	1.57	1.76	0.66 - 2.23	0.3	0.3
Nitrate (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	50	50
Lead (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	0.01	0.01

Key: WA1- School A drinking water; WA2- School A cooking water; WB- School B water; WC1- School C drinking water; WC2- School C cooking water; WD1- School D drinking water; WD2- School D cooking water, ND: Not Detected (below detection limit); WHO, 2014; NIS: National Industrial Standards (2007); all drinking water are from borehole

4.8 Microbiological analyses of water samples

Aerobic Plate Counts (APC) for water samples ranged from 1.76 logCFU/mL and 6.37 logCFU/mL. The values obtained from public schools were higher than that recorded from private schools. All tested water samples exceeded the allowable limit of 1.3 logCFU/mL. Total coliform count in boarding schools water samples ranged from 0 MPN/100 mL to 13 MPN/100 mL. Coliform counts for most schools' water samples were in the allowable limit of 10 MPN/100ml except School A cooking water (WA2) with 13 MPN/100mL. Only school C cooking water (WC2) recorded presence of *E. coli* (2 MPN/100mL), indicating presence of faecal contaminants (Table 4.23).

4.9 Phenotypic characterisation of isolated bacteria from schools' FSEs

The percentage frequency of occurrence of isolated bacteria from boarding schools' FSEs is given in Figure 4.2. One hundred and fifty-four bacteria belonging to twelve different bacterial species were presumptively characterised by phenotypic methods. *Escherichia coli* was the most prevalent organism 43(27.9%), *Salmonella paratyphi* 27(17.5%), *Staphylococcus aureus* 26(16.9%), *Salmonella typhi* 25(16.2%), *Proteus mirabilis* 14(9.1%), *Pseudomonas aeruginosa* 6(3.9%), *Klebsiella pneumoniae* 5(3.2%), *Serrattia marcescens* 3 (1.9%), and *Proteus vulgaris* 2(1.3%). The lowest were single isolates of *Staphylococcus epidermis*, *Bacillus subtilis* and *Bacillus cereus* (0.6%) each.

The distribution of various bacteria genera isolated from selected schools' FSEs are given in Table 4.24. The highest incidence of contaminated samples was recovered from food handlers' hands (21.4%); chopping board (18.8%); knives (10.4%); tray (9.1%); countertops and grinders (7.1%); fish stew (5.8%) and dining table (3.9%). Others were yam flour and fried egg (3.2% each); bread and cooking water (2.6% each); semolina and drinking water (1.3% each); while lowest value was from rice (0.6%). Details of biochemical characterisation are given in Appendix 7.

Table 4.23: Aerobic Plate count, Total coliform count and *E. coli* counts of boarding schools water samples

School	Water sample	APC (LogCFU/mL)	Total Coliform (MPN/100 mL)	*E. coli* (MPN/100 mL)
Public				
WA1	Drinking	5.52	2	0
WA2	Cooking	6.37	13	0
WB	Drinking/Cooking	4.06	2	0
Private				
WC1	Drinking	1.76	0	0
WC2	Cooking	3.00	2	2
WD1	Drinking	3.30	2	0
WD2	Cooking	3.73	2	0

Key: WA1- School A drinking water; **WA2**- School A cooking water; **WB**- School B water; **WC1**- School C drinking water; **WC2**- School C cooking water; **WD1**- School D drinking water; **WD2**- School D cooking water; **APC**- Aerobic Plate Count; **MPN/100 mL**- Most Probable Number in 100 Ml; Allowable APC limits-1.3logCFU/Ml; Total coliform- < 10MPN/100 mL; E. coli- Zero MPN/100mL (NIS, 2007)

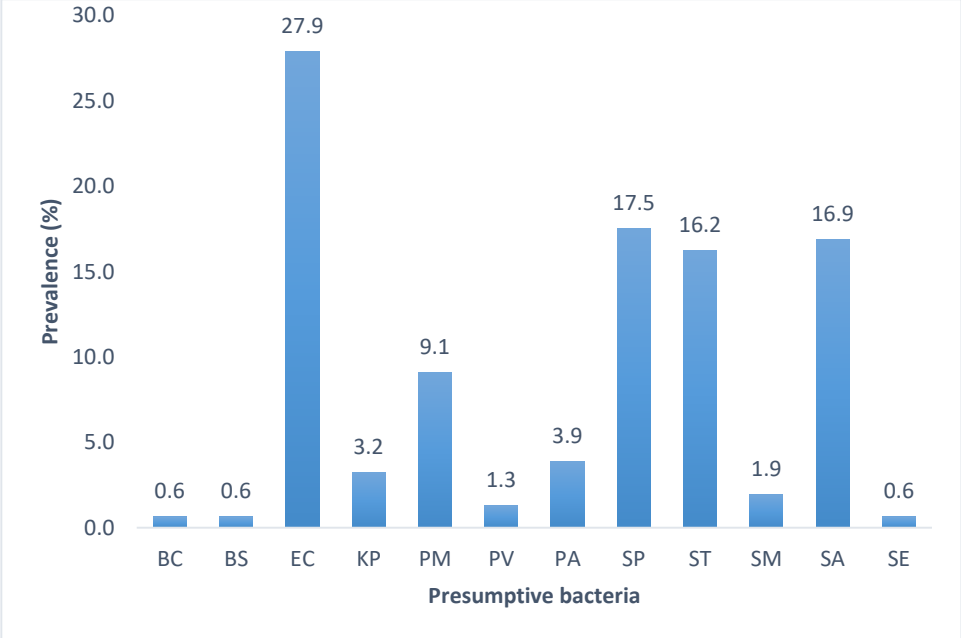


Figure 4.2: Phenotypic diversity of bacteria in selected boarding schools' FSEs

Key: BC: *Bacillus cereus;* BS: *Bacillus subtilis;* EC: *Escherichia coli; KP: Klebsiella pneumoniae;* PM: *Proteus mirabilis;* PV: *Proteus vulgaris;* PA: *Pseudomonas aeruginosa;* SP: *Salmonella paratyphi;* ST: *Salmonella typhi;* SM: *Serratia marcescens;* SA: *Staphylococcus aureus;* SE: *Staphylococcus epidermidis*

Table 4.24: Distribution of isolated bacteria and their sources from boarding schools' FSEs

Sample Category	Source/ Organism	BC	BS	EC	KP	PM	PV	PA	SP	ST	SM	SA	SE	School Stauts		Total
														Private	Public	
Food Contact Surfaces	Chopping Board	1	0	8	1	1	0	1	2	5	0	10	0	17	12	29(18.8)
	Countertop	0	0	4	0	0	0	0	2	3	1	1	0	11	0	11(7.1)
	Dining Table	0	0	1	0	0	1	0	2	2	0	0	0	4	2	6(3.9)
	Drinking water tap	0	0	1	0	0	0	0	1	0	0	0	0	2	0	2(1.2)
	Grinder	0	0	4	0	2	0	1	1	0	0	3	0	5	6	11(7.1)
	Knife	0	0	3	1	1	0	0	2	5	1	3	0	8	8	16(10.4)
	Tray	0	0	4	1	1	0	0	2	2	0	4	0	7	7	14(9.1)
Food handlers hands	Food handler Hands	0	1	12	1	4	0	0	8	4	1	2	0	19	14	33(21.4)
RTE foods	Amala	0	0	0	0	0	0	1	3	0	0	1	0	0	5	5(3.2)
	Bread	0	0	1	1	2	0	0	0	0	0	0	0	2	2	4(2.6)
	Fish stew	0	0	3	0	0	0	1	2	2	0	0	1	9	0	9(5.8)
	Fried Egg	0	0	0	0	1	1	1	0	1	0	1	0	0	5	5(3.2)
	Rice	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1(0.6)
	Cooked Semolina	0	0	0	0	1	0	1	0	0	0	0	0	2	0	2(1.3)
Water	Cooking Water	0	0	1	0	1	0	0	1	0	0	1	0	3	1	4(2.6)
	Drinking water	0	0	0	0	0	0	0	1	1	0	0	0	2	0	2(1.3)
Total (N)		1	1	43	5	14	2	6	27	25	3	26	1	91	63	154(100.0)
Total (%)		1	1	28	3	9	1	4	18	16	2	17	1	59	41	

Key: BC: *Bacillus cereus;* BS:*Bacillus subtilis;* EC:*Escherichia coli; KP:Klebsiella pneumoniae;* PM:*Proteus mirabilis;* PV:*Proteus vulgaris;* PA:*Pseudomonas aeruginosa;* SP:*Salmonella paratyphi;* ST: *Salmonella typhi;* SM:*Serratia marcescens;* SA:*Staphylococcus aureus;* SE:*Staphylococcus epidermidi*

4.10 Antibiotic susceptibility testing of phenotypic bacteria

Antibiotic susceptibility profiles of the presumptively identified bacterial is shown in Table 4.25. Gram-negative bacteria level of resistance to cefuroxime, cefixime, nitrofurantoin gentamicin, augmentin, ceftazidime, ciprofloxacin and ofloxacin were 80%, 62.4%, 53.6%, 52%, 44.8%, 33.6%, 2.4%, and 2.4%, respectively. *Salmonella* spp. showed high resistant to cefuroxime (80.8%), cefixime (73.1%) and gentamicin (69.2%). Almost all bacterial isolates were susceptible to ciprofloxacin except few *Salmonella spp*. with 5.8% and 3.8% resistance to ciprofloxacin and ofloxacin, respectively. Over two-third (60%) of each bacteria isolate were resistant to cefuroxime.

Also, Gram-positive isolates (Table 4.26), most antibiotics showed high resistance to ceftazidime (89.7%), cloxacillin (86.2%) and erythromycin (69%). The antibiotics to which most Gram-positive bacteria tested in this study showed lowest resistance to gentamicin (37.9%) and augmentin (48.2%). S*taphylococcus* spp. were highly resistance to ceftazidime (92.5%), cloxacillin (85.2%) and erythromycin (66.7%). Only 18.5% of *Staphylococcus* spp. were resistant to ofloxacin, while no *Bacillus spp*. showed resistance to ofloxacin.

4.11 Genotypic characterisation of bacteria isolates

A combination of Random Amplified Polymorphic DNA-PCR and partial sequencing of the 16S rRNA gene of the identified bacterial isolates were used for molecular analyses.

4.11.1 Random Amplified Polymorphic DNA-PCR

A total of 154 bacterial isolates from boarding schools' FSEs were analysed in this study. The band sizes produced by the isolates ranged from 100 to 1100 basepairs. The electrophoretic gel images are shown in Appendix 9. The construction of the genetic distance phylogenetic tree (Figure 4.3) and the subsequent cluster analysis were conducted using the Unweighted Pair-Group Method with Averaging (UPGMA) and ape 5.0, an R package. Most bacteria were genetically varied and comprised of a heterogeneous population at the 75% similarity index. The phylogenetic analysis of bacteria from RAPD analysis separated the isolates into seven clusters with four main clusters having highest isolates (large black, red, green and purple colours). Each cluster contained at least five isolates.

Table 4.25: Resistance patterns of Gram-negative bacteria from boarding schools' FSEs

Organism	Frequency	Antibiotic							
		CPR(%)	**NIT(%)**	**CXM(%)**	**OFL(%)**	**AUG(%)**	**CAZ(%)**	**CRX(%)**	**GEN(%)**
Escherichia coli	43	0(0.0)	16(37.1)	16(37.1)	1(2.3)	16(37.1)	5(11.6)	32(74.4)	13(30.2)
Klebsiella pneumoniae	5	0(0.0)	2(40.0)	2(40.0)	0(0.0)	2(40.0)	2(40.0)	5(100.0)	1(20.0)
***Proteus* spp.**	16	0(0.0)	8(57.1)	14(87.5)	0(0.0)	10(62.5)	8((50.0)	13(81.3)	11(68.8)
Pseudomonas aeruginosa	6	0(0.0)	5(83.3)	6(100.0)	0(0.0)	4(66.7)	4(66.7)	6(100.0)	4(66.7)
***Salmonella* spp.**	52	3(5.8)	34(65.4)	38(73.1)	2(3.8)	22(42.3)	21(40.4)	42(80.8)	36(69.2)
Serratia marcescens	3	0(0.0)	2(66.7)	2(66.7)	0(0.0)	2(66.7)	2(66.7)	2(66.7)	0(0.00)
Total	125	3(2.4)	67(53.6)	78(62.4)	3(2.4)	56(44.8)	42(33.6)	100(80.0)	65(52.0)

Key: Ciprofloxacin-**CPR**; Nitrofurantoin –**NIT**; Cefixime- **CXM**; Ofloxacin- **OFL**; Augmentin-**AUG**; Ceftazidime-

CAZ; Cefuroxime-**CRX**; Gentamicin –**GEN**;

Table 4.26: Resistance patterns of Gram-positive bacteria from boarding schools' FSEs

Organism	Frequency	Antibiotics							
		CTR	**ERY**	**CXC**	**OFL**	**AU6G**	**CAZ**	**CRX**	**GEN**
***Bacillus* spp.**	**2**	1(50)	2(100)	2(100)	0(0.0)	1(50)	1(50)	1(50)	1(50)
***Staphylococcus* spp.**	**27**	17(63.0)	18(66.7)	23(85.2)	5(18.5)	13(48.2)	25(92.5)	15(55.6)	10(37.0)
Total	**29**	18(62.1)	20(69.0)	25(86.2)	5(17.2)	14(48.3)	26(89.7)	16(55.1)	11(37.9)

Key: Ceftriaxone –**CTR**; Erythromycin –**ERY;** Cloxacillin- **CXC**; Ofloxacin **OFL-**Augmentin-**AUG**; Ceftazidime- **CAZ;**

Cefuroxime-**CRX**; Gentamicin –**GEN**

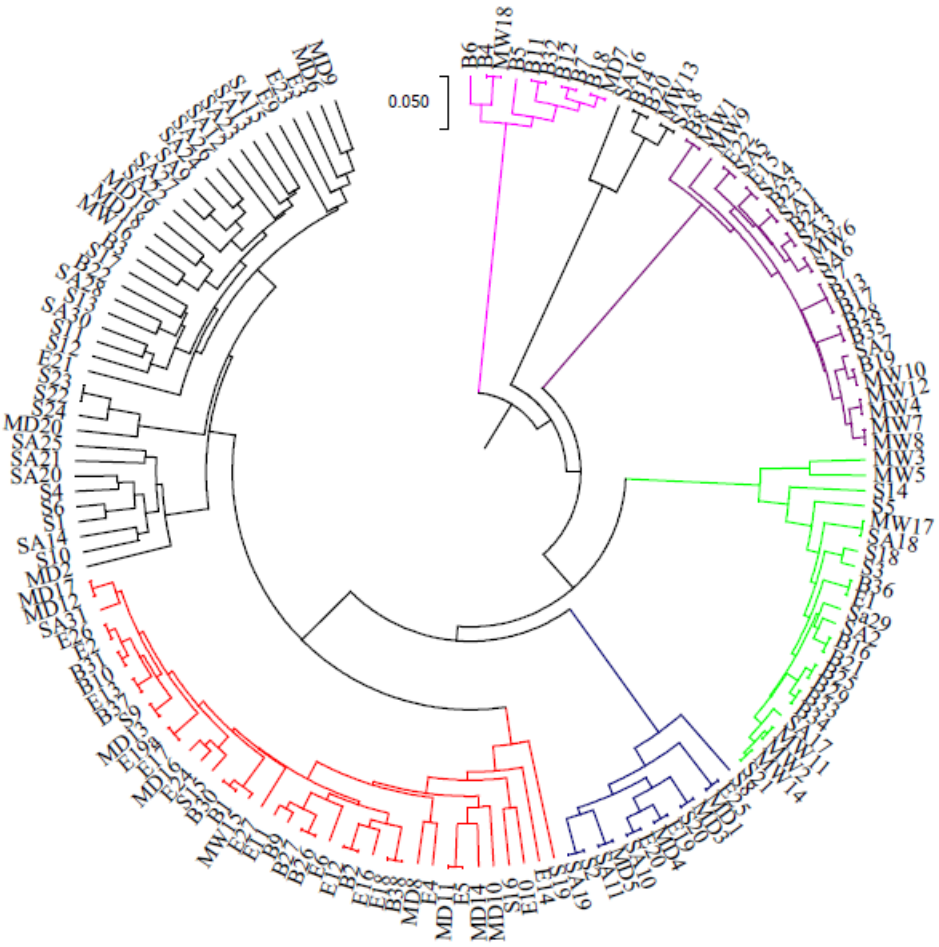


Figure 4.3: Phylogenetic analysis of RAPD-PCR fingerprints among bacteria isolated from boarding schools' FSEs

Key: E-*Escherichia coli*, S- *Salmonella* species, MW- Enterobacteria (Wet season), MD-Enterobacteria (Dry season), SA- *Staphylococcus aureus*, B-*Bacillus cereus*

Cluster I (lilac) consisted of 10 bacterial isolates, namely B- 4, 5, 6, 7, 11, 12, 18, 32; MD7 and MW18. The dominant isolates of cluster I was B isolates with 8 of 10 isolates. Cluster II (small black) consisted of 5 isolates, namely B14, B20, MW13, SA16 and SA8. Cluster III (purple) consisted of 25 isolates of bacteria, namely B- 8, 13, 17, 19, 23, 24, 28, 35; MW-1, 4, 6, 7, 8, 9,10,12; SA- 1,3,4,5,6,7; E15 and E22 and S7. B and MW isolates dominated Cluster III.

Cluster IV (green) consisted of 22 isolates of bacteria, namely B-16,21. 25, 29, 33, 34, 36; MW-2, 3, 5, 11, 14; S- 3, 5, 14, 18, 21; SA- 2, 17, 18, 29 and E1. Isolates in cluster IV were highly diverse. Cluster V (blue) consisted of 13 isolates, namely E- 20, 25; MD-1, 3, 4, 5; S- 2, 8, 20; SA-10, 11, 18. Cluster VI (red) is made up of 40 bacterial isolates namely B-1, 2, 9, 10,26,27, 30, 31, 37,38; E- 2,4, 5, 6, 10, 11, 12, 13, 14, 16,17, 18, 19A, 24, 26,2; MD-8, 10, 11, 12, 13, 14, 16, 17; S-9, 15, 16, 19; MW15; SA31. The dominant isolate was E isolates with 15 of 40 isolates. Cluster VII (large black) consisted of 39 isolates, namely B-22, 3; E-3, 9, 21, 23; MD- 2, 6, 9, 18, 19 ,20; MW16; S-1,2,4, 6, 10, 11, 12, 13, 17, 22, 23, 24; SA- 9,12, 13, 14,15, 20, 21, 22 23, 24, 25, 26, 27, 28, 30. The dominant isolate was SA isolates with 15 of 39 isolates.

Based on the source of bacterial isolated from the selected boarding schools' FSEs, isolates derived from one school or sample source category were not found in a specific cluster. The RAPD profile showed high diversity among the isolates. Seventy representative bacterial isolates from all clusters were selected and prepared for 16S rRNA gene sequencing using the Sangers method.

4.11.2 Partial 16S rRNA sequencing and evolutionary relationships of taxa

The bacterial sequences were analysed with the Basic Local Alignment Search Tool (BLAST) to find sequence similarities that were not random and searches were limited to the 16S ribosomal RNA database. The BLAST results with the lowest expect-value (e-value), highest query cover and highest percent identity were selected. The frequency of occurrence of sequenced bacteria from selected boarding schools' FSEs is represented by Figure 4.4. Percent identity was set between 75% and 100% (Table 4.27). Out of the seventy selected isolates, thirty-seven bacterial isolates had significant sequences while the remaining 33 samples gave non-significant results.

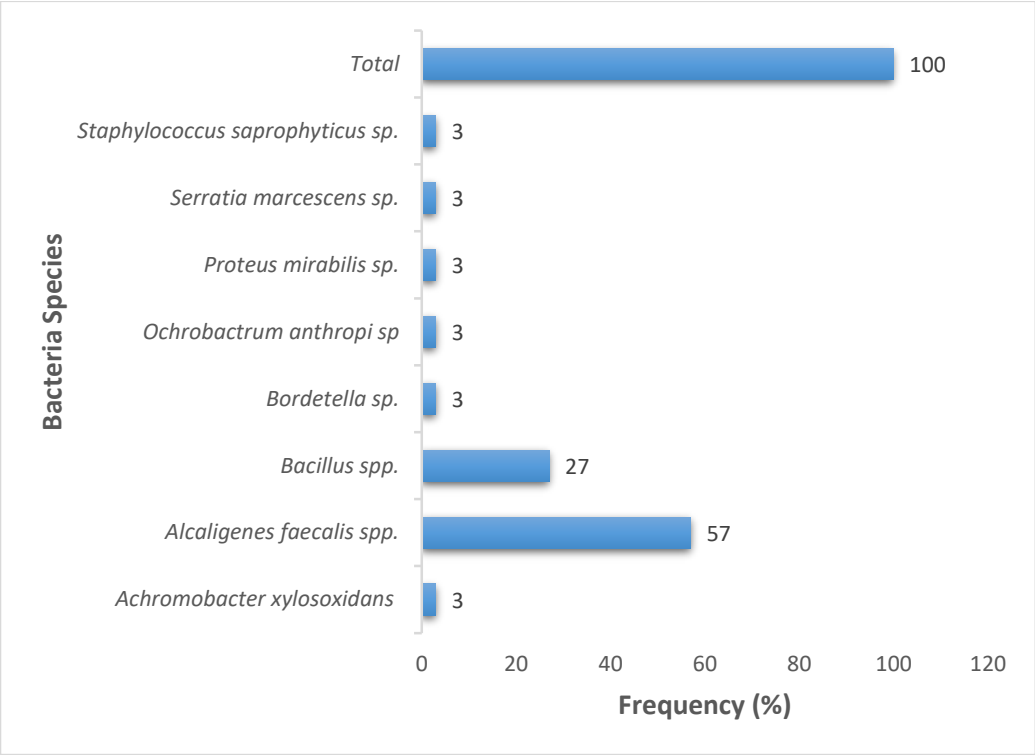


Figure 4.4: Prevalence of sequenced bacteria from boarding schools' FSEs

Table 4.27: Blast results for 16S rRNA sequences from bacteria from boarding schools' FSEs

Isolate Code	Source	School	Type species	Query length (Bp)	Similarity (%)	Accession No
PAM2	Amala	A	*Ochrobactrum anthropi* ICMM9	238	91.9	MN945430.1
TCHB2	Chopping Board	C	*Alcaligenes faecalis* strain GX19	404	99.47	KU937382.1
MVCHB3	Chopping Board	D	Alcaligenes faecalis strain GX28	442	99.3	KU937391.1
MCHB1	Chopping Board	D	*Bacillus cereus* strain S5	936	98.8	KU927490.1
MCHB2	Chopping Board	A	*Bacillus cereus* AJK3	933	98.59	MF187726.1
GCHB2	Chopping Board	B	*Bacillus* sp. Strain BA	434	95.38	KY379943.1
GBCHB	Chopping Board	B	*Alcaligenes faecalis* strain sihong_738_1	279	81.29	MN309919.1
MCW	Cooking water	A	*Bacillus cereus* strain ASU1	432	93.88	MG515188.1
MVCW2	Cooking water	D	Bacillus cereus strain K22	198	87.29	KF641833.1
MVCTT1	Counter top	D	*Proteus mirabilis* strain PS2	316	98.07	MT470980.1
TDNT2	Dining Table	C	*Alcaligenes faecalis* strain SH 15	326	99.67	KC!72022.1
TDNT	Dining Table	C	*Bacillus* sp XJZ123	404	97.93	KJ175238.1
TFS2	Fish Stew	C	*Bacillus cereus* strain Ht7-4	321	98.44	JF899283.1
MVFH2	Food Handler	D	*Serratia marcescens* strain NPKI_32	518	99.22	MN691641.1
MVFH1	Food Handler	D	*Alcaligenes faecalis* strain DST213	999	98.89	MH.793409.1
PFH4	Food Handler	A	*Alcaligenes faecalis* strain B26	405	97.47	KF641855.1
PFH1	Food Handler	A	*Alcaligenes faecalis* strain CD- 232	207	91.23	JQ724535.1
GFH2A	Food Handler	B	*Alcaligenes faecalis* strain dn3	131	90.36	MH393204.1

Table 4.27: Blast results for 16S rRNA sequences from bacteria from boarding schools' FSEs (Continued)

MVFH5	Food Handler	D	*Alcaligenes faecalis* strain CCZ1	145	86.92	KT868883.1
MVFH3	Food Handler	D	*Achromobacter xylosoxidans* strain CUMB AAJ 03	599	77.66	MN197738.1
PFEG2	Fried Egg	A	*Alcaligenes faecalis* strain sihong_749_2	445	99.27	MN309920.1
PFEG1	Fried Egg	A	*Alcaligenes faecalis* strain GX19	294	98.23	KU937382.1
PGRM1	Grinder	A	*Bacillus flexus* strain 0075	133	100	KP236211.1
MVGRM	Grinder	D	*Alcaligenes faecalis* sp A23	399	98.48	KT316401.1
MGRM	Grinder	D	*Bacillus cereu*s strain TO-11	932	97.93	MN330085.1
TGRM	Grinder	C	*Alcaligenes faecalis* strain SH15	255	96.75	KC172022.1
TGRM2	Grinder	C	*Bacillus cereus* sp. BC3	431	93.08	LT630453.1
PGRM	Grinder	A	*Alcaligenes faecalis* strain B11	360	96.33	KF641842.1
TKN	Knife	C	*Alcaligenes faecalis subsp faecalis* strain PK 13	282	100	KC790255.1
GKN2	Knife	B	*Alcaligenes faecalis subsp phenolicus* strain DHL 32	1427	98.89	MN833525.1
MKN2	Knife	D	*Alcaligenes faecalis* strain NIOER346	209	98.29	MG206054.1
PKN1	Knife	A	*Alcaligenes faecalis* strain TZQ4	212	96.04	HQ143627.1
GKN1	Knife	B	*Alcaligenes faecalis* strain SS-1	387	91.96	JN804562.1
MVSML	Semolina	D	*Alcaligenes faecalis* strain SH 100	365	95.75	KC172042.1
TTRY1	Tray	C	*Alcaligenes faecalis subsp faecalis* strain PK 13	357	98.3	KC790255.1
PTRY3	Tray	A	*Staphylococcus saprophyticus* strain CJ-5	233	94.64	HQ455044.1
TTRY2	Tray	C	*Bordetella* sp. AC3	204	85.23	EU043370.1

BLAST analysis categorised the 37 isolates into eight distinct genera listed below: *Alkaligenes faecalis* (21), *Achromobacter xylosoxidans* (1), *Bacillus species* (10), *Ochrobactrum anthropi* (1), *Proteus mirabilis* (1), *Serratia marcescens* (1), *Staphylococcus saprophyticus* (1), and *Bordetella sp.* (1). *Alcaligenes faecalis species* and *Bacillus species* were the most identified with 57% and 27%, respectively. All other organisms were all singly isolated from the schools' FSEs. The distribution of the sequenced isolates from schools' FSEs were (food handlers (n = 7); food contact surfaces (n = 23); RTE foods (n = 5) and cooking water (n = 2). The prevalent bacteria on food contact surfaces food handlers' hands, RTE foods and water were 62%, 19%, 14% and 5%, respectively as shown in Table 4.28.

A total of 34 distinct nucleotide sequences were analysed in this study. First, Second, Third, and Noncoding codon locations were covered. For each sequence pair, all unclear positions were eliminated (pairwise deletion option). There were a total of 1437 positions in the final dataset. All of the nucleotide sequences generated in this work can be found in GenBank.

The result of phylogenetic tree construction of the 37 sequences (with three organisms appearing twice) obtained from Genbank is given in Figure.4.5. The 34 unique bacterial sequences were placed in two main clades (upper Clade A and lower Clade B). Upper Clade A also consists of two subclades: First subclade is made up o*f Proteus mirabilis and Serratia marcescens* (from genus Enterobacteriaceae of class Gamma-proteobacteria) and *Ochrobactrum anthropi* which belong to class Alpha-proteobacteria, while the second subclade consists of *Alcaligenes species*, *Bordetella sp.* and *Achromobacter xyloxidans* (class Beta-proteobacteria). The lower Clade B consists of all *Bacillus species* and *Staphylococcus saprophyticus* (belonging to the phylum Firmicutes).

Table 4.28: Distribution of sequenced bacteria from boarding schools' FSEs

Organisms/Source	Frequency (%)			
	Alcaligenes faecalis	***Bacillus* spp.**	**Others**	**Total**
Food Handlers hands	14	0	5	19
Food contact surfaces	35	19	8	62
Ready-to-Eat foods	8	3	3	14
Water	0	5	0	5

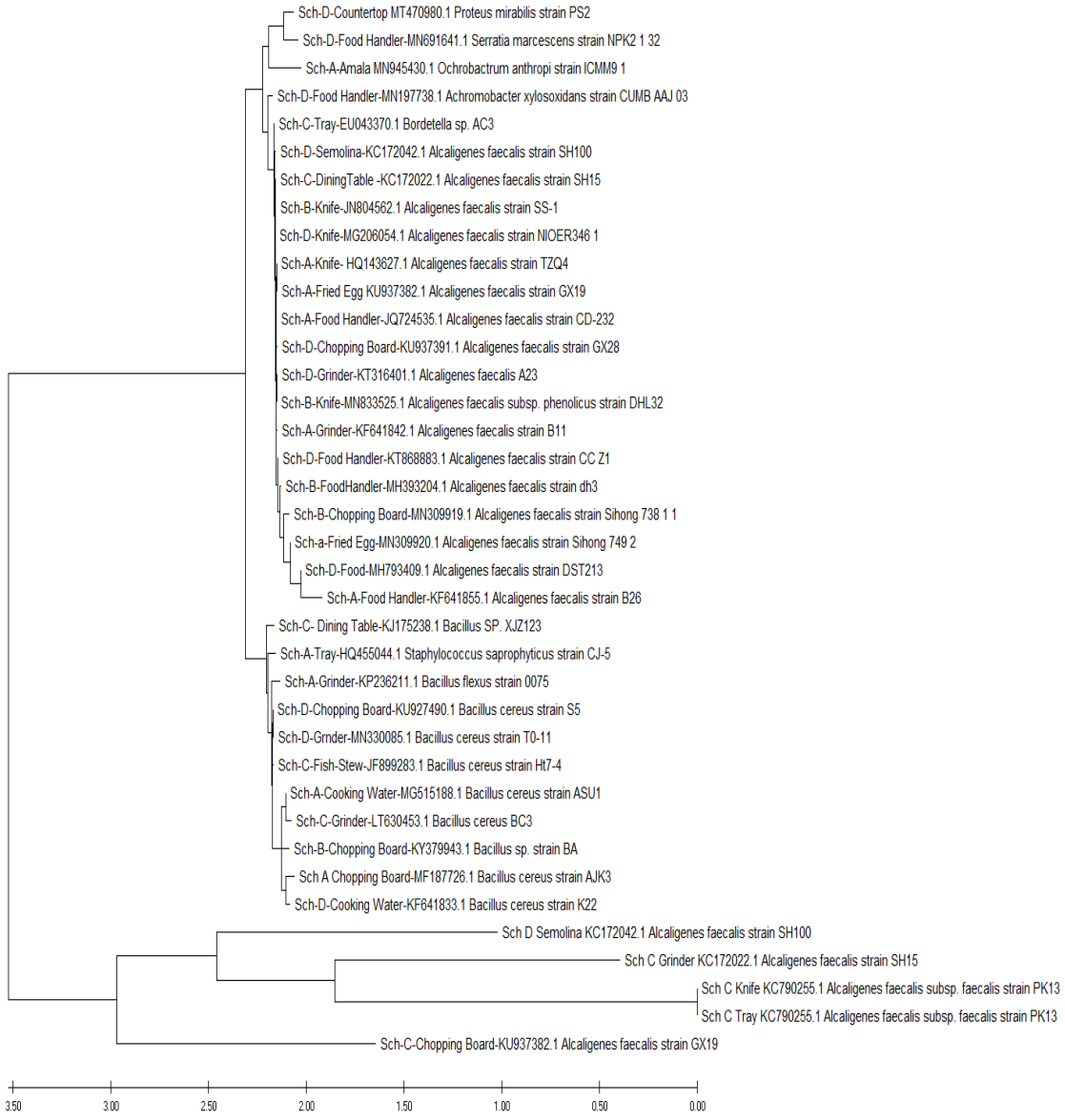


Figure 4.5: Phylogenetic structure of the bacteria isolated from Schools' FSEs

The tree is constructed using partial 16S rRNA gene sequences obtained through the neighbour joining method combined with the Maximum Composite Likelihood method (MEGA X software package). The species names are followed by the GenBank accession number. Node numbers are bootstrap values calculated using 1000 resamplings. 0.00 substitutions per site, bar.

The isolated bacteria are naturally found in soil, animal and the environment. They are referred to as opportunistic pathogens and have been linked to several human health problems. The phylogenetic tree showed that results of all the isolated species have significant sequence identities and were grouped into three main phylogenetic groups. (between 78% and 100%) with typed strains. Taxonomic analysis showed that the isolates were members of the phyla Proteobacteria (70%) and Firmicutes (30%).

Figure 4.6 shows the relationship between the *Alcaligenes faecalis* spp. isolated in this study in comparison with the sequences of other *Alcaligenes faecalis* spp. that have been deposited in the NCBI database. Figure 4.7 represents the evolutionary relationship between the Gram- positive (*Bacillus* species and *S. saprophyticus* sp.) isolated from this study with the nucleotide sequences of other related in the NCBI database. The last figure (Figure 4.8) is showing the relatedness among the other Gram-negative bacteria (Enterobacteriaceae) isolated from boarding schools' FSEs in this study.

The taxonomic identity of the isolates is summarised in Table 4.29, the majority of isolates (94 percent) belonged to the genera Alcaligenes (57 percent) and Bacillus (27 percent). The isolates were found in such a wide variety of sample sources across the four schools that there was no particular pattern of isolate prevalence in any tested material.

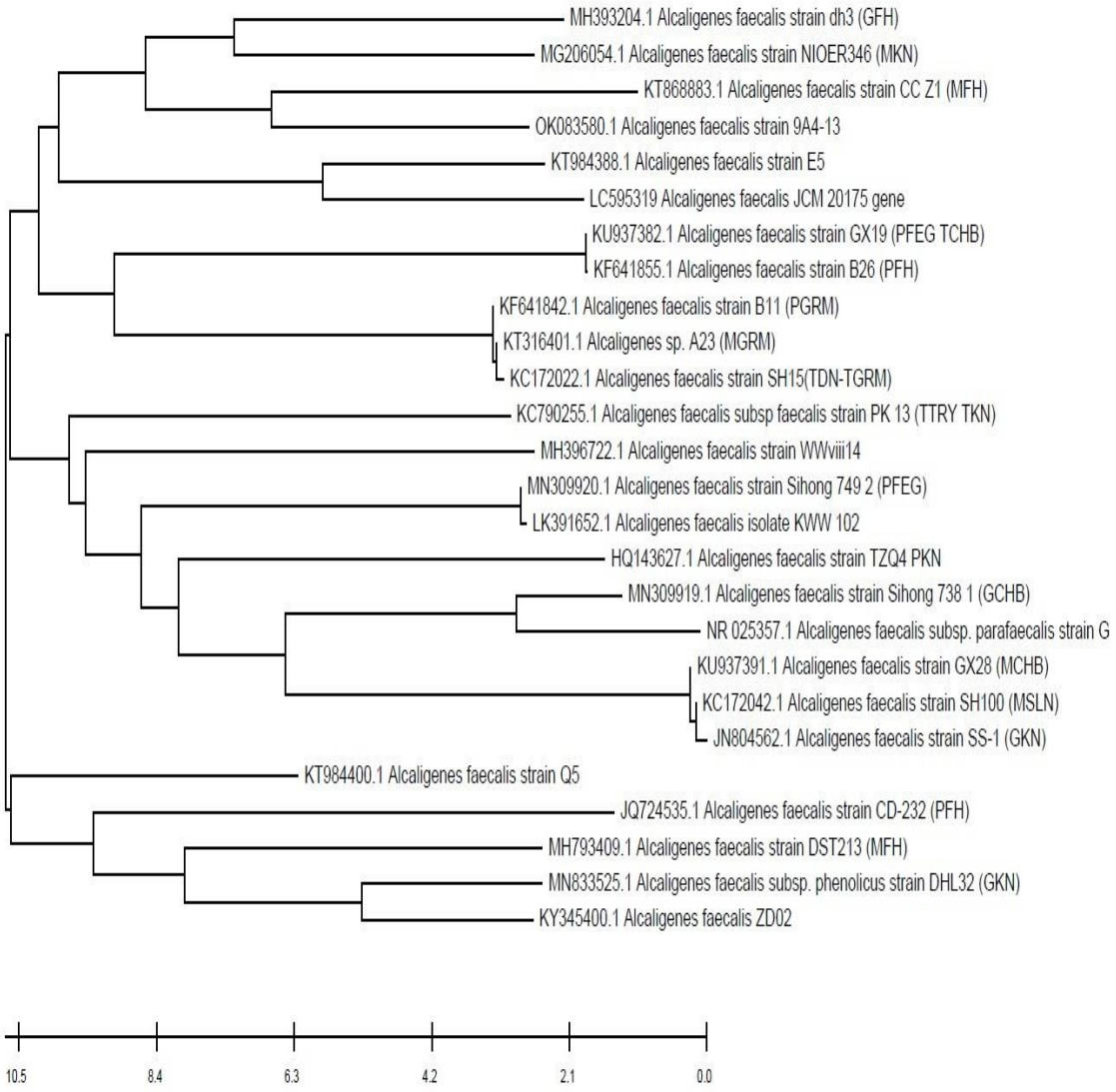


Figure 4.6: Phylogenetic tree showing the evolutionary relationship between the *Alcaligenes faecalis* spp. isolated from schools' FSEs and other sequences deposited in NCBI database

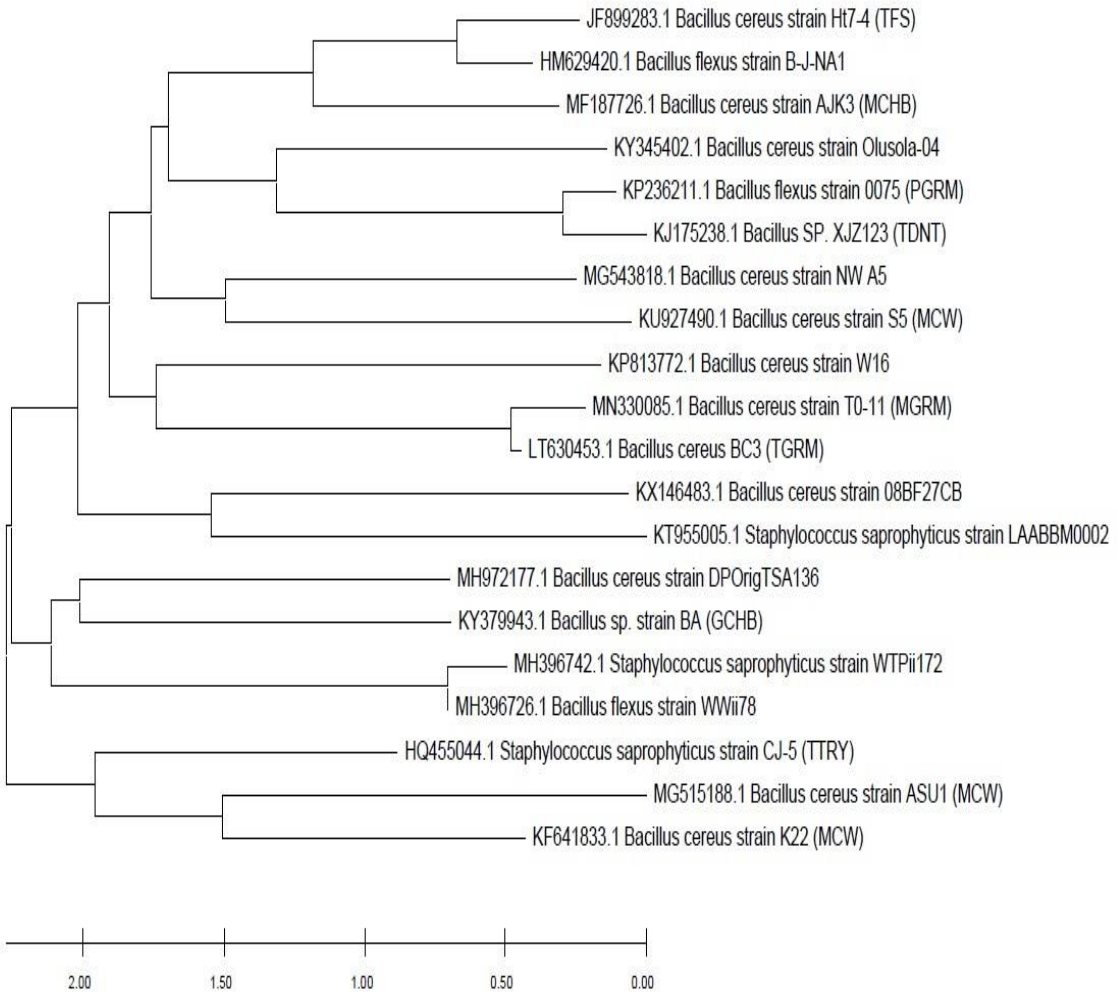


Figure 4.7: Phylogenetic tree showing the evolutionary relationship between the *Bacillus* spp. isolated from schools' FSEs and other sequences deposited in NCBI database

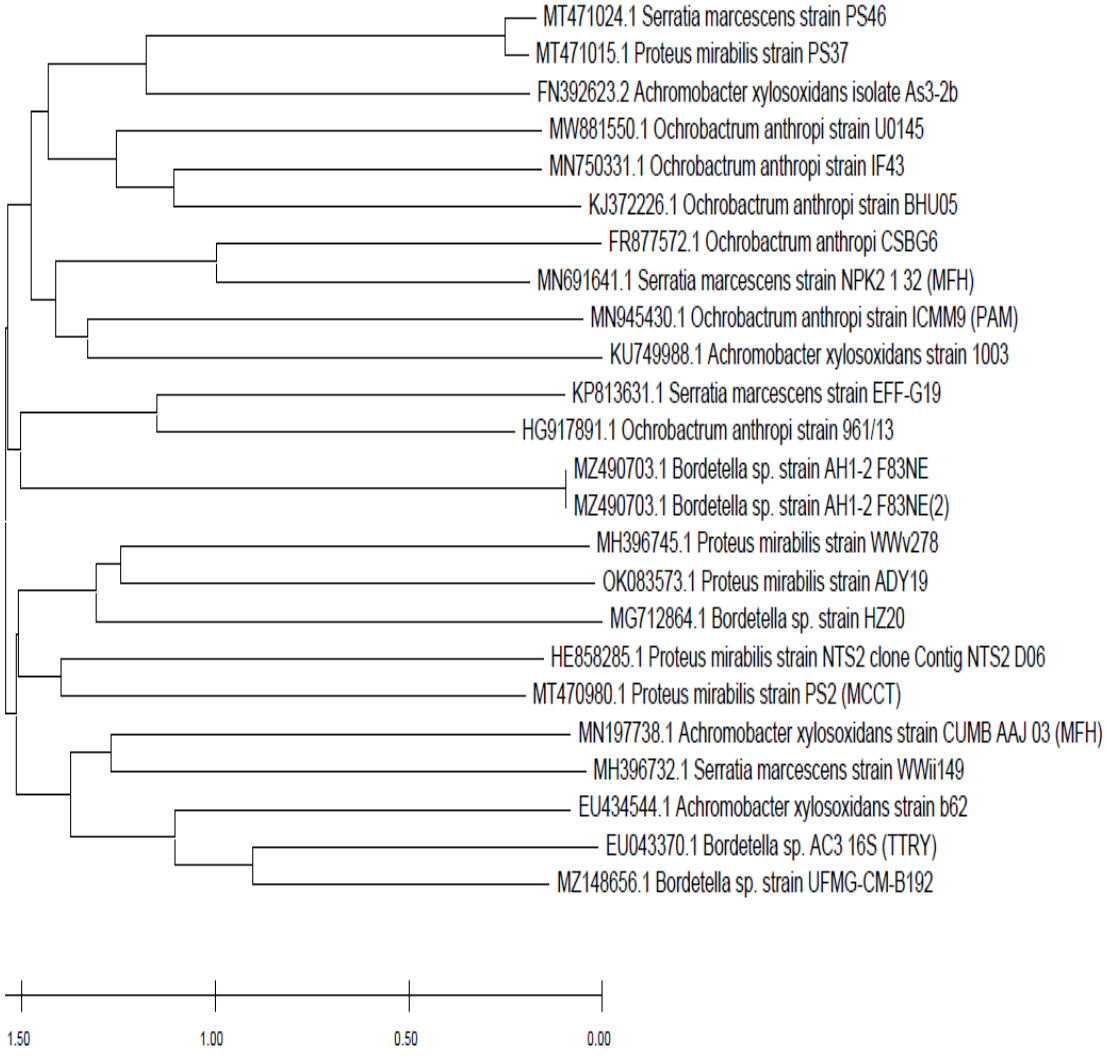


Figure 4.8: Phylogenetic tree showing the evolutionary relationship between other Gram-Negative bacteria isolated from schools' FSEs and other sequences deposited in NCBI database

Table 4.29: Phylogenetic identities of resistant bacteria from boarding schools' FSEs

Bacteria Classification	Best match by BLAST	Accession No	Per. Ident (%)	No of isolates	Source
∝-Proteobacteria	*Ochrobactrum anthropi* ICMM9	MN945430.1	91.9	1	Sch A (Amala)
β-Proteobateria	*Achromobacter xylosoxidans strain* CUMB AAJ 03	MN197738.1	77.66	1	Sch D (Food handler)
	Alcaligenes faecalis strain sihong_738_1	MN309919.1	81.29	1	Sch B (Chopping board)
	Alcaligenes faecalis sp A23	KT316401.1	98.48	1	Sch D(Grinder)
	Alcaligenes faecalis strain B11	KF641842.1	96.33	1	Sch A (Grinder)
	Alcaligenes faecalis strain B26	KF641855.1	97.47	1	Sch A(Food handler)
	Alcaligenes faecalis strain CCZ1	KT868883.1	86.92	1	Sch D(Food handler)
	Alcaligenes faecalis strain CD- 232	JQ724535.1	91.23	1	Sch A (Food handler)
	Alcaligenes faecalis strain dn3	MH393204.1	90.36	1	Sch B (Food handler)
	Alcaligenes faecalis strain DST213	MH793409.1	98.89	1	Sch D (Food handler)
	Alcaligenes faecalis strain GX19	KU937382.1	99.47	2	Sch A (Fried egg); Sch C (Chopping board)
	Alcaligenes faecalis strain GX28	KU937391.1	99.3	1	Sch D (Choping board)
	Alcaligenes faecalis strain NIOER346	MG206054.1	98.29	1	Sch D(Knife)
	Alcaligenes faecalis strain SH 100	KC172042.1	95.75	1	Sch D(Semolina)
	Alcaligenes faecalis strain SH 15	KC172022.1	99.67	2	Sch C (Dining table); Sch C (Grinder)
	Alcaligenes faecalis strain sihong_749_2	MN309920.1	99.27	1	Sch A (Fried egg)
	Alcaligenes faecalis strain SS-1	JN804562.1	91.96	1	Sch B (Knife)

Table 4.29: Phylogenetic identities of resistant bacteria from boarding schools' FSEs (Continued)

	Alcaligenes faecalis strain TZQ4	HQ143627.1	96.04	1	Sch A (Knife)
	Alcaligenes faecalis subsp faecalis strain PK 13	KC790255.1	100	2	Sch C (Knife);Sch C (Tray)
	Alcaligenes faecalis subsp phenolicus strain DHL 32	MN833525.1	98.89	1	Sch B (Knife)
	Bordetella sp. AC3	EU043370.1	85.23	1	Sch C(Tray)
γ-Proteobacteria	*Proteus mirabilis strain* PS2	MT470980.1	98.07	1	Sch D (Countertop)
	Serratia marcescens strain NPKI_32	MN691641.1	99.22	1	Sch D (Food handler)
Firmicutes	*Bacillus cereus* AJK3	LT630453.1	98.59	1	Sch D (Chopping board)
	Bacillus cereus BC3	MF187726.1	93.08	1	Sch C (Grinder)
	Bacillus cereus strain ASU1	MG515188.1	93.88	1	Sch D (Cooking water)
	Bacillus cereus strain Ht7-4	JF899283.1	98.44	1	Sch C (Fish stew)
	Bacillus cereus strain K22	KF641833.1	87.29	1	Sch D (Cooking water 2)
	Bacillus cereus strain S5	KU927490.1	98.8	1	Sch D (Chopping board)
	Bacillus cereus strain TO-11	MN330085.1	97.93	1	Sch D (Grinder)
	Bacillus sp. XJZ123	KJ175238.1	97.93	1	Sch C (Dining table)
	Bacillus sp.*strain* BA	KY379943.1	95.38	1	Sch B (Chopping board)
	Bacillus flexus strain 0075	KP236211.1	100	1	Sch A (Grinder)
	Staphylococcus saprophyticus strain CJ-5	HQ455044.1	94.64	1	Sch A (Tray)

4.12 Antibiotic susceptibility by disc diffusion of genotypic bacteria

The antibiotic resistance profile with disc diffusion assay is presented in Table 4.30 and Table 4.31. The species *Alcaligenes faecali*s represented the most prevalent bacteria in this study. *Alcaligenes faecalis* showed resistance to cefixime (76.2%), cefuroxime (71.4%), ceftazidime (66.7%), gentamicin (61.9%), augmentin (57.1%), nitrofurantoin (42.9%) and ofloxacin and ciprofloxacin (4.8%) each, respectively. Almost all *A. faecalis* isolates were susceptible to ciprofloxacin and ofloxacin except *Alcaligenes faecalis* PKNI and *A. faecalis* CDNT. More than half of *Alcaligenes faecalis* isolated were resistant to at least four tested antibiotics with cephalosporins showing the highest resistance. The high resistance of *A. faecalis* to tested antibiotics may be as a result of antibiotic misuse in human, animal and the environment.

Bacillus cereus species showed resistance to cefuroxime (100%), cefixime and gentamicin (85.7%) each, ceftazidime and nitofurantoin (57.1%) each and augmentin (28.6%), but totally (100%) sensitive to ofloxacin and ciprofloxacin. The single isolate of *Bacillus flexus* was 100% sensitive to augmentin and ofloxacin but completely resistant to ceftazidime, cefuroxime and gentamicin. *Staphylococcus saprophyticus* was resistant to augmentin and cefuroxime while being susceptible to other tested antibiotics. All other singly isolated NF-GN bacteria (*Achromobacter xylosoxidans, Bordetella spp*, and *Ochrobactrum anthropi*) did not show a particular trend in resistance pattern but were resistant to cephalosporins (cefuroxime and cefixime) and sulphonamide (nitrofurantoin). The overall antibiotic resistance pattern of identified bacteria by the disc diffusion method is given in Figure 4.9. High level of antibiotic resistance recorded were cefuroxime (83.8%), cefixime (75.7%), and gentamicin (67.6%), respectively, while the low resistance of 2.7% was exhibited on ofloxacin and ciprofloxacin (both of which are fluoroquinolones). All identified bacterial showed resistance of 56.8% 48.7% and 45.9% to ceftazidime, augmentin, and nitrofurantoin, respectively. The resistance of Alcaligenes ranged betwwen 42.9 % and 76.2% except for the fluoroquinolones with 4.8% ciprofloxacin and ofloxacin 0% respectively. The resistance of *A. faecalis* among the antibiotics was relatively similar except with the fluoroquinolones. *Bacillus cereus* isolates showed higher resistance to tested antibiotics compared with *A. feacalis* (Figure 4.10)

Table 4.30: Antibiotic resistant pattern of sequenced bacteria from boarding schools' FSEs

Organisms	AUG	CAZ	CRX	GEN	OFL	CXM	CPR	NIT	CXC	CTR	ERY	ABR (N)
Alcaligenes faecalis strain sihong_738_1 (BCHB)	S	R	R	R	S	NA	NA	NA	R	R	R	6
Alcaligenes faecalis sp A23 (DGRM)	R	R	R	R	S	R	S	R	NA	NA	NA	6
Alcaligenes faecalis strain B11 (AGRM)	R	R	R	R	S	R	S	S	NA	NA	NA	5
Alcaligenes faecalis strain B26 (AFH)	R	R	S	S	S	R	S	S	NA	NA	NA	3
Alcaligenes faecalis strain CCZ1 (DFH)	R	R	R	R	S	R	S	R	NA	NA	NA	6
Alcaligenes faecalis strain CD- 232 (AFH)	R	R	S	S	S	R	S	R	NA	NA	NA	4
Alcaligenes faecalis strain dn3 (BFH)	S	R	S	R	S	NA	NA	NA	R	R	R	5
Alcaligenes faecalis strain DST213 (DFH)	S	S	R	S	S	S	S	R	NA	NA	NA	2
Alcaligenes faecalis strain GX19A (CCHB)	S	R	S	S	S	NA	NA	NA	S	R	R	3
Alcaligenes faecalis strain GX19B (AFEG)	R	S	S	S	S	NA	NA	NA	R	S	R	3
Alcaligenes faecalis strain GX28 (DCHB)	R	R	R	R	S	R	S	R	NA	NA	NA	6
Alcaligenes faecalis strain NIOER346 (DKN)	S	S	R	R	S	R	S	R	NA	NA	NA	4
Alcaligenes faecalis strain SH 100 (DSM)	R	S	R	S	S	R	S	S	NA	NA	NA	3

Table 4.30: Antibiotic resistant pattern of sequenced bacteria from boarding schools' FSEs (Continued)

Organisms	AUG	CAZ	CRX	GEN	OFL	CXM	CPR	NIT	CXC	CTR	ERY	ABR (N)
Alcaligenes faecalis strain SH 15B (CDNT2)	S	S	R	S	S	R	R	R	NA	NA	NA	4
Alcaligenes faecalis strain SH15A (CGRM)	R	R	R	R	S	R	S	S	NA	NA	NA	5
Alcaligenes faecalis strain sihong_749_2 (AFEG2)	S	S	R	R	S	R	S	S	NA	NA	NA	3
Alcaligenes faecalis strain SS-1 (BKN)	S	R	S	S	S	R	S	S	NA	NA	NA	2
Alcaligenes faecalis strain TZQ4 (AKN)	R	R	R	R	R	R	S	R	NA	NA	NA	7
Alcaligenes faecalis subsp faecalis strain PK 13A (CTRY)	R	R	R	R	S	R	S	R	NA	NA	NA	6
Alcaligenes faecalis subsp faecalis strain PK 13B (CKN)	R	R	R	R	S	R	S	S	NA	NA	NA	5
Alcaligenes faecalis subsp phenolicus strain DHL 32 (BKN2)	S	S	R	R	S	R	S	S	NA	NA	NA	3
Resistance (%)	**12(57)**	**14 (67)**	**15(71)**	**13(62)**	**1(4.8)**	**16(77)**	**1(4.8)**	**9(43)**				
Bacillus cereus AJK3 (DCHB2)	S	S	R	R	S	R	S	S	NA	NA	NA	3
Bacillus cereus BC3 (CGRM)	R	S	R	R	S	R	S	R	NA	NA	NA	5
Bacillus cereus strain ASU1 (DCW)	S	S	R	R	S	R	S	R	NA	NA	NA	4
Bacillus cereus strain Ht7-4 (CFS)	S	R	R	R	S	R	S	R	NA	NA	NA	5

Table 4.30: Antibiotic resistant pattern of sequenced bacteria from boarding schools' FSEs (Continued)

Organisms	AUG	CAZ	CRX	GEN	OFL	CXM	CPR	NIT	CXC	CTR	ERY	ABR (N)
Bacillus cereus strain K22 (DCW2)	S	R	R	R	S	S	S	R	NA	NA	NA	4
Bacillus cereus strain S5 (DCHB)	R	R	R	S	S	R	S	S	NA	NA	NA	4
Bacillus cereus strain TO-11 (DGRM)	S	R	R	R	S	R	S	S	NA	NA	NA	4
Resistance (%)	**2 (29)**	**4(57)**	**7 (100)**	**6 (86)**	**0 (0)**	**6 (86)**	**0 (0)**	**4 (57)**	**NA**	**NA**	**NA**	
Other Bacteria												
Achromobacter xylosoxidans strain CUMB AAJ 03 (DFH)	R	S	R	R	S	R	S	R	NA	NA	NA	5
	100	0	100	100	0	100	0	100	NA	NA	NA	
Bacillus flexus strain 0075 (AGRM)	S	R	R	R	S	NA	NA	NA	R	R	R	6
	0	100	100	100	0	NA	NA	NA	100	100	100	
Bacillus sp. XJZ123 (CDNT)	R	R	R	R	S	R	S	S	NA	NA	NA	5
	100	100	100	100	0	100	0	0				
Bacillus sp. Strain BA (BCHB)	S	S	R	R	S	R	S	S	NA	NA	NA	3
	0	0	100	100	0	100	0	0				
Bordetella sp AC3 (CTRY)	S	S	R	R	S	R	S	S	NA	NA	NA	3
	0	0	100	100	0	100	0	0				
Ochrobactrum anthropi ICMM9 (AAM)	S	S	R	S	S	R	S	R	NA	NA	NA	3
	0	0	100	0	0	100	0	100				
Proteus mirabilis strain PS2 (DCTT)	R	S	R	S	S	S	S	R	NA	NA	NA	3
	100	0	100	0	0	0	0	100				
Serratia marcescens strainNPKI_32 (DFH)	S	R	R	R	S	R	S	R	NA	NA	NA	5
	0	100	100	100	0	100	0	100				

Table 4.30: Antibiotic resistant pattern of sequenced bacteria from boarding schools' FSEs (Continued)

Organisms	**AUG**	**CAZ**	**CRX**	**GEN**	**OFL**	**CXM**	**CPR**	**NIT**	**CXC**	**CTR**	**ERY**	**ABR (N)**
Staphylococcus saprophyticus strain CJ-5 (ATRY)	R	S	R	S	S	S	S	S	NA	NA	NA	2
	100	0	100	0	0	0	0	0				
Overall Resistance N (%)	**18 (49)**	**21 (57)**	**31 (84)**	**25 (68)**	**1 (3)**	**28 (76)**	**1 (3)**	**17 (46)**	**4 (11)**	**4 (11)**	**5 (14)**	

Key: Augmentin-**AUG**; Ceftazidime- **CAZ;** Cefuroxime-**CRX**; Gentamicin –**GEN**; Ofloxacin- **OFL**; Cefixime- **CXM**; Ciprofloxacin-**CPR**; Nitrofurantoin –**NIT**; Cloxacillin- **CXC**; Ceftriaxone –**CTR**; Erythromycin –**ERY**; Resistance-**R**; Sensitive-**S**; Not Available- **NA; ABR-** Antibiotic Resistance

Table 4.31: Overall antibiotic resistance profile of identified bacteria from boarding schools' FSEs

Antibiotics	*Achromobacter xylosoxidans* (n=1, n=21, n(%)	*Alcaligenes faecalis* (n=21, n(%)	*Bacillus cereus* (n=7, n(%)	*Bacillus flexus* (n=1, n(%)	*Bacillus spp* (n=2, n(%)	*Bordetella sp* (n=1, n(%)	*Ochrobactrum anthropi* (n=1, n(%)	*Proteus mirabilis* (n=1, n(%)	*Serratia marcescens* (n=1, n(%))	*Staphylococcus saprophyticus* (n=1,	Overall Resistance (n= 37, n(%)
Augmentin	1(100)	12(57.1)	2(28.6)	0(0)	1(50)	0	0	1(100)	0	1(100)	18(48.7)
Ceftazidime	0 (0)	14(66.7)	4(57.1)	1(100)	1(50)	0	0	0	1(100)	0	21(56.8)
Cefuroxime	1(100)	15(71.4)	7(100)	1(100)	2(100)	1(100)	1(100)	1(100)	1(100)	1(100)	31(83.8)
Gentamicin	1(100)	13(61.9)	6(85.7)	1(100)	2(100)	1(100)	0	0	1(100)	0	25(67.6)
Ofloxacin	0 (0)	1(4.8)	0	0(0)	0	0	0	0	0	0	1(2.7)
Cefixime	1(100)	16(76.2)	6(85.7)	NA	2(100)	1(100)	1(100)	0	1(100)	0	28(75.7)
Ciprofloxacin	0(0)	1(4.8)	0(0)	NA	0	0	0	0	0	0	1(2.7)
Nitrofurantoin	1(100)	9(42.9)	4(57.1)	NA	0	0	1(100)	1(100)	1(100)	0	17(45.9)

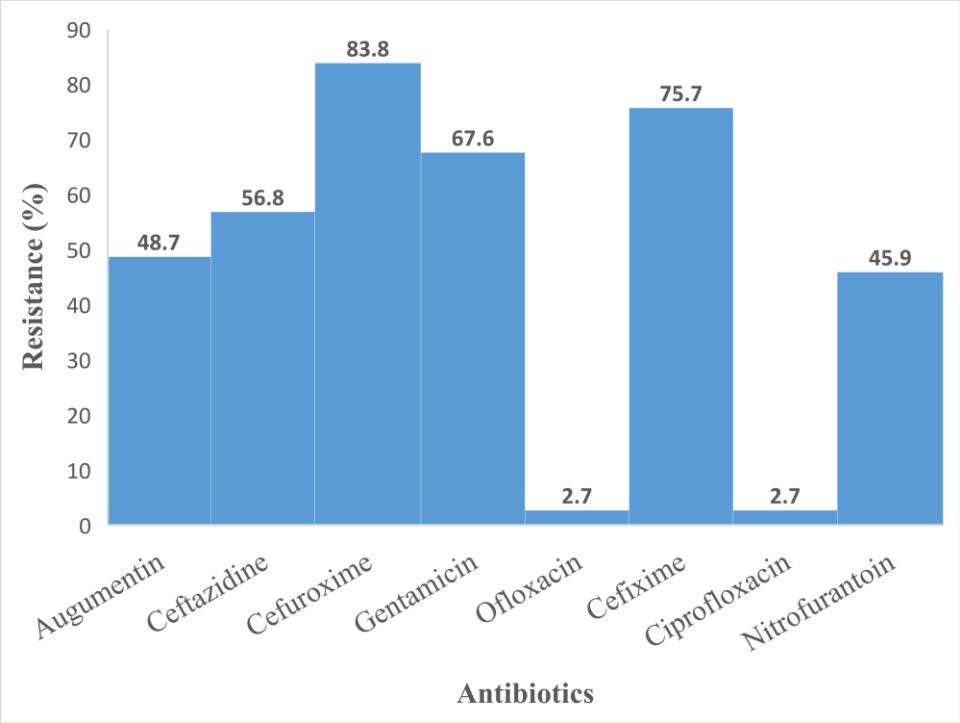


Figure 4.9: Overall antibiotic resistant pattern of identified bacteria by Disc Diffusion Method

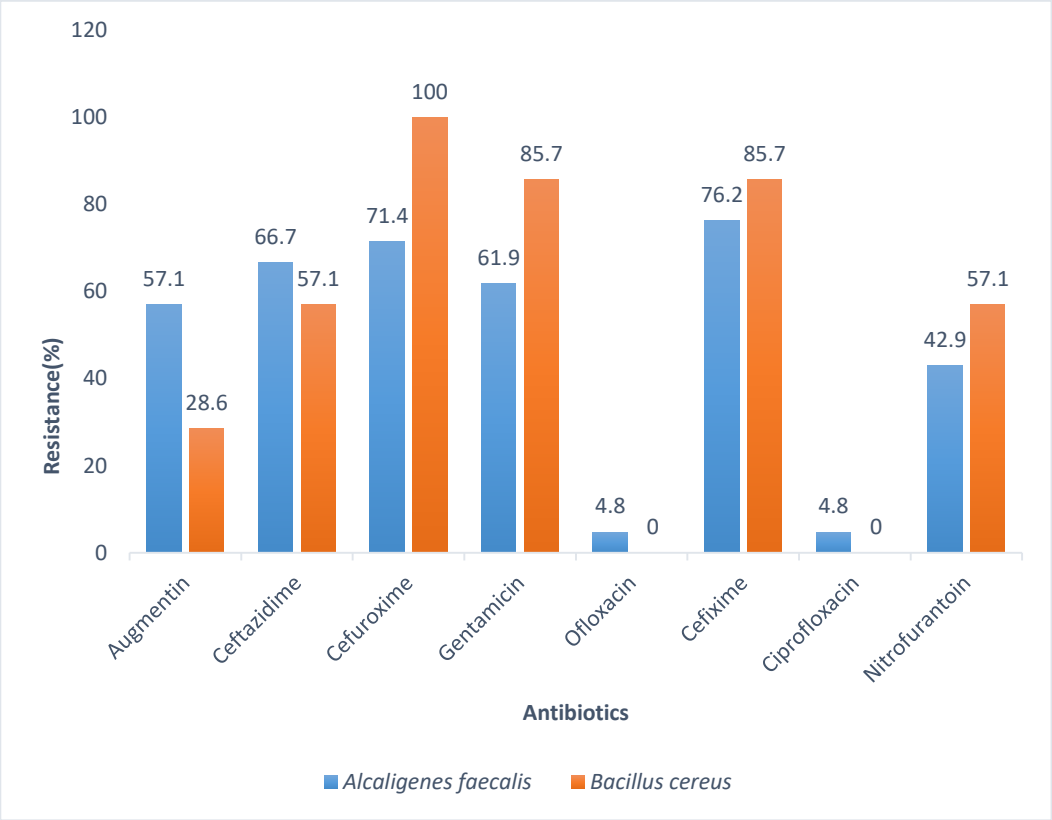


Figure 4.10: **Percentage resistance of *Alcaligenes faecalis* and *Bacillus cereus* to selected antibiotics**

4.13 Minimum inhibitory Concentration (MIC) by E-strip Method

Thirty selected bacterial isolates were tested for antibiotic susceptibility using the Minimum Inhibitory Concentration (MIC) method by E-strip technique. The EUCAST breakpoints were used to interpret the MIC values (See Table 4.32). MIC for *Alcalignenes faecalis* were interpreted to the EUCAST breakpoint for *Pseudomonas aeruginosa* spp. as no breakpoint was defined for *Alcaligenes faecalis* (Filipe *et al.*, 2017).The antibiotic resistance profile with MIC test for *Alcaligenes faecalis* is presented in Table 4.33. The concentration of ceftriaxone ranged between 0.016 µg/mL and 32 µg/mL with only four (25%) showing resistance at >24 µg/mL. All *A. faecalis* isolates were found to be susceptible to piperacillin/tazobactam and imipenem (100%) at concentrations between 0.016 µg/mL and 3µg/mL; 0.016 µg/mL and 2 µg/mL, respectively. More than half 9 (56.3%) of *Alcaligenes faecalis* were resistant to colistin. The resistance to colistin occurred among *Alcaligenes faecalis* PFH1, *Alcaligenes faecalis* PFH4, *Alcaligenes faecalis* MKN2, *Alcaligenes faecalis* PKN, *Alcaligenes faecalis* GKN2 and *Alcaligenes faecalis* FH2A (>256 µg/mL); *Alcaligenes faecalis* TGRM (24 µg/mL) and *Alcaligenes faecalis* PFEG (3 µg/mL).

Table 4.34 showed the resistance pattern of identified *Bacillus* species. Two isolates *Bacillus cereus* MCW and *Bacillus cereus* TGRM showed resistance to imipenem (> 0.5 µg/mL), while *Bacillus cereus* MGRM was resistant to ceftriaxone at 64 µg/mL. All *Bacillus* species were susceptible to piperacillin/tazobactam, but showed 100% resistance to colistin between 3µg/mL and > 256 µg/mL.

Among the singly isolated bacteria, *Bordetella* sp. MTRY was resistant to ceftriaxone, imipenem and colistin. *Proteus mirabilis* MVFH1 and *Serratia marcescens* MVCTT1 showed resistance to colistin. *Ochrobactrum anthropi* PAM2 and *Staphylococcus saprophyticus* PTRY3 were susceptible to all four tested antibiotics (Table 4.35). The summary of the antibiotic resistance of identified bacterial by MIC technique is given in Table 4.36. Antibiotic resistant profile of *Alcaligenes faecalis* species and *Bacillus cereus* species is summarised in Figure 4.11. The images of selected bacteria species showing antibiotic susceptability by MIC technique on agar plates are shown in Plates 4.1 and 4.2. Eighty-one percent of the bacteria were multi-antibiotic resistance and showed resistance to at least three classes of antibiotics (Table. 4.37).

Table 4.32: EUCAST MIC Breakpoints

Bacteria isolated	Reference organism	MIC (µg/mL)							
		CRO		IMI		CS		TZP	
		≤ S	> R	≤ S	> R	≤ S	> R	≤ S	> R
Alcaligenes faecalis spp.	*Pseudomonas aeruginosa*	-	-	0.001	4	2	2	0.001	16
Bacillus species/ Staphylococcus saprophyticus	*Bacillus* spp.	-	8	0.5	0.5	NA	NA	-	8
Serratia marcescens	Enterobacteriaceae	1	2	2	4	2	2	8	16
Proteus mirabilis	Enterobacteriaceae	1	2	2	4	2	2	8	16
Bordetella sp.	PK/PD	1	2	2	8	IE	IE	4	16
Ochromobacter anthropi	PK/PD	1	2	2	4	IE	IE	4	16

Source: www.eucast.org

EUCAST: The European Committee on Antimicrobial Susceptibility Testing

Key: CTR; Ceftriaxone; IMI: Imipenem; CS: Colistin; TZP: Piperacillin/Tozobactam; PK/PD: Pharmacokinetics/ Pharmacodynamics for non-species related breakpoint; ≤S: Sensitive, >R: Resistant; NA: Not Available; IE: Insufficient Evidence

Table 4.33: Antibiotic resistance pattern of *Alcaligenes faecalis* spp. from boarding schools' FSEs by MIC (µg/mL)

Isolate Code	CRO	S	R	IMI	S	R	CS	S	R	TZP	S	R
MVGRM	24	-	R	0.75	S	-	1.5	S	-	0.5	S	-
PGRM	2	S	-	0.032	S	-	2	S	-	2	S	-
PFH4	32	-	R	0.023	S	-	>256	-	R	1	S	-
PFH1A	0.19	S	-	3	S	-	>256	-	R	0.38	S	-
GFH2A	8	S	-	0.032	S	-	12	-	R	6	S	-
MVFHC1	0.19	S	-	0.38	S	-	1.5	S	-	0.5	S	-
PFEG1	0.125	S	-	0.75	S	-	3	-	R	0.19	S	-
MVCHB3	0.25	S	-	0.5	S	-	1.5	S	-	1	S	-
MKN2	32	-	R	0.32	S	-	>256	-	R	0.38	S	-
MVSML	32	-	R	0.016	S	-	>256	-	R	0.19	S	-
TDNT2	0.75	S	-	0.38	S	-	1.5	S	-	0.5	S	-
TGRM	0.032	S	-	0.016	S	-	24	-	R	0.016	S	-
PFEG2	0.125	S	-	0.5	S	-	1.5	S	-	1.5	S	-
PKN1	0.016	S	-	0.75	S	-	>256	-	R	0.25	S	-
TKN	0.5	S	-	0.016	S	-	0.016	S	-	0.19	S	-
GKN2	16	S	-	0.016	S	-	>256		R	0.19	S	-
Resistance (%) n=16		12 (75)	4 (25)		16 (100)	0 (0)		7 (44)	9 (56)		16 (100)	0 (0)

Key: CTR; Ceftriaxone; IMI: Imipenem; CS: Colistin; TZP: Piperacillin/Tozobactam ≤S: Sensitive >R: Resistant

Table 4.34: Antibiotic resistant pattern of identified *Bacillus* spp. from boarding schools' FSEs MIC (µg/mL)

Isolate Code	CRO	S	R	IMI	S	R	CS	S	R	TZP	S	R
MCHB2	0.19	S	-	0.38	S	-	4	-	R	0.25	S	-
TGRM	0.016	S	-	0.75	-	R	>256	-	R	0.125	S	-
MCW	0.016	S	-	4		R	>256	-	R	0.19	S	-
TFS2	0.016	S	-	0.5	S	-	>256	-	R	0.19	S	-
MCHB1	0.19	S	-	0.38	S	-	4	-	R	0.38	S	-
MGRM	64	S	R	0.023	S	-	>256	-	R	0.38	S	-
PGRM1	0.75	S	-	0.5	S	-	3	-	R	0.5	S	-
TDNT	1	S	-	0.023	S	-	32	-	R	0.38	S	-
GCHB2	0.094	S	-	0.38	S	-	4	-	R	0.25	S	-
Resistance (%)	n=9	8 (89)	1(11)		7 (78)	2(22)		0 (0)	9 (100)		9 (100)	0 (0)

Key: CTR; Ceftriaxone; IMI: Imipenem; CS: Colistin; TZP: Piperacillin/Tozobactam, ≤S: Sensitive, >R: Resistant

Table 4.35: Antibiotic resistant pattern of singly identified bacteria isolates from boarding schools' FSEs (µg/mL)

Bacteria Identity	CRO	S	R	IMI	S	R	CS	S	R	TZP	S	R
Bordetella sp. MTTRY	6	R	R	0.023	S	-	3	-	R	1	S	-
Ochrobactrum anthropi PAM2	0.125	S	-	0.38	S	-	2	S	S	0.19	S	-
Proteus mirabilis MVCTT1	0.38	S	-	0.38	S	-	6	-	R	6	S	-
Serratia marcescens MVFFHC1	0.094	S	-	0.016	S	-	16	-	R	0.38	S	-
Staphylococcus saprophyticus PTRY3	0.19	S	-	0.5	S	-	2	S	S	0.5	S	-
Resistance (%) n=5		4 (80)	1 (20)		5 (100)	0 (0)		2(40)	3(60)		5(100)	0(0)

Key: CTR; Ceftriaxone; IMI: Imipenem; CS: Colistin; TZP: Piperacillin/Tozobactam, ≤S: Sensitive, >R: Resistant

Table 4.36: Antibiotic resistance profile of identified bacteria by MIC Method

Organism/ Antibiotics	Ceftriaxone	Imipenem	Colistin	Piperacillin/ Tazobactam
***Alcaligenes faecalis* (n=16, n(%)**	4(25.0)	0	9(56.3)	0
***Bacillus cereus* (n=6, n(%)**	1(16.7)	2(33.3)	6(100.0)	0
***Bacillus flexus* (n=1, n(%)**	0	0	1(100)	0
***Bacillus spp* (n=2, n(%)**	0	0	2 (100)	0
***Bordetella sp*. (n=1, n(%)**	1(100)	0	0	0
***Ochrobactrum anthropi* (n=1, n(%)**	0	0	1(100)	0
***Proteus mirabilis* (n=1, n(%)**	0	0	0	0
***Serratia marcescens* (n=1, n(%)**	0	0	1(100)	0
***Staphylococcus saprophyticus* (n=1, n(%)**	0	0	1(100)	0
Overall Resistance (n= 30, n(%)	6(20)	2(6.6)	21(70)	0

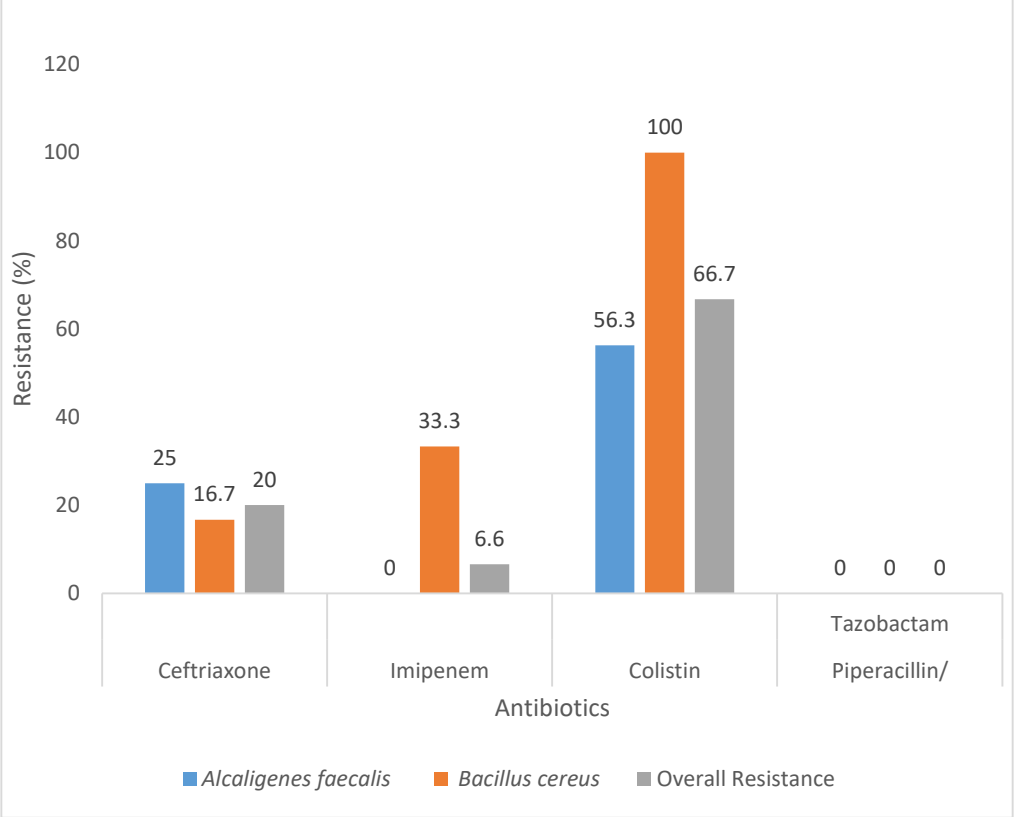
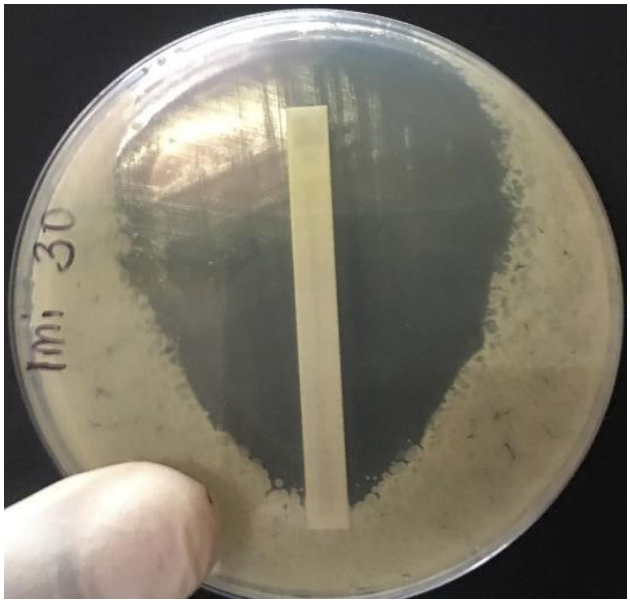
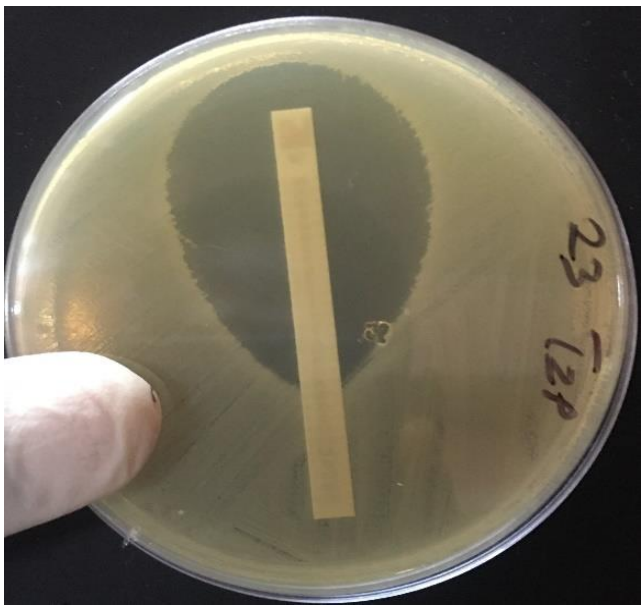


Figure 4.11: Antibiotic resistant profile of prevalent bacteria by MIC



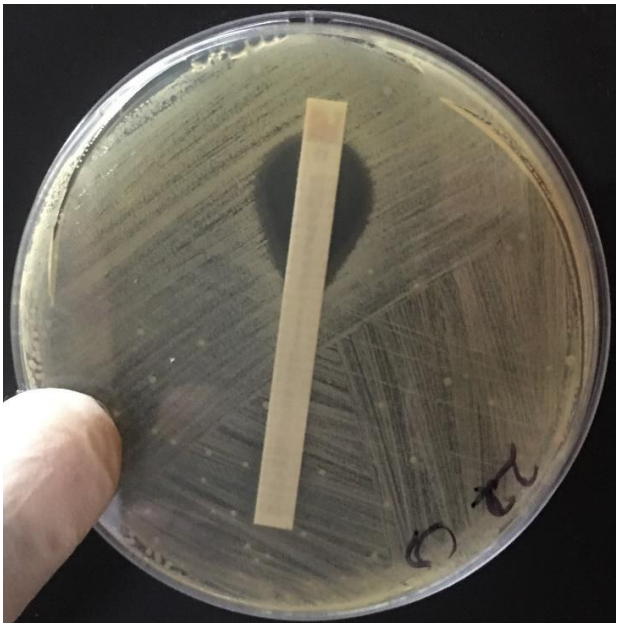
Bacillus sp. XJZ123 on Imipenem (0.023mg/L)



A*lcaligenes faecalis* sp. A23 on Piperacillin/Tozobactam (0.5mg/L)

Plate 4.1: Agar plates showing bacteria susceptibility to Imipenem and Piperacillin/Tozobactam

Alcaligenes.faecalis on Colistin >256mg/L



Alcaligenes faecalis on colistin (1.5mg/L)

Plate 4.2: Agar plates showing Alcaligenes faecalis susceptibility to Colistin

Table 4.37: Multi-antibiotic resistant profile of identified bacteria from boarding schools' FSEs

Identified Bacteria	Antibiotic Classes							MAR
Alcaligenes faecalis strain TZQ4 (PKN)	Ceph	Col	Pen	Fluo	Sul	Amy	-	6
Bacillus cereus BC3 (TGRM)	Ceph	Col	Pen	Fluo	Sul	-	-	5
Bacillus cereus strain ASU1 (MCW)	Ceph	Col	-	Fluo	Sul	-	Cab	5
Alcaligenes faecalis sp A23 (MGRM)	Ceph	-	Pen	-	Sul	Amy	-	4
Alcaligenes faecalis strain CD- 232 (PFH)	Ceph	Col	Pen	-	Sul	-	-	4
Alcaligenes faecalis strain GX28 (MCHB)	Ceph	-	Pen	-	Sul	Amy	-	4
Alcaligenes faecalis strain NIOER346 (MKN)	Ceph	Col	-	-	Sul	Amy	-	4
Alcaligenes faecalis strain SH15A (TGRM)	Ceph	Col	Pen	-	-	Amy	-	4
Alcaligenes faecalis subsp faecalis strain PK 13B (TKN)	Ceph	-	Pen	-	-	Amy	-	4
Bacillus cereus strain Ht7-4 (TFS)	Ceph	Col	-	Fluo	Sul	-	-	4
Bacillus cereus strain S5 (MCHB)	Ceph	Col	Pen	Fluo	-	-	-	4
Bacillus cereus strain TO-11 (MGRM)	Ceph	Col	-	Fluo	-	Amy	-	4
Proteus mirabilis strain PS2 (MCTT)	Ceph	Col	Pen	Fluo	-	-	-	4
Proteus mirabilis strain PS2 (MCTT)	Ceph	Col	Pen	-	Sul	-	-	4
Serratia marcescens strainNPKI_32 (MFH)	Ceph	Col	-	Fluo	Sul	-	-	4
Alcaligenes faecalis strain B11 (PGRM)	Ceph	-	Pen	-	-	Amy	-	3
Alcaligenes faecalis strain B26 (PFH)	Ceph	Col	Pen	-	-	-	-	3
Alcaligenes faecalis strain dn3 (GFH)	Ceph	Col	-	-	-	Amy	-	3
Alcaligenes faecalis strain SH 100 (MSM)	Ceph	Col	-	-	-	-	-	3
Alcaligenes faecalis subsp phenolicus strain DHL 32 (GKN2)	Ceph	Col	-	-	-	Amy	-	3
Bacillus cereus AJK3 (MCHB2)	Ceph	Col	-	Fluo	-	-	-	3
Bacillus sp. strain BA (GCHB)	Ceph	Col	-	Fluo	-	-	-	3
Bordetella sp AC3 (TTRY)	Ceph	Col	-	Fluo	-	-	-	3
Ochrobactrum anthropi ICMM9 (PAM)	Ceph	-	-	Fluo	Sul	-	-	3

Key: Sul: Sulfonamide; Amy: Amyloglycoside; Pen: Penicillin; Ceph: Cephalosphorin; Col; Colistin: Cab: Carbapenem**;** Fluo: Fluoroquinolone

CHAPTER FIVE

DISCUSSION

5.1 Implementation of school health programme and school feeding service

The results showed that most respondents had general knowledge about SHP. It was obvious that proper implementation of SHP was a challenge because there were no clear directives from the government. Most key informants focused on school feeding services and healthful school environment as duo were based on school infrastructure and students' welfare. Their responses were quite varied because of differences in their career, backgrounds and years of experience in school system. Earlier research findings identified lack of adequate government support, poor funding and inadequate facilities as major factors affecting the implementation of SHP in schools (Ademokun *et al*., 2014, Sarkin-Kebbi and Kwashabawa, 2016).

The provision of nutritious and safe food in a healthy environment was a common theme among all respondents. Every school had a menu table, but no fruit was found on any of them. The majority of respondents stated that only fruits in season were served occasionally and expressed a desire for regular fruit service during meals. Ababio *et al*, (2016) reported no fruits on the menu plans in all the schools visited with the exception of one in a Ghanaian study of boarding secondary high school.

Majority of respondents carried out bulk food purchase but most schools did not have functional cold storage facilities. There should be functional refrigerators and freezers to ensure storage, preparation and service of safe food. Unsafe food could increase the risk of foodborne infections and probably food poisoning. These infections have a wide range of negative effects on children's health, including absenteeism, low productivity, long-term illnesses, malnutrition, poor immunity, and increased health-care costs (Marzano and Balzaretti, 2013; De Oliveira *et al.,* 2014; Ababio *et al.,* 2016; Walson and Berkley, 2018). Community members can participate in fresh local food supplies

and adoption of school gardens can proffer solution to availability of fresh fruits and also vegetables.

This study suggests that the boarding schools prioritise the well-being and working conditions of their food handlers which is similar to reports by Castaneda-Ruelas and Jiménez-Edeza (2020). The recruitment approach ensures that the recruited individuals already have relevant experience in the mass catering industry. Regarding accommodation, only schools located in the rural area have housing facilities for their food workers. This would be advantageous in terms of convenience and proximity to work. The irregular running taps in the toilets can be seen as a potential area for improvement, as proper hygiene practices is crucial towards achieving food safety. Also, working shifts and days off approach will afford the food handlers necessary rest and leisure time, while contributing to higher retention rates. The findings support previous research indicating that workers' welfare and training interventions can significantly enhance food safety and hygiene among boarding school food handlers in Nigeria (Afolaranmi *et al.,* 2015).

Water supply was adequate in all schools but there were very few distribution channels, making students queue before getting water to drink. This could be probably due to the storage tanks that were not cleaned on regular bases. Dirty water receptacles can be a source of contamination with cumulative effects if not discovered on time. Schools water must be adequately treated and water storage tanks cleaned to reduce risk of microbial contamination to consumers (Rossi *et al.,* 2018). Waste disposal by deep pit and burning was reported by three respondents whose schools were located in rural communities with no presence of waste collectors. The others use organised waste collectors twice in a month because of their urban location. There must be adequate waste disposal of both liquid and solid wastes to achieve food safety through environmental hygiene (Olumakaiye and Bakare, 2013).

In summary, respondents desired larger, fully enclosed kitchens with adequate facilities. They wished that government health workers would come around to inspect, identify non-compliance, and provide regular training for food handlers. It is suggested that the school feeding service supervisory team include hostel supervisor, home economics teacher, the vice principal, environmental health officer from local government and the PTA as community representative. The collaborative efforts will have long-term effects

on all students (boarding and day), preparing them for a healthy life and a better approach to making healthy food choices. For optimal performance, there should be government working document where the roles of school FSEs managers are clearly defined with better coordination by stakeholders and adequate human resources, since there was no clearly defined implementation plan of school health programme to guide their decision-making. With better informed schools FSEs managers, there could be better use of resources, improved infrastructures, student awareness, food handlers' commitment and government policy change.

5.2 Environmental hygiene of boarding schools' FSEs

There was no national standard grading system for school FSEs, it was challenging to categorise the cumulative hygienic conditions among all schools as acceptable or poor. Seventy-five percent of the schools' FSEs scored more than 50% pass mark in their sanitary condition assessment. This is better than the findings by Kibret and Abera (2012) where only 21.3 % of the investigated FSEs had good sanitary facilities in a study in Bahir Dar Town, Ethiopia. This study reported only 25% school FSEs had handwashing facility with soap in dining areas. Lack of handwashing facilities will reduce the food handlers' ability to maintain good personal hygiene, which could increase the spread of infectious microorganisms through cross-contamination (Oranusi *et al*., 2007). The reports on specific areas of the school FSEs showed that kitchen, dining area and toilet need to be better maintained. This highest score of 82.2% for toilets could be because the toilets were not used by students but only food handlers. This contradicts the findings of Imam (2013), who found that bathrooms received the lowest marks (43 %) in a survey among public boarding schools in Ibadan, Nigeria. Toilets are mostly reported as dirty in many public places like schools, markets, and parks. Lack of running tap and proper cleaning are factors contributing to the dirty conditions of toilets. It is important to define the roles of cleaners and provide them with necessary cleaning and sanitising materials alongside specific cleaning procedures.

Most schools scored below 50% in the dining area assessment except School C (where dining hall sits all students at once). This could be due to insufficient cleaning time in between meals since the dining areas do not sit students at once. There should be provision for a bigger dining areas that can accommodate all students at once and cleaning will be effectively done. Kitchens had overall lowest score of 52.8%. Most

unsanitary practices were found in the kitchens because it is the busiest part of FSEs and the layout is not appropriate for easy cleaning. The layout of FSEs should be designed to make work flow and general maintenance easy to manage by food handlers.

Presence of domesticated animals reported in 50% of schools' FSE is worrisome because these animals are often implicated in the transmission of zoonotic diseases. This agrees with the works of Oranusi *et al*. (2007) and Imam (2013) who both reported presence of domestic animals in boarding schools' kitchen in Zaria and Ibadan, respectively. Presence of coliforms in cooked food was attributed to poor food handling and animal faecal droppings in the kitchen and its environment. Dish washing was done in basins and buckets in all the selected schools. Research findings indicated that institutional mass catering services often use basins for dish washing (Oranusi *et al*., 2007; Imam, 2013; Nomakhushe and Wilkenson, 2018). This was due to lack of running tap water in food preparation areas and poor kitchen layout. It is critical to provide deep/wide sinks in FSEs and train food handlers on their benefits; otherwise, dish washing will be ineffective and can be a source of cross-contamination. Also, non-availability of sanitising agents in all schools implied that no procedure was in place for thorough cleaning and disinfection.

Cleaning and sanitising of food contact surfases are important procedures in food preparation. All kitchen utensils and surfaces should be adequately sanitised after cleaning in order to kill food poisoning bacteria. Food residues on FCSs are prone to biofilm formation, which can be difficult to remove (Kim *et al.,* 2019). Solid waste receptacles must be covered at all times in order to reduce distribution of germs and infectious agents in to the air. Recontamination of food items by air could become a significant issue if food products remain in places exposed to airborne infection for an extended period of time (Rodríguez-Caturla *et al.,* 2012). In a Mexican school study, lack of Good Hygiene Practices (GHP) and cleaning/sanitising protocols were high risk factors that threaten food safety (Castañeda-Ruelas and Jiménez-Edeza, 2020). There should be a working document prepared by Ministry of Education in collaboration with School Health Unit of Ministry of Health based on the WHO five keys to safer food guidelines. The document should be included in requirements for school's accreditation.

5.3 Socio-demographic characteristics of food handlers

This study documented food handlers' knowledge, attitudes, and practices regarding food hygiene and safety in selected boarding private and public secondary schools in Ibadan, Nigeria. The mean age of food handlers was 40.7 ± 11.2 years, which agrees with average age of 41.5 ± 9.5 years in a Ghana institutional study (Akabanda *et al.*, 2017). Numerous food handlers (43.3%), had finished secondary education in this study. This finding is comparable with study in Brazil by Da Cunha *et al.* (2012) and Kibret and Abera (2012) where 39.8% and 33.6% had completed secondary school respectively, but lower than findings by Rossi *et al.* (2018) where 81.25% had finished secondary education. Most food handlers lack higher degrees in specific training but mostly work for income (Da Cunha *et al*., 2012). Most of the food handlers were female (90%) and ever married (86.7%). Married and older people were frequently employed as food workers in institutional FSEs, most likely because of their skills, experience and work ethics. Many studies also reported over 70% of food handlers as married (Olumakaiye and Bakare, 2013, Afolaranmi *et al.*, 2014). Furthermore, in a Brazilian school kitchen study, 100% of food handlers were all female (Rossi *et al.*, 2018). School managements may prefer married female food handlers that will be more responsible with students healthy feeding, and make dining time more interesting. More than half of the food handlers (56.7%) acquired knowledge of food preparation through mentoring. Akabanda *et al.* (2017), reported that 86.7% learnt through personal intuition and mentoring. This strategy might be the most effective because it would guarantee continuous learning since the mentee could contact the mentor whenever a need arose. Also, evidence of training is necessary to issue license to the workers and probability of a short test should be considered before given such licence. In order to reduce the risk of food contamination, WHO (2014) recommend that all people who handle food receive the proper basic training in food safety and hygiene. About half (46.7%) of food handlers reported that they took part in pre-medical examination and were examined for tuberculosis, urine culture and HIV screening, but none were screened for Hepatitis A virus. The Hepatitis A virus primarily spreads through water and food, predominantly via the oral-fecal route. Therefore, it is essential to include it in the medical screening requirements for food handlers. Medical examination should be carried out twice in a year on food handlers. This is to prevent the spread of infectious organisms which could

cause contamination in the FSEs. Food handlers' medical screening should be made compulsory since it is one of the criteria to obtain medical certificate of fitness.

5.4 Food service establishments water supply and sanitation facilities

The major sources of water mentioned by the respondents were motorised borehole, protected well and hand pump borehole. Availability of potable water is important in food preparation. Most infectious organisms are waterborne and can easily be transmitted to food during preparation. World Health Organisation recommends that only potable water should be usedfor cooking and dish washing. Out-sourced water must meet the required standards and all distribution/storage facilities properly maintained. It is important to monitor water supply points for schools' population (Sanches *et al.,* 2015). Large proportion of the food handlers (80%) reported that pour flush toilet is the main method of sewage disposal in the school. This is comparable with an Ethiopian FSE study (Kibret and Abera, 2012) who reported 66% pour flush toilet. Only few (13.3%) reported throwing human waste into the bush (due to poor toilet facility). Inadequate sanitation is associated with the transmission of diseases such as cholera. diarrhoea, hepatitis A, poliomyelitis and typhoid (Akabanda *et al.,* 2017, WHO, 2019).

5.5 Knowledge about risk factors associated with food handling

The food handlers reported high knowledge scores in the four different categories which ranged between 62.86% and 70.42%. The respondents had good knowledge about risk associated with food handling (>50%). This was lower than knowledge scores of 83.3 % among studies at Terengganu Hospital, Lebanon and 88.2% reported in a study on consumers from developing countries, respectively (Bou-Mitri *et al*., 2018, Odeyemi *et al*., 2019).

The highest knowledge score of food handlers was separation of cooked and raw material during cooking and storage (90%) which agrees with work of Dora-Liyana *et al.* (2018) with 93.3% knowledge score in a boarding school kitchen study in Malaysia. This is important in prevention of cross-contamination because pathogenic organisms in raw food can easily contaminate fresh vegetables or food that will not be heat treated during food preparation. Foodborne pathogens such as *Escherichia coli, Bacillus cereus* and *Salmonella* species have been detected in raw meat/chicken and even raw vegetables (Marzano and Balzaretti, 2013). Majority of the respondents (83.3%) revealed that food

can get contaminated through improper handling by food handlers. Cross-contamination can occur through direct contact between different types of food especially high-risk food like meat and poultry, or indirect through food handlers hand and contact surfaces (chopping boards, trays, and knives). Safe food handling will minimise the possibility of food contamination. Food preparatory surfaces should also be regularly cleaned with effective cleaning agents and appropriate procedures.

Most respondents (80%) and about half (53.3%) agreed that typhoid and cholera are foodborne illnesses but majority (86.7%) did not know that Hepatitis A was foodborne pathogen. Akabanda *et al.* (2017) in his study found that 87.7% food handlers had knowledge about typhoid while 70.6% had no knowledge about Hepatitis A. Typhoid is a common infection that is caused by *Salmonella typhi* and cholera is a very deadly diarrhoeal disease caused by *Vibrio cholera*, that can kill in multitude in a short period. It can be easily spread by cross-contamination from food handlers with poor personal hygiene. Hepatitis A is foodborne and should be included in food handlers' medical tests.

Food handlers' assessment of Good Hygiene Practices (GHP) were influenced by proper knowledge of hand hygiene, personal hygiene, and health fitness during food preparation. In this study, about one fifth (23.3%) believed that handwashing was not necessary at the onset of cooking. This indicates poor knowledge about periods of handwashing for food handlers. Rossi *et al*. (2018) reported that a boarding school kitchen's food handlers had a hetero-bacterial count of >4.0 logCFU/cm^2. Food handling is the easiest pathway of introducing contaminants during food preparation. Odeyemi *et al.* (2019) reported that a cut or abrasion on food handlers' hand can be a source of cross-contamination. This is because opened sore can be easily infected with *S. aureus* and other pathogens. Handwashing should be done as frequently as possible to reduce the spread of contaminants, particularly pathogens. Hand hygiene is always essential during food preparation to prevent the spread of pathogens that cause foodborne illnesses. About half (46.6%) did not agree long nails and uncovered hair can affect food hygiene, this agreed with work of Abdul-Mutalib *et al.* (2012).

High percentage (70%) of the food handlers stated that prolong service period (holding conditions) can contribute to food contamination. Long holding temperature could lead to altered temperature that can increase the growth of microorganisms. Only a third of

the respondents were informed about time/temperature control, while most food handlers did not respond to the question on temperature control. This agrees with a study by Elsherbiny *et al.* (2019) in Ismaila Hospital, Egypt where only 31.8% food handlers knew the holding temperature of cooked food. A very few food handlers (6.7%) stated that cooked food should not be kept between 5°C and 65°C to avoid growth of food pathogens while majority (93.3%) lack knowledge about food temperature control and danger zones. This was mainly due to lack of temperature checking devices (thermometers) in all food establishments involved in this study. Dora-Liyana *et al*. (2018) reported 56% of food handlers lacked knowledge about temperature control in a study of boarding school kitchens in Malaysia. Food safety measures must be put in place from procurement of raw materials till the prepared food get to its consumers. There was a significant difference among food handlers in terms of methods of skill acquisition and but not in terms of years of experience ($p<0.05$). This agrees with Norhaslinda *et al.* (2016) and Bou- Mitri (2018) hospital studies where methods of skill acquisition influenced knowledge scores significantly, though they both reported age and years of experience being significant as well.

5.6 Attitude of food handlers about risk factors associated with food handling

Attitudes are the behaviours that is a reflection of your clear understanding about an action. Most (93.3%) of the food handlers agreed that in order to prevent cross-contamination, cooked food should be preserved separately from uncooked food. This is similar to a study on Lebanese hospital food handlers, where 94.1% agreed that raw and cooked food should be separated (Bou-Mitri *et al.,* 2018). But the results were higher than 59.1% reported in an Egyptian hospital study (Elsherbiny *et al*., 2019). More than half of the food handlers agreed that refreezing already-thawed food can result in food contamination. The method of thawing influences the microbial load and growth of food pathogens. While it is possible to thaw food in the microwave or refrigerator, it should never be done at room temperature. More than seventy percent of food handlers defrost foods at room temperature. The proliferation of foodborne microorganisms is reactivated by a long-term, gradual decrease in temperature. Freezing temperatures do not kill microorganisms; rather, they slow their rate of growth. Food should be kept in smaller portions so that it can be eaten immediately and avoid being put back in the freezer.

About two third (63.3%) disagreed on the use of protective equipment such as gloves, caps, and apron to ensure food safety which is similar to the findings by Bou-Mitri *et al*. (2018). Personal protective equipments (gloves, apron, caps, face masks and foot wears) are very essential in food service facilities as they can reduce the risk of food contaminations. Most earlier studies recorded about 60% on use of PPE (Akabanda *et al*., 2017; Elsherbiny *et al*., 2019; Odeyemi *et al*., 2019 and). Also, during food service to students, only the waiters or servers wore face masks in a boarding school kitchens (Dora-Liyana *et al*., 2018). The use of face masks has been found to reduce aerosol contaminants due to cough, sneezing and during talking and should be a precaution against cross-contamination during food processing. During the coronavirus (COVID-19) pandemic, the importance of face masks usage even in food establishments was also reiterated. This was to minimise transmission of Severe Acute Respiratory Syndrome Coronavirus- 2 (SARS-CoV-2) virus through respiratory droplets dispersed via talking, sneezing and coughing. Hair coverings such as hair nets, beard covers are essential in reducing physical contaminants like hair strands. Dora-Liyana *et al.* (2018) reported that 76.9% of food handlers agreed that hair contained bacteria that can contaminate food. Gloves must be worn whenever high-risk food are handled and must not be used without changing for more than two hours. Aprons and coverall have been found to carry high loads of microorganisms.

The majority of food handlers concurred that pest control is necessary to achieve food safety. Inadequate waste management may be a source of infection because pests like cockroaches, rodents, and houseflies spread pathogens. In any food establishment, environmental hygiene must be taken seriously and closely monitored. The performance of food handlers in terms of hygiene can be improved by regular inspection, pest control, and hygiene compliance checks by environmental health officers on sanitary facilities in FSE (Olumakaiye and Bakare, 2013; Suryani *et al.*, 2019). Due to the fact that approximately 50% of food handlers perceive foodborne outbreaks as natural occurrences, there exists a deficiency in understanding the root causes of such outbreaks. In 2018, an outbreak of FBD occurred at Queens College in Lagos, resulting in the death of two students. It was traced back to the use of non-potable water for cooking due to insufficient water treatment (Ezeamalu, 2018.). The media primarily reports on food poisoning incidents, especially when the fatality rate is high (Ababio *et al*, 2014). There is need for government regulatory bodies to conduct regular surveillance and

documentation of FBDs in boarding schools. Foodborne outbreaks are community disaster and all efforts must be put to prevent their occurrence. Food safety implementation plans should be readily available and must include regular training and monitoring for all stakeholders in food service facilities and among students.

5.7 Food safety training and medical examination

About half (53.3%) of the food handlers received food hygiene training which lasted mostly for a day. This agrees with the findings of Suryani *et al.* (2019) where 56% of food workers had attended food hygiene training. There was a significant difference in trained and untrained food handlers and this agrees with work of Kibret and Abera (2012) and Baluka *et al*. (2015). Food handling is a continuous work and one-off training cannot be sufficient to achieve good food safety practices. Regular trainings have been reported to be beneficial since only knowledge do not often translate into good practices (Lee *et al.,* 2017). This study showed that informal training is the most practiced. In-house training can be a better option because it is on-site and food handlers can learn in their regular work setting. Lack of food safety training manual in FSEs in public schools has been reported earlier (Imam, 2013). Also, Odeyemi *et al.* (2019) reported 97% respondents supported the importance of adequate knowledge and training on food safety. He proposed that food handlers and vendors should have on-going education with adequate monitoring in terms of food safety.

On-job food safety training had significant association on food safety practices. while level of education was not significantly related in a study among food vendors in Ghana (Monney *et al.*, 2013). According to Afolaranmi *et al*. (2015), food safety training was shown to be an effective technique for enhancing food handler's knowledge and practice in boarding school study in Jos, Plateau State. The present study indicated that about half of food handlers were never screened before employment. This is similar to 56.3% food vendors that never did medical screening in Abeokuta study (Bankole *et al*., 2009). Pre-employment medical screening should be a prerequisite for licensing all school food handlers as specified by NAFDAC under Federal Ministry of Health. This is to ensure that people with communicable diseases are excluded from handling foods (Monney *et al*., 2013). Asymptomatic food handlers can transmit infections like tuberculosis, Hepatitis A and typhoid if not properly diagnosed and treated. Since HIV is not transmitted through food, the screening should not be included in test to be done by food

handlers. The WHO five keys to safer food can be a baseline manual for food safety training at all schools' FSEs, the training should be adequately supervised and monitored by School Health team (Mwamakamba *et al.,* 2012).

5.8 Handwashing and reported food hygiene practices of food handlers

Handwashing is the most important singular practice in FSEs that is effective in minimising spread of foodborne infections (Oranusi *et al.,* 2013). Hands are easily contaminated with microbial contaminants during food preparation which can spread to other foods and/or FCSs by cross-contamination (Bankole *et al.*, 2009; Lee *et al.*, 2017). In this study, food handlers had poor hand hygiene practices when compared to their knowledge of handwashing. This could be due to a lack of clean water, handwashing stations, and soap. To ensure food safety, all food handlers must be adequately trained on procedure, frequency, and duration of handwashing. Audio-visual messages can be used to improve food handlers' practice of handwashing (Castañeda-Ruelas and Jiménez-Edeza, 2020).

Ready-to-Eat foods should be handled with clean hands since they will not be further processed or heated before consumption. *S. aureus* is a food pathogen that causes severe foodborne diseass and human body surfaces carrying this organism include skin, face, and ears. Food handlers should avoid touching their body parts while preparing food and wearing of gloves can help reduce cross-contamination. Most of the participants stated that there is no need to wash their hands after sneezing or coughing, despite the fact that hands play a direct role in transmitting pathogens. Odeyemi *et al*. (2019) reported 58% of food handlers covered their mouths with hands after sneezing and coughing which is far higher than 6.7% from this study. Hands must be washed after using the toilet to minimise transmission of faecal organisms through the faecal-oral route. Although, most respondents washed their hands after using the toilet, the correct handwashing techniques were often not implemented. WHO (2020) recommends at least twenty seconds for washing hands with soap and under running taps. Ayoade and Ardern (2018) tested the reasons for non-compliance during handwashing. They reported lack of awareness of health implications, laziness and unavailability of soap as major factors.

The reported pattern of handwashing by food handlers varied and were found to be dependent on available resources. Many studies reported inadequate handwashing

caused by lack of soap, potable water, handwashing stations, awareness and lack of time. Soap (bar or liquid) and detergent will sufficiently remove pathogens from hands. Use of alcohol-based sanitisers is very important in clinical setting and when water is not immediately available in general. It became a major intervention during the onset of viral haemorrhagic fever like Ebola, Lassa and recently COVID-19 (Nnaji *et al*., 2021).

Majority (80%) of respondents normally check date of expiry of food items at point of purchase. This is comparable with the findings reported from primary schools' canteen study in Indonesia. Expired products should not be cooked nor used during food preparation and should be thrown away (Suryani *et al*., 2019). The high level of literacy (90%) among the participants could be responsible for the good performance. It is important to take time off by food handlers. Food handlers who are ill must be allowed to fully recover before returning to work because they can still be asymptomatic carriers of foodborne causing pathogens (Santos, 2008).

Inadequate cleaning and disinfection of FCSs can be a source of cross-contamination with spoilage bacteria like *S. aureus, E. coli, L. monocytogenes* which have ability to attach to inert surfaces as a result of biofilm formation (Castañeda-Ruelas and Jiménez-Edeza, 2020). Most food handlers do not check refrigerator temperature because of non-availability of thermometer. No thermometer was available in Portugal school canteen study (Santos *et al.,* 2008). Poor time and temperature control is often implicated in food processing failures. Storage temperature of RTE foods is critical factor for food safety. Most organisms must not be kept within the danger temperature zones. Lack of temperature control will increase growth of food pathogens and risk of cross-contamination (De Oliveira *et al.,* 2014).

The positive correlation between knowledge and attitude (r = 0.22), as well as knowledge and practices (r = 0.321), indicates that food handlers' attitudes and practices toward food safety improve as their knowledge about food safety increases. According to Dora-Liyana *et al.,* 2018 in a Malaysian boarding school study, food safety attitude had a positive significant relationship with overall food safety knowledge (r = 0.196, p=0.040). This finding revealed a negative correlation between attitude and practices (r = - 0.113), while none of the associations showed a significant correlation at the p=0.05 level of significance. This was inversely similar to studies by Dora-Liyana *et al.* (2018)

and Suryani *et al*. (2019), where they discovered that there was a significantly positive correlation between food safety attitude and practice.

Food handlers reportedly have very high knowledge about food hygiene and handwashing, which were not translated into practice because of inadequate infrastructure, knowledge of proper handwashing procedure and poor supervision (Akabanda *et al.*, 2017; Rossi, *et al.* 2018). Also, most food handlers did not relate handwashing to their personal health and there should be continuous training on the importance of hand hygiene to human health.

5.9 Contributions of food contact surfaces to incidence of foodborne infections

The level of hygiene of FCSs determine the quality of food prepared on them. Grinders, countertops, drinking-water taps and chopping boards were grossly unsatisfactory (>2logCFU/cm^2). This agrees with findings by De Oliveira *et al*. (2014) and Castañeda-Ruelas and Jiménez-Edeza (2020) where similar contaminated surfaces results were reported. The chopping boards in this study were all made of wooden materials. Chopping boards could be a major contributor of foodborne pathogen contamination (Yoon *et al*., 2008). Wood is the most difficult surface from which to extract bacterial cells due to its low surface charge, intrinsic crevices, and inherent surface roughness (Adetunji and Isola, 2011). Regardless of raw food contamination, utensils such as an industrial blender and beef grinder may be sources of harmful organisms due to their built construction, making adequate cleaning and disinfection impossible (Gholam-Mostafaei *et al.*, 2017). This has shown that the cleaning and disinfection practices in the selected school kitchens were insufficient to protect working surfaces. Cleaning and sanitising food preparation surfaces would reduce the rate of food contamination (Rossi *et al*., 2018).

A South African study found that 16.2% of tested FCSs school kitchens that prepare food for school lunch programmes were satisfactory, with aerobic colony counts of less than 2 logCFU/cm^2 (Sibanyoni and Tabit, 2019). Proper cleaning and sanitization of FCSs is required during food preparation because food remnants on the surfaces can form biofilms that are often difficult to remove from the surfaces (Castañeda-Ruelas and Jiménez-Edeza, 2020).

5.10 Contributions of food handlers' hands to incidence of foodborne infections

The presence of gross contamination on the hands of food handlers is consistent with the findings of Lee *et al*. (2017), who reported high total coliform and Salmonella counts among Malaysian university food handlers. These results highlight a significant concern regarding hand hygiene practices among food handlers. The high bacterial counts and detection rates suggest a potential risk of transmitting pathogens through contaminated hands. This underscores the crucial importance of implementing effective handwashing procedures and strict adherence to hygiene protocols in food handling environments.

The identification of Enterobacteriaceae, which are gastrointestinal organisms, is indicative of poor personal hygiene among food handlers. Inadequate handwashing practices before and during food preparation can significantly increase the transmission of pathogens and the risk of foodborne infections, as highlighted by previous studies conducted by Bankole *et al.* (2009) and Lee *et al.* (2017). Given that food handlers play a vital role in ensuring safe food preparation, it is imperative that they receive proper training. Reports emphasize the significance of prioritising the welfare of competent food handlers, as they are considered predictors of safe food practices (Rossi *et al.*, 2018; Castañeda-Ruelas and Jiménez-Edeza, 2020).

Food handlers have been linked to the spread of many pathogens through their skin, hands, and sneezing/coughing (Marzano and Balzaretti, 2013). Most food handlers claimed to wash their hands, but on-site observations revealed that many did not practice adequate handwashing. Lack of basic infrastructure like soap and handwashing stations have affected proper handwashing especially in public municipal schools. Adequate washing and sanitising hands can reduce diseases transmissible during food preparation (Rossi *et al*., 2018).

5.11 Contributions of Ready-to-Eat foods to incidence of foodborne infections

The bacterial counts from RTE foods indicated 72% positive contamination for aerobic plate count with fried egg, fish stew, meat stew being highly unsatisfactory (≥5 logCFU/g). APC values greater than 4 logCFU/g could be a health risk due to possible presence of food pathogens (Marzano and Balzaretti, 2013). The results indicating high incidences of bacteria counts agree with the findings of Nyenje *et al.* (2012), where enterobacteriaceae was detected in RTE foods from roadside cafetaria in Alice, South

Africa. This study revealed higher faecal coliform counts when compared to a previous study by Petruzelli *et al.* (2018) that investigated RTE meals served in school-based mass catering in Italy. High bacterial counts for *Bacillus* cereus, *S. aureus*, *Salmonella-Shigella* and *E. coli* were also detected in RTE food samples and have been reported earlier (Nyenje *et al.,* 2012; Marzano and Balzaretti, 2013). The high rate of contamination may be due to cross-contamination by food handlers, uncleaned utensils or prolonged service period. By following GMP guidelines and implementing appropriate food safety measures, the risk of pathogens in RTE foods can be minimised, thus reducing the likelihood of foodborne infections and their associated negative consequences. Pathogenic *E. coli* strains can cause travellers' disease and also life threatening infections such as Haemolytic Uremic Syndrome (HUS).

5.12 Physico-chemical and microbiological characteristics of water

Water used by FSEs for food preparation must be potable, meet the required water quality standards and be free of pathogens. Water usage can be through direct addition as an ingredient, washing of food contact surfaces, washing of raw produce like vegetables, fruits, animal carcasses and as a cleaning agent. The physico-chemical results of water samples from the selected schools were mostly within the allowable limits specified by Nigeria Industrial Standards (NIS, 2007). Low pH (which measures the concentrations of hydrogen ions present within the water samples) can cause corrosion of plumbing system and affect human health (Eseigbe *et al*., 2018). Increased water acidity could be as a result of acid rainfall, run off from industrial activities and other airborne pollutants causing underground water to be acidic (Afolabi *et al*., 2012). Turbidity (the measure/extent of clarity or cloudiness) was high in samples from public schools, despite the fact that the samples were collected from boreholes, indicating more suspended particles, most likely due to human activities such as farming. The borehole pipes that transport water to the surface may have developed some form of leakage.

Total Dissolved Solids (TDS) is a measure of the amount of materials dissolved in water All water samples tested were within the standard limit of 500 mg/L. The elevated TDS in WA1 could be due to household waste water, road runoff, and dumpsite runoff washing into the underground water. Conductivity is the extent of electric current transmission, due to the ionic concentration. Water samples with decomposed organic

matter and inorganic dissolved particles can increase water conductivity (Leong *et al*., 2018).

Total hardness values (which give a measure of the (Ca^{2+} and Mg^{2+}) mineral contents in the form of trioxocarbonates (V) in water samples were all within the allowable limit of 150mg/L. Hard water often make cleaning difficult because more soap is needed to form lather and can cause a lot of scale formation. The high iron content for all tested water samples may be due to the fact that iron is a relatively plentiful metal in the earth's crust. Storage of excessive iron in the body can be dangerous to health. Iron stains clothing and plumbing fixtures at quantities greater than 0.3 mg/l; at values less than 0.3 mg/l, there is usually no detectable taste.

For microbiological examination, APC was higher in public school when compared with private schools. WA2 had a high coliform count (13MPN/100 mL) but no *E. coli* was found in the water sample. This could be due to location of the borehole beside the dormitory and possibility of contaminated underground water and irregular cleaning of water storage vessels. Water sample WC2 was positive for *E. coli*, whose presence in water indicates faecal contamination, implying the presence of more pathogenic organisms. Yoon *et al*. (2008) reported no *E. coli* in tap water samples but APC results were in the range of 2.6 to 4.9 logCFU/mL which is similar to the findings in this study and could be caused by cross-contamination from water distribution channels.

If water is outsourced, schools' water safety plans should include a requirement that third-party suppliers be certified. Storage tanks, distribution pipes, and vessels should also be cleaned on a regular basis in accordance with standard operating procedures (De Oliveira *et al.*, 2014). Water samples should be tested at least once a year to detect any quality failures as soon as possible. To avoid contamination of underground water, the environment must be kept clean of faecal waste. It is proposed that schools implement a safe water supply plan in order to meet the minimum safe water requirements for physical, chemical, and microbiological parameters. To effectively assure the quality of water intended for school consumption, a quality monitoring, control and assurance system must be developed.

5.13 Phenotypic characterisation of isolated bacteria

The high diversity in the bacteria associated with boarding schools' FSEs indicates that schools' FSEs are complex ecological niche for bacteria proliferation. Most organisms were Enterobacteriaceae and are found in human gastrointestinal tract. The dominance of enteric bacteria signifies the extent of faecal contamination. These bacteria are often spread directly or indirectly through cross-contamination such as inadequate food handlers' personal hygiene and unsafe food hygiene practices. Some of these organisms are of public health importance and have been implicated in foodborne infections (Kibret and Abera, 2012; Norhaslinda *et al.,* 2016; Bou-Mitri *et al.,* 2018). Improper handling during food preparation is responsible for high level of bacteria detection. Hand hygiene is a singular act that can prevent food contamination (Oranusi *et al*., 2013). Prevalence of *E. coli* on hand swabs reflects faecal contamination that can easily be transmitted by asymptomatic food handlers (Lee *et al*., 2017). The presence of enteric organisms in water samples is a cause for alarm. There should be proper storage and distribution channels for water sources (Sanches *et al*., 2015). Most characterised organisms were opportunistic in nature. All schools' FSEs need to develop and implement simple food safety plans that could be used for routine assessment and detection of non-compliance during food preparation.

5.14 Molecular characterisation of bacteria isolates

5.14.1 Random Amplified Polymorphic DNA-PCR

The RAPD- PCR was used to classify the bacteria isolated into clusters and generate an overall estimate on the groups, similarity relatedness and genetic diversity. The method has been found useful in identifying bacteria (Alsanie, *et al*., 2018). The RAPD profile revealed high diversity among the isolates, but there was no significant relationship between clusters and bacterial source. These disparities could be explained by factors influencing food product and process safety, such as available infrastructure, school location, and monitoring plans in each school (Gholam-Mostafaei *et al.,* 2017). Transmission of bacteria by cross-contamination from contaminated water, food contact surfaces and food handlers to RTE foods vice versa could be a major problem in mass catering settings (Jaffee *et al*., 2019).

RAPD-PCR technique can be a useful tool for assessing genetic relatedness among the bacteria isolated from schools' FSEs. This will aid in the examination of potential epidemiological problems caused by these bacteria pathogens by tracing their origins of infection, allowing for the identification of important sites and implementation of suitable management measures to protect public health.

5.14.2 Partial 16S rRNA Sequencing and Evolutionary relationships of taxa

The study discovered a high diversity in the source of bacteria contaminants, which could be a risk factor for food safety. Although the genotyping results were not identical to the conventional morphological and biochemical methods, the bacteria identified with both techniques belonged to the same phyla (Proteobacteria and Firmicutes). This demonstrates the limitations of using only conventional methods, which could have resulted in species misidentification, making it less suitable. It is critical to confirm the identity of bacteria using a genotyping technique that provides a higher level of specificity and sensitivity (Law *et al.,* 2015; Aruwa and Ogunlade, 2016).

The two main genera namely Alcaligenes and Bacillus were most identified species from this study. In 1919, *Alcaligenes faecalis* was identified for the first time in faeces and is frequently found in water, natural habitats, and soil. Despite its presence alimentary canal, the bacterium does not cause systemic infections in majority of humans. People with weakened immune systems, as well as those with normal immune systems, have been found to contract *A. faecalis* infections (Al-Zhakari *et al.,* 2020). They are catalase and oxidase positive and are motile via peritrichous flagella. Colonies have a thin, uneven border that is not pigmented. The organism is an important zoonotic pathogen that has been reported in human infections. The organism had been isolated from blood, urine, tonsils, pus and faeces (Tena *et al.,* 2016). This pathogen is typically spread by droplets via ventilation equipment and nebulizers. However direct contact transmission has been recorded in a few case reported. Although, several cases of *A. faecalis* infections exist, they have not been extensively described in the literature. The pathogen can cause rare but fatal infections including appendicitis, abscesses, cystic fibrosis, meningitis, bloodstream infection, endocarditis, and post-operative endophthalmitis (Huang, 2020).

The foodborne organism appears to be of public health relevance because its resistance to conventional antibiotics and occurs frequently in nosocomial infections and water

contamination (Ethica, 2017). Ayandiran and Dahunsi (2017) reported that about 10% of bacteria isolated from *Clarias* species (Catfish) from Oluwa Rivers, Ondo Nigeria were *Alcaligenes faecalis.* Fresh leafy vegetables in Elele Market, Nigeria were contaminated with *Alcaligenes faecalis* among other pathogenic bacteria (Kemajou *et al*., 2017). The study concluded that poor handling from farm to cooking with use of poor water for irrigation and washing during preparation contributed to the microbial load. Adedeji, *et al.* (2017) also identified *Alcaligenes faecalis* in two Nigerian fermented condiments (iru and ogiri) and their raw seeds (locust bean and melon). *Alcaligenes* spp. were isolated from a river used as a waste dump by a poultry farm in Ogbomoso, Nigeria and were extremely resistant to antibiotics (Adelowo and Fagade 2012). Also, virulent and highly resistant *Alcaligenes faecalis* was isolated from untreated borehole water in a South African study (Horn, *et al.,* 2016). The study conducted by Bankole *et al.* (2009) found that fast-food handlers in Abeokuta, Nigeria had *Alcaligenes faecalis* present on their hands.According to Ogundipe *et al*. (2012), 10% of all bacteria isolated from *Lycopersicon esculentum* (Tomato) sold in Lagos State, Nigeria were *Alcaligenes* species.

The genera Achromobacter and Bordetella are closely grouped with Alcaligenes in the family Alcaligenaceae. They are opportunistic human pathogens and is found in soil, plant and natural environment. *Bordetella pertussis* causes whooping cough, a severe infection in human. *Achromobater xylosoxidans* is associated with cystic fibrosis, bacteremia and urinary tract infection. It is an emerging threat to immunocompromised patients (Wittmann *et al.,* 2014).

Bacillus species are ubiquous in nature and found in the environment such as soil, plant, water and intestinal tract of invertebrates. This study isolated *Bacillus* species (*Bacillus cereus* and *Bacillus flexus*) from FCSs (tray, chopping board, dining table and grinder). This could have been due to improper cleaning of the surfaces, since none of the studied schools carry out sanitation of surfaces as reported in the observational checklists, which can lead to biofilm formation. *Bacillus cereus* is able to adhere to surfaces, form biofilm and become a possible source of cross-contamination during and after processing of finished products (Kim *et al,* 2019). *Bacillus cereus* was found in RTE foods and water (Oranusi *et al*., 2007; Aruwa and Ogunlade, 2016). *Bacillus cereus* was a major contaminant in a University campus study of food handlers hand and FCSs (Oranusi *et al*., 2013). Enteric pathogens can be spread by a variety of routes, the most common of

which being direct contact with contaminated surfaces and hands. In an earlier study, Petruzzelli *et al.*, (2018) reported high load of *B. cereus* in RTE vegetable salads in mass catering facilities and suggested proper sanitation plan of washing machines so as to reduce possibility of cross-contamination. In a study of drinking water borehole in South Africa, *B. cereus* with high pathogenity was documented (Horn *et al.*, 2016). *Bacillus species* are well-known spore formers that cause diarrhoea and food poisoning. They can withstand the severe conditions of food storage and processing (Adimpong *et al.*, 2012). Implementing effective HACCP, GMP and handwashing could greatly reduce the rate of foodborne illnesses.

Staphylococcus saprophyticus is a coagulase-negative, Gram-positive organism that is found in marine environment and food derived from fish (Sousa *et al*., 2017). The organism is a normal flora of slaughtered animal and can contaminate food, FCSs when there is poor hygiene (GHP, GMP) and eventually colonise humans and is responsible for uncomplicated urinary tract infection (Lawal *et al.*, 2021). Its presence on tray, a FCS could be as a result of improper cleaning and subsequent cross-contamination by other raw food materials like meat and fish being processed on the tray.

Ochrobatrum anthropi is a non-fermenting, oxidase, urease, citrate-positive Gram-negative rods. The organism was isolated from various ecological niches (Adelowo and Fagade, 2012). It has been increasingly reported as emerging pathogens capable of infecting both immune-competent and immune-compromised individuals. It isolation from tray could have occurred as result of cross-contamination from animal source and the environment. The organism is found in soil, water, human waste and medical devices. They have been reported with low virulence factor. They have been associated with endocarditis and catheter-related bloodstream infections (Khan *et al.*, 2014).

Proteus mirabilis and *Serratia marcescens* are the only Enterobacteria species isolated from this study. They are enteric organisms found in human faeces, soil, and the environment. They have been associated with nosocomial and other opportunistic infections in human and veterinary medicine. They were isolated from School D counter top and food handlers' hands. They were both isolated from food handlers hand in a Nigerian University study (Ayoade and Ardern, 2017) and food condiments (Adedeji *et al.*, 2017). *Serratia marcescens* was also found on food handlers' hands (Bankole *et al.*, 2009). It was attributed to poor handwashing, hygiene practices and its transmission

which could be through faecal-oral routes. *Proteus mirabilis* have been commonly found in foods and FCSs (Nyenje *et al.*, 2012; Ogundipe *et al.*, 2012). It was responsible for food poisoning outbreak in Beijing (Wang *et al*., 2010), which was traced to infected cooks and waiters. The organism is a mobile swarmer and have the ability to form biofilm. Biofilm development is a significant issue in the food and medical industries, posing considerable economic and health consequences. This ability depends on surface type, bacteria species, growth conditions, and gene regulation (Chen, 2017). The biofilm microbial community is highly resistant to antibiotics, sanitisers and confers sustained survival, which makes it difficult to eradicate (Sadekuzzaman, 2015).

Alcaligenes faecalis spp that were 100% similar were not isolated from the same schools nor same sample sources. This shows the extent of distribution of the organism across multiple niches in food preparation environments, as most of the raw food materials were obtained from a central local wet market. Transmission to food handlers, food contact surfaces and RTE foods could be due to cross-contamination. In this study, FCSs was most implicated for *Alcaligenes faecalis* spp (62%) with knife, chopping board and grinding machines recording 38% and 23% each respectively. With less effective cleaning and poor sanitation of FCSs (worn equipment, inside machine), large clusters of cells may interact as a result of biofilm formation and are important for growth, development and survival (Sadekuzzaman, 2015).

5.15 Antibiotic susceptibility of identified bacteria

Globally, multi-antibiotic resistance to all currently active antibiotics has emerged. Most of the organisms were highly resistant to diverse antibiotics, cefuroxime (83.8%), cefixime (75.7%), gentamicin (67.6%) and ceftazidime (56.8%), respectively. These antibiotics belong to cephalosporin group apart from gentamicin which is an aminoglycoside. The organisms were all susceptible to ofloxacin and ciprofloxacin except one isolate each. *Alcaligenes faecalis* showed high resistance to all cephalosporins. This agrees with Ayandiran and Dahunsi (2017) who reported that *Alcaligenes faecalis* showed highest antibiotic resistance in catfish organs even higher than Clostridium and Bacillus species. *Alcaligenes faecalis* from the *Clarias gariepinus* skin and *Clarias buthopogon* muscle exhibits 80% and 90% resistant to most antibiotics but were all sensitive to ciprofloxacin and highly sensitive to ofloxacin. According to Junejo *et al*. (2018), a rare case of pneumonia caused by extensively drug-resistant *A.*

faecalis was reported. The study findings indicated that immune-compromised patients are more susceptible to such infections, and the presence of contaminated medical devices and solutions played a role in the transmission of the bacteria.

Filipe *et al.* (2017) evaluated *A. faecalis* isolated from otitis media cases in Angola against a variety of selected antibiotics and discovered that the organism was completely resistant to cephalosporins and colistin. In contrast, more than half (57%) of the bacterial was resistant to colistin as revealed in this study. According to Ngbede *et al*. (2020), a Nigerian study on bacteria isolated from animal and human samples reported *mcr-1* gene in *Alcaligenes faecalis* which transferred colistin resistance successfully by conjugation. Colistin is the last-resort antibiotic used to treat infections caused by carbapenem-resistant multi-antibiotic resistant Gram-negative bacteria. It kills bacteria by specifically targeting the Lipopolysaccharide (LPS) in both the outer and cytoplasmic membranes, causing rupture of the cell envelope and subsequent bacterial lysis. *Alcaligenes faecalis* MUB14 was discovered from a patient in Poland and confirmed to be resistant to all antibiotic classes examined, causing the patient's death (Majewski *et al.*, 2020).

Development of increasing colistin resistance could be as a result of mutation and other adaptive mechanisms. In Africa, the presence of mobile colistin resistance (mcr) genes in *Alcaligenes* species has been reported (Anyanwu, 2021). These mcr genes, specifically mcr-1 to mcr-9, which are transmitted through plasmids, have been implicated in conferring resistance to colistin (Anyanwu, 2021). In African strains, plasmids carry genes responsible for multi/extensive drug resistance and pathogenicity, which are found together with the mcr gene. The mcr epidemic in Africa is driven by various mobile genetic elements (MGEs) such as insertion sequences, class 1 integrons, and transposons (Anyanwu, 2021). *A. faecalis* can cause serious disease and even death, despite the fact that these organisms are classified as gut flora. It is argued that *A. faecalis* should be considered a pathogen rather than a contaminant, as global cases of life-threatening infections caused by *A. faecalis* are emerging (Majewski *et al.*, 2020).

Bacillus cereus from the gills of *Clarias gariepinus* (Catfish) was susceptible to ofloxacin, ampicillin and augmentin (Ayandiran and Dahunsi, 2017). *Bacillus cereus* are producers of heat-stable cereulide and diarrhoeal enterotoxins (hemolysins, cytotoxins). In this study, *Bacillus cereus* was isolated from a dining table and cooking

water. Tirloni *et al* (2020) reported *B. cereus* on surface of table grind-box and water hose in a dairy processing plant in Italy. The organism was found to be resistant to cefuroxime (100%), gentamicin (85%), ceftazidime (57%) and augmentin (29%) but were susceptible to quinolones: ofloxacin and ciprofloxacin (100%).

Two *Bacillus cereus* isolates (*Bacillus cereus* MCW *and Bacillus cereus TGRM*) were resistant to imipenem. This resistance could have been acquired from the ground water or the environment through transmission of resistance genes. As earlier reported, *B. cereus* were resistant to β-lactam antibiotic like penicillin and cephalosporins because of the bacteria ability to synthesise a beta-lactamase, an enzyme that attacks the β-lactam ring (Fiedler, 2019). For multi-antibiotic resistance, all *Bacillus* species displayed resistance to three or more antibiotics. The spread of resistance can be through Horizontal Gene Transfer (HGT) by mobile genetic elements (plasmid and transposon) or other intrinsic properties such as efflux system and alteration of target sites (Adimpong *et al*., 2012).

Ochrobactrum anthropi resistance to cephalosporins agrees with the work of Alonso *et al.* (2017) who reported 100% resistant to ceftriaxone and about 50% resistance to fluoroquinolones. The resistance could be due to their intrinsic ability to produce beta-lactamase. *Alcaligenes faecalis, Achromobacter xylosoxidans* and *Ochrobactrum anthropi* are classified as non-fermentative Gram-negative bacilli (NF-GNB). These organisms are being increasingly isolated from different environment. This is due to availability of improved identification techniques. They are often multi-antibiotic resistant, thus can be a potential source of infections. Proper screening and antibiotic susceptibility studies should be carried out (Grewal *et al*, 2017). These NF-GNB are innately resistant to many antibiotics and are known to produce extended spectrum ß-lactamases and metallo ß-lactamase (Gales *et al*., 2005).

Since there is a lack of substantial data regarding the prevalence and antibiotic susceptibility profile of *A. faecalis* due to its limited pathogenic role and rare isolation, results obtained from recent studies may help guide the choice of antibiotics for its infection treatment (Grewal *et al*., 2017)

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary and Conclusion

The occurrence of foodborne diseases in schools' food service establishments in Nigeria is on the rise, and while the adverse health effects of such diseases are becoming more evident, they are often underreported. This study evaluated the environmental and food hygiene status; and microbiological contamination among food handlers, food contact surfaces and Ready-to-Eat foods from selected boarding schools Food Service Establishments in Ibadan, Nigeria.

A survey, through a checklist and questionnaire was conducted on thirty food handlers' food safety knowledge, attitudes and practices. Eighty-seven samples were collected from randomly selected sources and subjected to aerobic plate count, salmonella/shigella count, *Bacillus cereus* count (BCC), *Staphylococcus aureus* count (SAC), total and faecal coliform counts on selective growth media. Bacteria isolated were identified by polyphasic approach through both conventional and molecular techniques and investigated for antibiotic susceptibility using Kirby-Bauer disc diffusion and minimum inhibitory concentration by E-strip techniques.

The mean age of food handlers was 40.7 ± 11.2 years, 22 (73.3%) were 30 years and above while most of the food handlers had more than five years of experience. About half 16 (53.3%) of the total respondents had food hygiene training, while many 14 (46.7%) never had pre-employment medical examination. The mean knowledge score was 26.0±4.58 (range=18-32) with most respondents having good knowledge about risk factors associated with food handling. The mean attitude score was 19.5.5±2.86 (range=14-24) with most respondents having positive attitude about risk factors associated with food handling.

The food handlers lack knowledge about food storage and preparation temperatures. There was a significant association between school status: level of food handler's

education, knowledge acquisition, pre-employment medical examination and on-job food hygiene training ($p<0.05$). Food handlers' good knowledge and positive attitude of food safety did not translate into practice. Most schools lack basic infrastructure including handwashing facilities and standard operating manuals. The incidences of contamination for APC on food contact surfaces for all categories of samples were very high. The general indication of the microbiological quality (APC) was out of the acceptable limit (<2 logCFU/cm^2). Concerning the food handlers' hands, the results showed that the APC, *Staphylococcus aureus* count and total coliforms exceeded the reference standards in 40%, 50%, and 35% samples respectively. Fourteen (78%) RTE foods conformed with the acceptable limits of <4 logCFU/g for aerobic plate count.

Bacteria (n=154) were isolated using the biochemical identification schemes with enteric organisms in prevalence. RAPD-PCR analysis was used to determine the genetic diversity among the bacteria. Thirty-seven bacterial species belonging to *Alcaligenes faecalis, Bacillus spp, Bordetella spp, Proteus mirabilis, Serratia marcenscens. Achromobacter xylosoxidans, Ochrobactrum anthropi* and *Staphylococcus saprophyticus* were identified by 16srRNA sequencing. *Alcaligenes faecalis* showed high resistance to cefixime (76.2%), cefuroxime (71.4%), ceftazidime (66.7%), gentamicin (61.9%), augmentin (57.1%) and colistin (56%). *Alcaligenes faecalis* were sensitive to ofloxacin and ciprofloxacin (95.2% each), ceftriaxone (75%), imipenem and piperacillin/tozobactam (100%). FCSs were most implicated materials with bacterial contaminants. The resistance to colistin, a last resort antibiotic is alarming and showed the extent of transmission of resistant genes in the ecosystem.

In conclusion, environmental hygiene compliance was poor in terms of kitchen design, food storage, dining space and non-availability sanitising agents. Most food handlers did not know about temperature control during food storage, majority did not put on personal protective equipment as required and training was mainly done by formal and in-house method. Food handlers' practices were significantly different among schools while handwashing practice was determined by available resources and manager's supervision. Extensive contamination of food contact surfaces was as a result of poor cleaning and lack of sanitising procedure. Food handlers' welfare is a predictor factor to provision of safe food at schools. Diversity of bacterial isolated from school FSEs indicate a complex ecological niche that should be closely monitored. *Alcaligenes faecalis* was highly resistant to colistin, while *Bacillus cereus* showed resistance to

imipenem. The bacteria exhibited multi-antibiotic resistance implying serious public health issue that must be given adequate attention.

6.2 Recommendations

1. Schools food service managers should ensure regular cleaning, sanitation, proper drying, storage and even replacement of food contact materials. Since wood and plastic surfaces have high porosity and hydrophilic properties, more inert material like stainless steel is recommended for use as food contact material.
2. The federal and state public health agencies should conduct epidemiological monitoring and microbiological risk assessment at institutional catering services and food preparation facilities in order to establish a better approach to food safety assurance and understand health risks to consumers.
3. There should be holistic approach involving the state and local government, Parents and Teachers Association (PTA), community leaders and school administrators in order to achieve food safety at schools' FSEs. They should provide functional school health programme, improved infrastructures, training intervention and even, a small school garden that will provide fresh food items at reduced cost while engaging students and community to be more productive.
4. Researches on the production of numerous virulent and resistant genes such as metallo-beta-lactamases and the mobile colistin resistance potential of *Alcaligenes faecalis* should be further investigated through Whole Genome Sequencing (WGS).
5. There must be aggressive and well-coordinated campaign about the consequences of antibiotics misuse along the food chain. All stakeholders in school's management including students should be informed about antibiotics resistance and its prevention.
6. The school health programme coordinating body (the Ministry of Education) should implement the national school health policy and collaborate with other agents (ministries of health, agriculture and environment) and develop a workable school health manual with adequate performance monitoring.

6.3 Contributions to Knowledge

1. Highly contaminated food contact surfaces were identified as a significant source of cross-contamination in boarding high school food service establishments (FSEs).
2. The predominant organism found in the FSEs of schools was *Alcaligenes faecalis*, an opportunistic pathogen.
3. *Alcaligenes faecalis* exhibited high resistance to colistin, which is considered a last-resort antibiotic. Additionally, two strains of *Bacillus cereus* displayed resistance to imipenem, raising concerns about antibiotic resistance.
4. The lack of Good Hygiene Practices (GHP) protocols emerged as the primary risk factor. Implementing an effective food hygiene program and comprehensive health education can greatly improve the situation.

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APPENDICES

APPENDIX 1: LIST OF VISITED SCHOOLS

List of visited Private and Public Boarding High Schools in Ibadan

LGA	NAME OF SCHOOL	NO OF BOARDERS	FH	PRIVATE/ PUBLIC
AKY	Crown Height College, Arulogun	150	2	Private
	Ogunsanya Girls Academy, Arulogun	103	3	Private
	Deeper Life High School, Oyo Road	193	8	Private*****
	School of Science, Pade	622	9	Public******
EGD	Providence High School, Ife Ibadan way	80	4	Public
	David- Joel College	21	1	Private
	Lasting Glory Comp. Sch. Erunmu	32	1	Private
	Adelayo Academy, Iyana Church	29	4	Private
	Medmina College, Ife/Ibadan Road	29	3	Private
IBN	Maverick College, Bodija	70	7	Private******
	Bishop Akinyele Memorial School	116	4	Private
	God Blessing College,Yemetu	300	7	Private*****
	All Souls High School, Bodija	370	4	Private
	Bodija International College, Bodija	50	3	Private
	Kolmor Metropolitan College, Ashi	8	2	Private

List of visited Private and Public Boarding High Schools in Ibadan (Contd)

LGA	NAME OF SCHOOL	NO OF BOARDERS	FH	PRIVATE/ PUBLIC
	The Apostolic College, Samonda	51	2	Private
	Walbrook College, Samonda	10	2	Private
	Goshen Height College, Bodija	5	1	Private
	St.Loius College, Mokola	50	4	Public
IBNE	Nil			
IBNW	Nil			
IBSE	George and Duke Int. School, Elebu	30	3	Private
	Pislib de Varsity, Soka	100	4	Private
	Bolade Model, Eleta	15	2	Private
	Fola Model College, Academy	30	3	Private
	Idikan Baptist College, Felele	30	2	Private
	Wesley Sch. Of Science, Elekuro	1085	12	Public*****
	St. Annes Sec. Sch, Molete	35	3	Public
IBSW	United Mission College, Molete	334	10	Private*****
	The Vale College, Iyakaugu	85	6	Private*****

List of visited Private and Public Boarding High Schools in Ibadan (Contd)

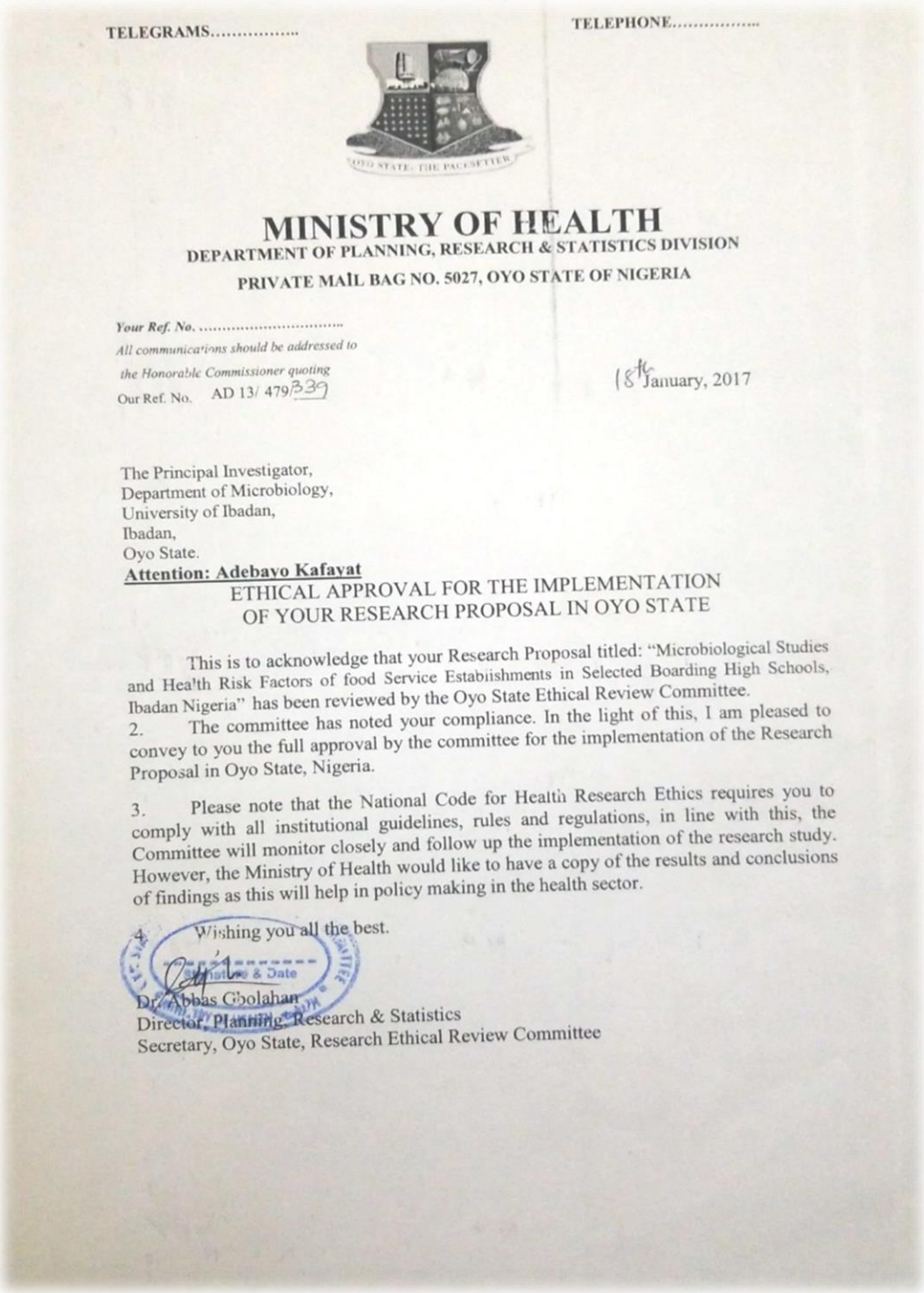
LGA	NAME OF SCHOOL	NO OF BOARDERS	FH	PRIVATE/ PUBLIC
IBSW	Victory Christian Academy, Oluyole	60	5	Private
	SunShine Int. Sch, Oke Bola	210	4	Private
	St. James Cathedral, Oke Bola	31	2	Private
	Alayande School of Science, Oke Ado	60	3	Public
	Queens School, Apata	55	2	Public
	Government College, Apata	51	2	Public
IDO	TAAS College, Omi Adio	200	8	Private**
LGL	Beulah Academy	30	2	Private
	Nickdel College	110	8	Private*
	Rosebud Collegel	16	2	Private
	Valaint Havinah Col.	28	2	Private
	Valencia College	28	2	Private
	School of Sci. Igbo Elerin	300	5	Public**
OLY	TSPC Int. College	190	8	Private*
	CTY College, Alomoja	150	5	Private
ONA	Nil			

Key: AKY: Akinyele; EGD: Egbeda; IBN: Ibadan North; IBNE: Ibadan North East; IBNW: Ibadan North West; IBSE: Ibadan South East; IBSW; LGL: Lagelu; OLY: Oluyole; ONA: Ona Ara; LGA: Local Government Area; FH: Food handlers

***** - Schools that met selection criteria

**- Randomly Selected schools

APPENDIX 2: ETHICAL APPROVAL



TELEGRAMS..... TELEPHONE.....

OYO STATE: THE PACESETTER

MINISTRY OF HEALTH

DEPARTMENT OF PLANNING, RESEARCH & STATISTICS DIVISION

PRIVATE MAIL BAG NO. 5027, OYO STATE OF NIGERIA

Your Ref. No. .....

All communications should be addressed to the Honorable Commissioner quoting

Our Ref. No. AD 13/ 479/339

18th January, 2017

The Principal Investigator,
Department of Microbiology,
University of Ibadan,
Ibadan,
Oyo State.

Attention: Adebayo Kafayat

ETHICAL APPROVAL FOR THE IMPLEMENTATION OF YOUR RESEARCH PROPOSAL IN OYO STATE

This is to acknowledge that your Research Proposal titled: "Microbiological Studies and Health Risk Factors of food Service Establishments in Selected Boarding High Schools, Ibadan Nigeria" has been reviewed by the Oyo State Ethical Review Committee.

2. The committee has noted your compliance. In the light of this, I am pleased to convey to you the full approval by the committee for the implementation of the Research Proposal in Oyo State, Nigeria.

3. Please note that the National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations, in line with this, the Committee will monitor closely and follow up the implementation of the research study. However, the Ministry of Health would like to have a copy of the results and conclusions of findings as this will help in policy making in the health sector.

4. Wishing you all the best.

Signature & Date

Dr. Abbas Gbolahan
Director, Planning, Research & Statistics
Secretary, Oyo State, Research Ethical Review Committee

APPENDIX 3: KEY INFORMANT INTERVIEW GUIDE

ENVIRONMENTAL HYGIENE AND MICROBIOLOGICAL STUDIES OF FOOD SERVICE ESTABLISHMENTS IN SELECTED BOARDING HIGH SCHOOLS IN IBADAN, NIGERIA

KEY INFORMANT INTERVIEW FOR KITCHEN HEAD, KITCHEN SUPERVISOR AND MATRON

School name:…… Local Government Area:…….School Population:……..

School Representative:……..

1. What is your position in the school?
2. How long have you been in the school?
3. Do you know about school health programme?
4. Can you tell me about your school feeding programme, when it started and its implementation?
5. How do you ensure raw materials are sourced from safe sources and are stored properly?
6. How do you recruit your kitchen staff and how long do they stay?
7. How do you ensure water is safe for use in the school environment, type of treatment and distribution?
8. What are the plans or facilities for waste/refuse disposal?
9. What sort of accommodation is available in school for kitchen staff?
10. Do you think there is need to improve your school food service programme and what will be your roles to accomplish this?
11. What is the most essential takeaway from this interview for you to share with us?
12. Is there anything else that you would like to add about what we have discussed or other areas we did not discuss but you think are important?

APPENDIX 4: OBSERVATIONAL CHECKLIST

Checklist for evaluating the sanitation of the kitchen, dining area, toilet, and observed food handlers at work in boarding schools' FSEs.

1. Name of School…….2. Type of School (day/boarding or full boarding) …… 3. Private or Government managed…….

4. Urban/Rural of community……5. Local Government Area…….

6. Total Boarders Population…… 7. Total number of food handlers……

8. **Kitchen Sanitation (P1)**

Item	Description	Yes	No
1.	Food preparation takes place in the kitchen		
2.	The kitchen is enclosed		
3.	Cooking materials, such as firewood and other unwanted objects, might be stored in a kitchen.		
4.	Untidy and unclean kitchen interior		
5.	The presence of domesticated animals within the kitchen		
6.	Signs of pest infestation (rats and cockroaches) in the kitchen		
7.	Toilets located in separate building		
8.	Kitchen has water distribution pipes		
9.	Availability of handwashing stations		
10.	Dirty dishes are washed in a basin or bucket.		
11.	Provision of on-site sink for dish washing		
12.	Kitchen flooring is made of concrete		

13.	Cobwebs found on walls of the kitchen		
14.	Lighting and ventilation are adequate		
15.	Kitchen floor is clean		
16.	Covered solid waste storage bins		
17.	Ceilings and walls are properly maintained		
18.	Availability of sanitising agents		

9. **Toilet Hygiene (P2)**

Item	Description	Yes	No
1	Toilet is available		
2	Taps are available Running tap are working		
3	Water storage vessels are available		
4	Flooded toilet floor		
5	Worn out materials like old items are kept in the toilet		
6	Wash hand basins are available		
7	Overall hygiene assessment is good		

10. Dining Area Assessment (P3)

Item	Description	Yes	No
1.	Separate dining hall is available		
2.	Dining hall is adequately furnished		
3.	Dining hall is enclosed		

4.	All students are sited in the dining hall at meal time		
5.	Dining hall floor are not wet nor littered		
6.	Floor and tables are littered with food remnants and unwashed plates		
7.	Ceilings are walls are adequately maintained		
8.	Availability of potable water for drinking		
9.	Handwashing stations are available		
10.	Soap is available at handwashing stations		
11.	Animals and pests are present in the dining area		

11. Observed Food Handlers at work (P4)

Item	Description	Yes	No
1	The food handlers put on the proper hair covering, clean overall/uniform		
2	The food handlers wear rings watches fingernail polish bandages		
3	The food handlers show signs of: illness cough boils cuts or wound		

4	Availability of handwashing facilities for food handlers near their work stations		
5	The food handlers wash their hands often when soiled or contaminated		
6	The food handlers Practice handwashing frequently Hair cover is properly worn Face, nose and ear is minimally touched		
7	There is controlled traffic in the kitchen		
8	Visitors are properly dressed in personal protective equipment		

APPENDIX 5: FOOD HANDLERS QUESTIONNAIRE

Consent Form

ENVIRONMENTAL HYGIENE AND MICROBIOLOGICAL STUDIES OF FOOD SERVICE ESTABLISHMENTS IN SELECTED BOARDING HIGH SCHOOLS IN IBADAN, NIGERIA

Dear Participant,

My name is Kafayat Adebayo, am a graduate student in the Department of Microbiology at the University of Ibadan. This research is on environmental hygiene and microbiological studies of Food Service Establishments in selected boarding high schools in Ibadan, Nigeria.

The outcomes of the research will be very useful in furthering knowledge, improving safety and hygiene, and decreasing the risks of foodborne disease in boarding schools. Your sincere answers to the questions below will be highly valued.

You may choose not to participate in this survey. You have the option to leave the study at any time if you so desire. If you want to participate, please be aware that all information you provide is confidential not to be disclosed to individuals, schools, administrators, or school officials.

Your assistance has been much appreciated.

The above information is all clear to me, and I am prepared to partake in the survey.

Signature……. Date ……

.

LOCATION …… SERIAL NO ……

NOTE: DO NOT PROVIDE YOUR PERSONAL NOR SCHOOL DETAILS ON THIS QUESTIONNAIRE

Directions

- Answer correctly
- Fill in the blanks
- Check the appropriate boxes

SECTION A SOCIO-DEMOGRAPHIC INFORMATION

1. Age as at last birthday ---------- (in years)
2. Gender (1) Male ( ) (2) Female ( )
3. Marital Status (1) Single ( ) (2) Married ( ) (3) Separated ( ) (4) Divorced ( ) (5) Widow
4. Religion (1) Christianity ( ) (2) Islam (3) Traditional ( ) (4) Others, please specify…….
5. Ethinicity (1) Yoruba ( ) (2) Hausa ( ) (3) Igbo ( ) (4) Others ( )
6. Level of Education (1) No formal Education ( ) (2) Primary ( ) (3) Secondary ( ) (4) Tertiary ( )
7. Designation/rank (a) Cook ( ) (b) Kitchen Assistant ( ) (c) Matron (d) Others ( )
8. Years of experience as food handler ----------
9. Employment status (1) Permanent ( ) (2) Contract ( ) (3) Volunteer ( ) (4) Others, please specify…….
10. Skill acquisition methods (a) Catering school ( ) (b) Home ( ) (c) Mentoring ( )
11. Reasons for choice of profession (a) Personal interest ( ) (b) No other option( ) (c) Others, please specify…….

SECTION B: FOOD SAFETY TRAINING AND MEDICAL EXAMINATION

ON-JOB FOOD HANDLERS' TRAINING

12. Have you been trained on hygienic handling food and its safety? (1) Yes ( ) (2) No ( ). If no, go to question 17.
13. What kind of food hygiene and safety training did you receive?? (1) Formal training at which you were given certificate ( ) (2) Informal training at which you were not given certificate ( ) (3) In–House training organised by your employer ( ) (4) Others, kindly state……
14. Who organised the training? (1) Your Institution (2) Government agency in food safety (3) Non-Governmental Organisations (4) Others, kindly state……
15. How long did the training take? (1) 1 day (2) 2 days (3) 3 – 5 days (4) > 5 days
16. How many times have you gone through this type of training on the job?? (1) Once (2) Two to five times (3) More than five times

MEDICAL EXAMINATION OF FOOD HANDLERS

17. Before commencing your career as a food handler, did you have a pre-employment medical exam? (1) Yes ( ) (2) No ( )
18. Did you have any form of medical examination during your career? (1) Yes ( ) (2) No ( ) If No, go to question 23.
19. If yes to question 18, when was your most recent medical check? (Please indicate in months?)……..
20. Kindly select the types of tests you did? You can tick more than one option (1) Tuberculosis (2) HIV (3) Hepatitis A (4) X ray (5) Eye test (6) Stool culture
21. Did a trained medical professional certify that you were fit as a food worker? (1) Yes ( ) (2) No ( )
22. Have you had any FBD in the previous six months? (a) Yes ( ) (b) No ( )

SECTION C: ENVIRONMENTAL HYGIENE AND SAFE FOOD HANDLING

23. Which of the following water source does the school use for its students? You have the option of selecting multiple choices. (1) Spring ( ) (2) Stream ( ) (3) Unprotected Well ( ) (4) Protected Well ( ) (5)Hand pump Borehole ( ) (6) Motorised borehole ( ) (7) Tap ( ) (8) Others, kindly state……
24. What is the distance between where you get water and where the food is prepared? (1) Less than 250m ( ) (2) Between 250m and 500m ( ) (3) Between 500m and 1km ( ) (4) Greater than 1km ( )
25. What are your schools' method(s) of sewage disposal? (It is possible to select more than one option) (1) Pit Latrine ( ) (2) Septic Tank ( ) (3) Pour Flush ( ) (4) Throw into bush ( ) (5) Others, please specify…….
26. How far do you have to walk from the waste disposal location(s) to where meals are prepared? (1) Less than 250m ( ) (2) Between 250m and 500m ( ) (3) Between 500m and 1km ((4) Greater than 1km ( )
27. Are there domesticated animals within the space where food is prepared? (a) Yes ( ) (b) No ( )
28. If Question 27 is yes, what kind of domesticated animal(s) can you find? Kindly state……..
29. How does your school dispose of garbage? (You have the option of selecting multiple choices) (a) Throwing on empty land ( ) (b) Organised waste collector ( ) (c) Throwing in dug ground ( ) (d) Other, kindly state…….
30. How do you dispose of leftover food?.....
31. Do you have pest control techniques in place?? (1) Yes ( ) (2) No ( )
32. If Question 31 is yes, what techniques of controlling pest is available? Kindly state

    ……
    ……
33. Is there a separate location for preparing raw and cooked foods? (1) Yes ( ) (2) No ( )

34. Are the surfaces used to prepare raw and cooked foods separate? (1) Yes ( ) (2) No( )

35. What kind(s) of cooking fuels do you use? You have the option of selecting multiple choices. (1) Firewood ( ) (2) kerosene ( ) (3) Bottled Gas ( ) (4) Charcoal (5) Other, kindly state ……

SECTION D

KNOWLEDGE ABOUT RISK FACTORS ASSOCIATED WITH FOOD HANDLING

36. Kindly check as "Correct" or "Wrong" if the following diseases are caused by foodborne microorganisms. If you're unsure, mark "don't know."

NO.	**DISEASES**	**Correct**	**Wrong**	**Don't know**
1	Tuberculosis			
2	Dysentery			
3	Cholera disease			
4	HIV/AIDS			
5	Diabetes mellitus			
6	Malaria			
7	High Blood Pressure			
8	Hepatitis A			
9	Diarrhoea			
10	Typhoid fever			
11	Worm Infestation			

37. Symptoms of diseases are listed in the following table. State whether the disease(s) is (are) below are caused by foodborne pathogens by checking "True" or "False." If you're unsure, mark "don't know."

Symptoms	True	False	Don't know

Backache			
Chest pain			
Stomach-aches			
Vomiting			
Watery stool			
Sore throat			
Skin lesions (boils, cut)			

38. The following is a list of statements regarding food handling techniques. Examine each statement carefully and mark if it is True or False. If you're unsure, select "Don't know."

	STATEMENTS	**True**	**False**	**Don't Know**
1	Food contamination is unlikely to occur if food is prepared in advance before serving.			
2	Food contamination might occur as a result of a prolong service period.			
3	The risk of foodborne illness is not increased by the inadequate cleaning of equipment, such as a grinding machine.			
4	Handwashing is only necessary after food preparation process, not prior to food preparation.			
5	Frozen meat should be thawed at room temperature overnight.			
6	To prevent the growth of food pathogens, cooked food should not be kept between 5°C and 65°C.			

7	Insect like houseflies, ants and cockroaches can spread foodborne pathogens			
8	Food hygiene can be impaired by food handlers with long fingernails and exposed hair..			
9	Cooked and uncooked foods should be preserved separately			
10	Food can get contaminated through improper handling by food handlers.			
11	Meats should be placed on the lower shelves of the refrigerator, while vegetables should be kept at the top.			
12.	Handwashing before touching food helps to keep the food safe to eat.			

39. The diseases listed below must be reported by food workers because they can cause foodborne illness if infected. To indicate "Correct" or "Wrong" select a checkbox. Tick "Don't know" if you are unsure.

S/N	**DISEASES**	**Correct**	**Wrong**	**Don't know**
1	Skin Infection			
2	Infected Ear			
3	Diabetes mellitus			
4	Cough and Catarrh			
5	Tuberculosis			
6	High Blood Pressure			
7	Diarrhoea			
8	Hepatitis A			
9	Sore throat			
10	HIV/AIDS			

11	Dysentery			
12	Malaria			

SECTION E: ATTITUDES OF FOOD HANDLERS TOWARDS FOOD HYGIENE

40. The following table contains a list of statements describing food handler's perceptions on food safety and sanitation. Please indicate whether or not you "Agree" or "Disagree" by clicking the appropriate box. If you aren't sure what the response should be, you can check the "Uncertain" box.

S/N	STATEMENTS ABOUT ATTITUDES TOWARDS FOOD HYGIENE	Agree	Disagree	Uncertain
1	In order to prevent cross-contamination, cooked food should be preserved separately from uncooked food.			
2	Refreezing already-thawed food can result in food contamination.			
3	The risk of contaminating food decreases when personal protective equipment (PPE) such as a head scarves, face masks, and hand gloves are used			
4	Expired food must be thrown away.			
5	Knowledge and training are crucial inorder to assure food safety			
6	Incidences of foodborne disease are natural life events.			
7	Food handlers should not put on clean overall regularly during food preparation			
8	Same cutting board and knife should not be used for raw vegetables and meat			

9	Pest control is part of requirements to achieve food safety			
10	Unwrapped foods should not be touched by food handlers with open wounds			
11	Food handlers good personal hygiene will help to ensure food safety			
12	Apron can be used to wipe hands after washing them			

SECTION F: FOOD HYGIEENE PRACTICES INCLUDING HANDWASHING BY FOOD HANDLER

41. Hands are washed when food is being prepared (a) Yes ( ) (b) No ( )
42. If yes to question 41, Kindly score your handwashing technique on the following five criteria? Please indicate by choosing Always, Most times, Sometimes, Rarely or Never

S/N	CRITICAL POINTS	Always	Most times	Sometimes	Rarely	Never
1	Before you begin cooking					
2	After you finish cooking					
3	Prior to handling prepared or Ready-to-Eat meals					
4	After touching skin, ear and face					
5	After you have sneezed or coughed					

6	After cleaning tasks or refuse/waste disposal					
7	Before you leave place where food is prepared					
8	When entering the place where food is prepared					
9	Prior to removing wrappings on raw food					
10	After removing wrappings on raw food					
11	When you taste cooked meals					
12	After using the toilet					

43. The table below shows the many methods and materials for handwashing and drying. Please mark the boxes that apply to your handwashing habits.

S/N	**Methods of washing Hands**	**Always**	**Most times**	**Sometimes**	**Rarely**	**Never**
1	Water					
2	Water and soap (bar)					
4	Water and liquid soap					
5	Use of alcohol-based hand sanitizer					
6	Single-use paper towel					

7	Reusable Hand towel					
8	Automated hand dryer					

44. Do you look at the date of expiry on packaged food when being bought? (1) Yes ( ) (2) No ( )
45. What do you think about storing below foods? (1) Together ( ) (2) Separate ( ) (3) There is no specific storing method ( )
46. Is the temperature of refrigerator/freezer where uncooked foods are kept monitored? (1) Yes ( ) (2) No ( ) If no, go to question 50.
47. If yes to question 46, which device(s) is available to determine the temperature of food? .....
48. At what temperature is chilled food items kept? .....
49. At what temperature should prepared or reheated food be stored? …….
50. Do you keep your kitchen space and work surfaces clean? (1) Yes ( ) (2)No ( )
51. If yes to question 50, when do you clean the kitchen space and work surfaces? (1) Onset of cooking only ( ) (2) completion of cooking only ( ) (3) Onset and completion of cooking ( )
52. Are your meals prepared ahead of time before serving it? (1) Yes ( ) (2) No ( )
53. If yes to question 52, for how long is the food kept in warmers before service? (Please provide the time in hours)…….
54. Is your meal reheated before serving it? (1) Yes ( ) (2) No ( )
55. If yes in 54, which method do you use? ……..
56. Do you take time off work if you're sick or suffering from a specific illness?(1) Yes ( ) (2) No ( )
57. If yes to question 56, which illnesses did you suffer from that made you take some time off work? (Please specify) …….

58. Is there a pest management programme in place for the food preparation and dining area? (1) Yes (2) No ( )
59. If yes question 58, what kind of pest management programme does your school utilise? (Please specify) ……
60. When you're cooking or handling food, do you wear any personal protection equipment? (1) Yes (2) No ( )
61. If yes to question 61, which kind(s) of protection equipment do you put on? (Kindly mention) ……
62. School Status (1) Public (2) Private
63. School location (1) Rural (2) Urban

APPENDIX 6: CULTURE MEDIA AND REAGENTS

CULTURE MEDIA

Baird Parker Agar Base	**Ingredients g/L**
Casein enzymic hydrolysate	10.0
Meat extract	5.0
Yeast extract	1.0
Glycine	12.0
Sodium pyruvate	12.0
Lithium chloride	5.0
Agar	20.0

Final pH (at 25°C) 7.0±0.2

Peptone water	**Ingredients g/L**
Peptone Water	10.0
Peptic digest of animal tissue:	5.0

Final pH (at 25°C) 7.4±0.2

Buffered Peptone Water	**Ingredients g/L**
Casein enzymic hydrolysate	10.0
Sodium chloride	5.0
Disodium hydrogen phosphate.12H2O	9.0
Monopotassium hydrogen phosphate:	1.5

Final pH (at 25°C) 7.1±0.1

MacConkey Agar	**Ingredients g/L**
Peptic digest of animal tissue	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.07
Agar	15.0
Final pH at 25°C) 7.5±0.2	

Nutrient Agar	**Ingredients g/L**
Glucose	1.0
Peptic digest of animal tissue	5.0
Beef extract	3.0
Agar	15.0
Final pH at 25°C) 7.2±0.2	

Saline Peptone Water	**Ingredients g/L**
Peptone	1
NaCl	8.5
Sterile distilled water	1000 mL

Normal saline/ Physiological saline	**Ingredients g/L**
NaCl	0.85g
Distilled water	100 mL

Mueller Hinton Agar	**Ingredients g/L**
Peptone	17.5
Starch	1.5
Solids of meat infusion	2.0
Agar	15.0

Brain Heart infusion broth	**Ingredients g/L**
Calf brain, infusion	200.0
Beef heart, infusion	250.0
Proteose peptone	10.0
Dextrose	2.0
Sodium chloride	5.0
Sodium Phosphate (Na2HPO4)	2.5

pH 7.4 ± 0.1

Eosin Methylene Blue Agar	**Ingredients g/L**
Peptic digest of animal tissue	10.0
Dipotassium phosphate	2.0
Lactose	5.0
Sucrose	5.0
Eosin – Y	0.4
Methylene blue	0.065
Agar	13.5

Mannitol Egg Yolk Polymixin Agar	**Ingredients g/L**
Beef extract	1.0
Peptone	10.0
Mannitol	10.0
Sodium Chloride	10.0
Phenol red (1% solution in 95% ethanol)	2.5 ml
Agar	15.0
Distilled water	900 Ml

MYP agar was sourced commercially from Oxoid, UK.

Polymyxin B solution, 0.1%

A 500,000 units polymyxin B sulfate (Oxoid, UK) was dissolve in 50 ml distilled water. Filter-sterilised and stored in the dark at 4°C until needed.

Egg yolk emulsion, 50% (Oxoid, UK)

Final medium

A volume of 225 ml melted base was added to 2.5 ml polymyxin B solution and 12.5 ml egg yolk emulsion. The mixture was dispensed in 18 ml portions sterile petri dishes. The plates were dried at room temperature for 24 h before use.

Salmonella-Shigella Agar	**Ingredients g/L**
Lactose	10.0
Bile salts no.3	8.5
Sodium citrate	8.5

Sodium thiosulfate	8.5
Beef extract	5.0
Proteose peptone	5.0
Ferric Citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	13.5

Buffers and Reagents

a. TE Buffer

Tris – Cl (pH 8.0)	100 mM
EDTA (pH 8.0)	10 mM
Tris-Saturated phenol (pH 8.0)	100 µL

b. TBE Buffer

Tris Base	108g
Boric acid	55g
0.5M EDTA (pH 8.0)	40 mL

c. 0.5M EDTA

Disodium EDTA	186.1g
Distilled water	800 mL
NaOH (for pH adjustment)	18-20 g

Dilute solution to 1 litre with distilled water and filter through 0.5-micron filter.

d. McFarland Standard

0.048M $BaCl_2$	0.5 mL
0.18M H_2SO_4	99.5 mL

Acceptable Optical Density at 625nm is 0.08-0.13

e. Potassium chromate indicator solution

5 gm of potassium chromate was dissolved in a l00 ml distilled water, silver nitrate solution was added to obtain a definite red precipitate. It was allowed to stand for 12 hours, filtered and diluted to 1 liter with distilled.

water.

f. Standard silver nitrate titrant — 0.0141 N. 2.395 gm of silver nitrate was dissolved in distilled water and diluted to 1 liter. This was then standardised against 0.0141N sodium chloride solution and stored in a brown bottle. 1.00 mL = 500 μg of chloride.

g. Vanado-Molybdate reagent – 25 gm of ammonium molybdate (NH4)6 MoO24·4H2O was dissolved in 200 ml hot water and cooled. Also, 1.25 g of NH4 metavanadate was dissolved in 120 ml hot water. Cool and add 250ml to the vanadate solution add dilute to 1 litre.

Phosphate standard solution: stock 1000 ppm P. A known weight of 0.4374 gm dry anhydrous KH2PO4 dissolved in distilled water and diluted to 1 litre. The solution was stored in a dark brown Pyrex glass bottle in a cool place.

APPENDIX 7: BIOCHEMICAL CHARACTERISTICS OF ISOLATED BACTERIA

Biochemical characterisation of Bacterial isolated from selected schools' kitchen in Ibadan

Gram-Negative Bacteria

SCHOOL	SOURCE	TSI SLANT	TSI BUTT	H_2S	GAS	MOTILITY	INDOLE	UREASE	OXIDASE	CITRATE	PROBABLE BACTERIA IDENTITY
A	Amala	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
A	Amala	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
A	Amala	K	A	+	-	+	-	-	+	+	*Salmonella paratyphi*
A	Amala	K	K	-	-	+	-	+	+	+	*Pseudomonas aeruginosa*
A	Bread	A	A	-	+	+	+	-	-	-	*Escherichia coli*
A	Bread	A	A	-	-	-	-	-	-	-	*Klebsiella pneumoniae*
A	Chopping Board	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
A	Chopping Board	K	A	+	-	+	-	-	-	-	*Salmonella paratyphi*
A	Chopping Board	A	A	-	+	+	+	-	-	-	*Escherichia coli*
A	Chopping Board	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
A	Dining Table	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
A	Dining Table	A	A	-	+	+	+	-	-	-	*Escherichia coli*
A	Fried Egg	A	A	-	+	+	+	-	-	-	*Proteus vulgaris*
A	Fried Egg	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
A	Fried Egg	K	A	-	-	+	-	+	+	+	*Proteus mirabilis*
A	Fried Egg	K	K	-	-	+	-	+	+	+	*Pseudomonas aeruginosa*
A	Food Handler	A	A	-	+	+	-	-	-	-	*Escherichia coli*
A	Food Handler	K	A	+	-	+	-	+	+	+	*Proteus mirabilis*
A	Food Handler	K	A	+	-	+	-	-	+	+	*Escherichia coli*

Biochemical characterisation of Bacterial isolated from selected schools' kitchen in Ibadan (Continued)

A	Food Handler	A	A	-	+	-	-	+	-	-	*Klebsiella pneumoniae*
A	Food Handler	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
A	Grinder	A	A	-	+	+	+	-	-	-	*Escherichia coli*
A	Grinder	K	K	-	-	+	-	+	+	+	*Pseudomonas aeruginosa*
A	Grinder	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
A	Grinder	K	A	-	+	+	+	-	-	-	*Escherichia coli*
A	Knife	K	A	+	-	-	-	+	+	+	*Proteus mirabilis*
A	Knife	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
A	Spaghetti	A	A	-	+	+	+	-	-	-	*Escherichia coli*
A	Tray	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
A	Tray	A	A	-	+	+	+	-	-	-	*Escherichia coli*
A	Tray	A	A	-	+	+	+	-	-	-	*Escherichia coli*
B	Chopping Board	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
B	Chopping Board	A	A	-	+	+	+	-	-	-	*Escherichia coli*
B	Chopping Board	A	A	-	+	+	+	-	-	-	*Escherichia coli*
B	Food Handler	A	A	-	+	+	+	-	-	-	*Escherichia coli*
B	Food Handler	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
B	Food Handler	K	A	-	-	-	-	-	-	+	*Serratia marcescens*
B	Food Handler	A	A	-	+	+	+	-	-	-	*Escherichia coli*
B	Food Handler	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
B	Food Handler	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
B	Food Handler	K	A	-	+	+	+	-	-	-	*Escherichia coli*
B	Knife	A	A	-	-	-	-	-	-	-	*Klebsiella pneumoniae*
B	Knife	K	A	-	-	-	-	-	-	+	*Serratia marcescens*
B	Knife	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
B	Knife	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
B	Knife	A	A	-	+	+	+	-	-	-	*Escherichia coli*

Biochemical characterisation of Bacterial isolated from selected schools' kitchen in Ibadan (Continued)

B	Tray	A	A	-	+	+	+	-	-	-	*Escherichia coli*
B	Tray	A	A	-	+	+	+	-	-	-	*Escherichia coli*
C	Chopping Board	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
C	Chopping Board	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
C	Chopping Board	K	A	+	+	+	-	-	-	-	*Escherichia coli*
C	Countertop	A	A	-	+	+	+	-	-	-	*Escherichia coli*
C	Countertop	A	A	-	+	+	+	-	-	-	*Escherichia coli*
C	Dining Table	K	A	-	+	+	-	-	-	-	*Proteus vulgaris*
C	Dining Table	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
C	Dining Table	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
C	Dining Table	K	A	+	-	+	-	-	+	+	*Salmonella paratyphi*
C	Food Handler	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
C	Food Handler	k	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
C	Food Handler	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
C	Fish Stew	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
C	Fish Stew	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
C	Fish Stew	A	A	-	+	+	+	-	-	-	*Salmonella paratyphi*
C	Fish Stew	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
C	Fish Stew	K	K	-	-	+	-	+	+	+	*Pseudomonas aeruginosa*
C	Fish Stew	A	A	-	+	+	+	-	-	-	*Escherichia coli*
C	Fish Stew	A	A	-	+	+	+	-	-	-	*Escherichia coli*
C	Fish Stew	A	A	-	+	+	+	-	-	-	*Escherichia coli*
C	Grinder	A	A	-	+	+	+	-	-	-	*Escherichia coli*
C	Grinder	K	A	+	-	+	-	+	+	+	*Proteus mirabilis*
C	Knife	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*

Biochemical characterisation of Bacterial isolated from selected schools' kitchen in Ibadan (Continued)

C	Knife	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
C	Tray	K	A	+	-	+	-	-	+	+	*Salmnella typhi*
C	Tray	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
D	Chopping Board	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Chopping Board	K	A	+	+	+	-	-	-	-	*klebsiella pneumoniae*
D	Chopping Board	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Chopping Board	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Countertop	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
D	Countertop	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
D	Cooking Water	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Food Handler	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Grinder	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Knife	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
D	Knife	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Knife	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
D	Tray	A	A	-	+	+	-	-	-	-	*Klebsiella pneumoniae*
D	Bread	K	A	+	-	+	-	+	+	+	*Proteus mirabilis*
D	Bread	K	A	+	-	+	-	+	+	+	*Proteus mirabilis*
D	Countertop	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
D	Chopping Board	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
D	Chopping Board	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Chopping Board	k	A	-	+	+	-	-	+	+	*Salmonella typhi*
D	Chopping Board	K	A	+	-	+	-	-	+	+	*Proteus mirabilis*
D	Chopping Board	K	K	-	-	+	-	+	+	+	*Pseudomonas aeriginosa*
D	Countertop	K	A	-	-	-	-	-	-	+	*Serratia marcescens*
D	Countertop	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Countertop	A	A	+	-	+	-	-	+	+	*Salmonella typhi*

Biochemical characterisation of Bacterial isolated from selected schools' kitchen in Ibadan (Continued)

D	Countertop	K	A	-	+	-	-	+	-	-	*Salmonella paratyphi*
D	Countertop	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Cooking Water	K	A	+	-	+	-	+	+	+	*Proteus mirabilis*
D	Drinking Water	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
D	Drinking Water	K	A	+	-	+	-	-	+	+	*Salmonella typhi*
D	Drinking Water Tap	K	A	-	+	+	-	-	-	-	*Salmonella paratyphi*
D	Drinking Water Tap	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Food Handler	A	A	-	+	+	+	-	-	-	*Salmonella typhi*
D	Food Handler	K	A	+	-	+		-	+	+	*Salmonella typhi*
D	Food Handler	K	A	-	-	-	-	-	-	+	*Proteus mirabilis*
D	Food Handler	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Food Handler	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Food Handler	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Food Handler	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Food Handler	K	A	+	-	+	-	+	+	+	*Proteus mirabilis*
D	Food Handler	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
D	Food Handler	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Food Handler	K	A	+	-	+	-	+	+	+	*Proteus mirabilis*
D	Food Handler	K	A	+	+	+	-	-	-	-	*Salmonella paratyphi*
D	Food Handler	K	A	-	-	-	-	-	-	+	*Salmonella paratyphi*
D	Food Handler	A	A	-	+	+	+	-	-	-	*Escherichia coli*
D	Grinder	K	A	+	-	+	-	+	+	+	*Proteus mirabilis*
D	Knife	A	A	-	+	+	+	-	-	-	*Escherichia coli*

Biochemical characterisation of Bacterial isolated from selected schools' kitchen in Ibadan (Continued)

D	Soup	K	K	-	-	+	-	+	+	+	*Pseudomonas aeruginosa*
D	Semolina	K	A	+	-	+	-	+	+	+	*Proteus mirabilis*
D	Tray	K	A	+	+	+	-	+	-	-	*Salmonella paratyphi*
D	Tray	K	A	+	-	+	-	+	+	+	*Proteus mirabilis*

Gram-Positive Bacteria

SCHOOL	SOURCE	CATALASE	COAGULASE	MANNITOL	OXIDASE	IDENTIFIED BACTERIA
A	Chopping Board	+	+	+	-	*Staphylococcus aureus*
A	Tray	+	+	+	-	*Staphylococcus aureus*
A	Fried Egg	+	+	+	-	*Staphylococcus aureus*
A	Chopping Board	+	+	+	-	*Staphylococcus aureus*
A	Chopping Board	+	+	+	-	*Staphylococcus aureus*
A	Grinder	+	+	+	-	*Staphylococcus aureus*
A	Grinder	+	+	+	-	*Staphylococcus aureus*
A	Knife	+	+	+	-	*Staphylococcus aureus*
A	Tray	+	+	+	-	*Staphylococcus aureus*
A	Amala	+	+	+	-	*Staphylococcus aureus*
B	Food Handler	+	+	+	-	*Staphylococcus aureus*
B	Treay	+	+	+	-	*Staphylococcus aureus*
B	Chopping Board	+	+	+	-	*Staphylococcus aureus*
C	Food Handler	+	+	+	-	*Staphylococcus aureus*
C	Chopping Board	+	+	+	-	*Staphylococcus aureus*
C	Fish Stew	+	-	-	-	*Staphylococcus epidermidis*

C	Chopping Board	+	+	+	-	*Staphylococcus aureus*				
C	Chopping Board	+	+	+	-	*Staphylococcus aureus*				
C	Chopping Board	+	+	+	-	*Staphylococcus aureus*				
D	Tray	+	+	+	-	*Staphylococcus aureus*				
D	Grinder	+	+	+	-	*Staphylococcus aureus*				
D	Countertop	+	+	+	-	*Staphylococcus aureus*				
D	Chopping Board	+	+	+	-	*Staphylococcus aureus*				
D	Cooking Water	+	+	+	-	*Staphylococcus aureus*				
D	Chopping Board	+	+	+	-	*Staphylococcus aureus*				
D	Knife	+	+	+	-	*Staphylococcus aureus*				
D	Knife	+	+	+	-	*Staphylococcus aureus*				

		CATALASE	OXIDASE	INDOLE	CITRATE	IDENTIFIED BACTERIA	GLUCOSE	XYLOSE		GALACTOSE
C	Chopping Board	+	-	-	+	*Bacillus cereus*		+	-	-
D	Food Handler	+	+	-	+	*Bacillus subtilis*		+	+	+

APPENDIX 8: ZONES OF INHIBITION BY DISC DIFFUSION ASSAY

Zones of Inhibition by Disc Diffusion Assay for Gram-Positive (mm)

Lab Code	Presumptive Identity	CXM	OFL	AUG	NIT	CPR	CAZ	CRX	GEN
PCHB	*Salmonella typhi*	40	22	36	R	40	40	18	15
PCHB1	*Salmonella typhi*	40	25	24	10	40	44	34	24
PTRY3	*Salmonella typhi*	36	20	36	10	36	40	42	15
PFH5A	*Klebsiella pneumoniae*	R	25	R	R	30	R	R	30
PFH5B	*Salmonella paratyphi*	R	26	R	R	26	R	R	R
GFH2C	*Escherichia coli*	R	20	R	R	20	15	R	18
GKN1	*Klebsiella pneumoniae*	25	23	20	18	32	21	R	15
PBR2	*Klebsiella pneumoniae*	30	28	20	19	20	20	11	15
PFEG2	*Salmonella typhi*	40	22	34	R	36	30	26	12
MVFHA3	*Salmonella typhi*	R	16	20	R	16	16	16	18
MVFHC1	*Escherichia coli*	40	20	32	R	30	32	25	16
MVFHC2	*Escherichia coli*	20	2o	16	R	24	24	R	16
MVCTT2	*Salmonella typhi*	R	22	35	R	25	28	16	30
PGRM4	*Escherichia coli*	46	26	38	R	30	33	32	18
TFS	*Salmonella typhi*	R	33	R	R	43m	R	R	32
GFH2B	*Serratia marcescens*	R	38	R	R	37	R	R	30
GKN1	*Serratia marcescens*	R	38	32	40	40	R	36	40
MVCTT1	*Serratia marcescens*	28	32	R	R	40	38	R	20
PGRM1	*Salmonella paratyphi*	R	20	26	18	18	20	R	R
GFH2A	*Salmonella typhi*	30	30	30	30	42	33	R	18
GKN2	*Salmonella typhi*	R	26	R	R	32	25	R	22
TFS1	*Salmonella paratyphi*	R	20	38	44	28	30	20	R
TFS2	*Salmonella typhi*	40	20	40	12	35	R	25	28
TDNT1	*Salmonella paratyphi*	R	20	22	12	20	12	R	R

TDNT2	*Salmonella paratyphi*	R	15	17	R	12	20	R	20
TFH4	*Salmonella paratyphi*	R	20	30	25	25	22	R	R
TFH3	*Salmonella paratyphi*	R	20	28	18	18	21	16	R
TKN	*Salmonella paratyphi*	R	30	36	22	30	21	R	R
TTRY	*Salmnella typhi*	R	21	40	22	23	22	14	R
PAM1	*Salmonella paratyphi*	R	22	32	18	22	17	12	R
PAM2	*Pseudomonas aeroginosa*	R	17	20	R	17	26	R	20
GKN1	*Salmonella typhi*	R	20	323m	30	30	18	R	R
GKN2	*Escherichia coli*	R	25	30	16	32	18	R	R
TDNT3	*Salmonella typhi*	R	20	22	13	20	22	R	R
PFEG2	*Proteus mirabilis*	R	16	24	15	24	16	R	R
PSPG	*Escherichia coli*	R	R	R	R	20	16	R	R
PFH1A	*Escherichia coli*	R	16	40	12	22	R	40	32
PFH4	*Proteus mirabilis*	R	38	12	21	39	R	16	25
MVBR1	*Proteus mirabilis*	R	13	R	12	20	16	R	R
MVBR2	*Proteus mirabilis*	R	20	16	12	20	R	R	R
MVSMLN1	*Proteus mirabilis*	R	30	R	27	26	16	R	22
MVCCT2	*Salmonella paratyphi*	R	20	22	12	16	26	R	R
MVCHB2	*Escherichia coli*	R	16	23	12	22	18	R	R
MCW	*Escherichia coli*	R	20	36	12	20		R	R
MVFHC1	*Proteus mirabilis*	10	20	28	16	36	35	R	R
MVFHC2	*Proteus mirabilis*	R	18	R	R	20	22	R	R
MVTRY2	*Salmonella paratyphi*	R	19	R	13	18	16	R	R
MVSLP2	*Pseudomonas aeroginosa*	R	16	12	R	20	15	R	R
MVDW4	*Salmonella typhi*	R	15	15	12	20	20	R	R
MVFHD1	*Salmonella paratyphi*	R	40	22	29	22	37	30	14
MVCW2	*Proteus mirabilis*	28	20	20	R	40	R	R	R

MVDWT	*Salmonella paratyphi*	R	20	28	12	20	13	R	R
TFS2	*Pseudomonas aeriginosa*	R	15	33	R	16	R	R	R
TCHB1	*Salmonella typhi*	R	R	R	R	R	R	R	R
TFH4	*Salmonella paratyphi*	R	12	R	R	R	R	R	13
GFH3A	*Salmonella paratyphi*	R	20	28	15	25	25	R	R
GFH3B	*Salmonella typhi*	R	20	R	R	20	20	R	R
GCHB2	*Escherichia coli*	R	16	16	15	20	20	R	R
TGRM	*Escherichia coli*	R	22	R	12	20	20	R	R
PCHB	*Salmonella paratyphi*	R	20	R	12	20	R	R	R
PKNI	*Salmonella typhi*	R	20	R	R	18	R	R	R
MCHB1	*Escherichia coli*	R	22	12	26	26	R	12	20
MCHB2	*Escherichia coli*	R	18	22	20	20	20	R	R
MVCHB3	*Proteus mirabilis*	R	28	R	16	23	25	20	20
MCTT1	*Salmonella typhi*	R	20	25	20	20	23	R	R
MCTT2	*Salmonella typhi*	R	16	R	12	16	18	R	R
MKN1	*Salmonella paratyphi*	R	18	R	16	20	19	R	R
MKN2	*Salmonella typhi*	R	16	16	12	16	20	R	R
MGRM	*Escherichia coli*	R	15	14	22	21	R	R	R
PGRM	*Escherichia coli*	R	20	25	12	20	22	R	R
MTRY2	*Klebsiella pneumoniae*	33	32	28	16	30	30	12	14
PKN2	*Proteus mirabilis*	R	40	R	14	38	23	R	17
MKN1	*Escherichia coli*	40	40	36	40	40	36	17	23
TCTT1	*Escherichia coli*	40	40	28	40	40	37	16	25
MCHB	*Escherichia coli*	32	40	36	40	40	36	20	22
TCTT2	*Escherichia coli*	28	40	16	40	40	38	R	18
GTRY2B	*Escherichia coli*	33	40	22	29	40	30	18	18
GCHB2	*Escherichia coli*	15	36	R	29	40	32	R	20
PCHB	*Escherichia coli*	35	38	30	30	40	38	12	20

PDNT2	*Escherichia coli*	30	36	24	25	40	34	12	18
GTRY2	*Escherichia coli*	30	36	20	20	40	40	11	26
MVCHB1	*Salmonella paratyphi*	25	25	12	12	20	R	R	R
MVCHB2	*Salmonella typhi*	22	22	16	16	21	R	R	R
MVFHC1	*Salmonella paratyphi*	18	18	R	R	20	R	R	R
MVDW1	*Salmonella paratyphi*	28	28	27	27	25	R	R	R
PAM	*Salmonella paratyphi*	22	22	12	12	20	R	R	R
GBCW	*Salmonella paratyphi*	28	28	20	20	20	R	R	R
TTRY	*Salmonella paratyphi*	R	30	R	R	32	R	R	R
PFEG2	*Pseudomonas aeroginosa*	R	26	R	13	20	R	R	R
MVTRY2	*Proteus mirabilis*	R	30	R	12	36	R	R	R
MVGRM	*Proteus mirabilis*	R	30	R	13	30	R	R	R
TGRM	*Proteus mirabilis*	R	25	40	30	36	R	R	R
MVFHA5	*Proteus mirabilis*	R	18	R	R	20	R	R	12
MVCHB3	*Pseudomonas aeriginosa*	R	22	R	12	25	R	R	R
TCHB1	*Salmonella typhi*	R	30	R	13	28	R	R	R
PDNT	*Salmonella typhi*	R	18	R	11	20	R	R	R
MVCTT2	*Salmonella paratyphi*	R	40	R	R	30	R	R	24
MCHB	*klebsiella pneumoniae*	R	30	R	14	28	R	R	R
MVFHD1	*Salmonella paratyphi*	R	20	R	R	22	R	R	16
PGRM	*Pseudomonas aeroginosa*	R	26	R	16	30	R	R	28
TKN	*Salmonella typhi*	R	26	R	17	20	R	R	R
TDNT	*Proteus vulgaris*	R	24	R	26	26	R	R	R
PFH5	*Escherichia coli*	R	20	R	R	26	R	R	14
MVFHA	*Salmonella typhi*	20	18	R	13	30	32	R	20
MFHA2	*Escherichia coli*	36	36	18	30	40	31	14	16

MVFHB1	*Escherichia coli*	28	30	R	24	40	22	R	16
MVFHB2	*Escherichia coli*	30	38	R	30	40	32	R	18
MVCTT1	*Escherichia coli*	33	38	18	28	40	36	16	18
MVCTT2	*Escherichia coli*	30	28	R	R	40	28	R	20
MVDWT2	*Escherichia coli*	30	32	15	40	40	24	15	16
MVKN1	*Escherichia coli*	29	30	16	18	34	30	14	19
MVFHB4	*Escherichia coli*	28	30	R	14	36	34	R	26
MVFHD1	*Escherichia coli*	30	30	17	22	40	30	12	16
GFH2	*Escherichia coli*	R	24	16	15	30	22	R	R
GFH4	*Escherichia coli*	R	21	R	R	20	20	R	R
TFS1	*Salmonella paratyphi*	18	33	R	12	40	28	R	22
TFS2	*Escherichia coli*	30	35	R	15	40	36	R	24
TFS3	*Escherichia coli*	34	30	14	28	40	38	14	16
TFS4	*Escherichia coli*	R	20	R	13	18	18	R	R
TCHB1	*Escherichia coli*	24	20	R	R	16	12	R	R
PAM1	*Salmonella paratyphi*	R	20	25	16	24	18	R	R
PFEG	*Proteus vulgaris*	28	30	18	25	36	34	15	15
PBR	*Escherichia coli*	30	28	18	28	34	29	15	20
PTRY3	*Escherichia coli*	28	30	12	18	33	29	R	14
PTRY4	*Escherichia coli*	34	30	14	30	36	36	R	14

Zones of Inhibition by Disc Diffusion Assay for Gram-Positive Bacteria (mm)

Lab Code	Presumptive Identity	CTR	ERY	CXC	OFL	AUG	CAZ	CRX	GEN
PCHB1	*Staphylococcus aureus*	R	R	R	16	R	R	R	R
PTRY2	*Staphylococcus aureus*	20	35	16	20	40	R	30	40
GFH2A	*Staphylococcus aureus*	20	R	14	30	25	R	28	R
PFEG1	*Staphylococcus aureus*	22	R	16	30	40	R	32	40
MVCW1	*Staphylococcus aureus*	40	40	R	R	20	R	40	40
TCHB2	*Staphylococcus aureus*	15	20	R	21	28	R	28	30
TFS3	*Staphylococcus epidermidis*	40	R	R	20	R	R	R	R
MTRY1	*Staphylococcus aureus*	R	R	12	20	R	R	R	R
PCHB1	*Staphylococcus aureus*	37	R	R	40	40	20	23	18
PCHB2	*Staphylococcus aureus*	35	R	R	R	13	R	R	40
PGRM1	*Staphylococcus aureus*	R	32	R	10	R	R	R	R
PGRM2	*Staphylococcus aureus*	R	R	R	R	R	R	R	R
GTRY1	*Staphylococcus aureus*	18	R	R	32	36	R	R	37
MGRM2	*Staphylococcus aureus*	40	R	R	R	R	R	12	20
MVCCT	*Staphylococcus aureus*	20	R	15	30	40	R	40	40
MVCHB	*Staphylococcus aureus*	20	R	16	30	30	R	33	40
PKN1	*Staphylococcus aureus*	20	38	12	30	36	R	26	40
GBCHB	*Staphylococcus aureus*	22	30	12	20	30	R	30	38
TFH3	*Staphylococcus aureus*	16	28	19	23	30	R	24	28
MVCHB3	*Staphylococcus aureus*	R	12	R	14	30	R	R	R
MKN	*Staphylococcus aureus*	R	R	R	20	R	14	R	R
MKN2	*Staphylococcus aureus*	16	28	14	26	40	R	R	38
PTRY2	*Staphylococcus aureus*	10	20	R	28	30	R	R	36

TCHB1	*Staphylococcus aureus*	40	R	R	23	R	R	R	16
TCHB2	*Staphylococcus aureus*	40	R	R	20	R	R	R	R
TCHB3	*Staphylococcus aureus*	40	R	R	30	R	19	19	15
PAM2	*Staphylococcus aureus*	40	R	R	16	R	R	R	R
MVFHC2	*Bacillus subtilis*	38	R	R	34	R	18	18	15
TCHB2	*Bacillus cereus*	40	R	R	30	R	R	R	R

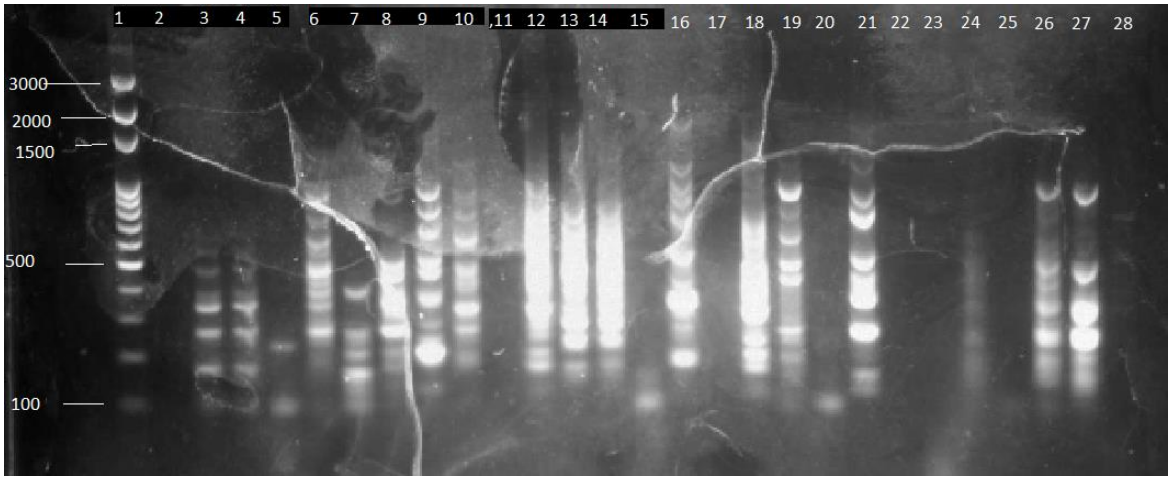
Antibiotics for Gram-Negative Bacteria

Code	Antibiotics	Concentration (µg)	Resistance Value ()
CXM	Cefixime	5	< 14
OFL	Ofloxacin	5	<12
AUG	Augumentin	30	<13
NIT	Nitrofurantoin	300	<14
CPR	Ciprofloxacin	5	<15
CAZ	Ceftazidime	30	<14
CRX	Cefuroxime	30	<14
GEN	Gentamicin	10	<12

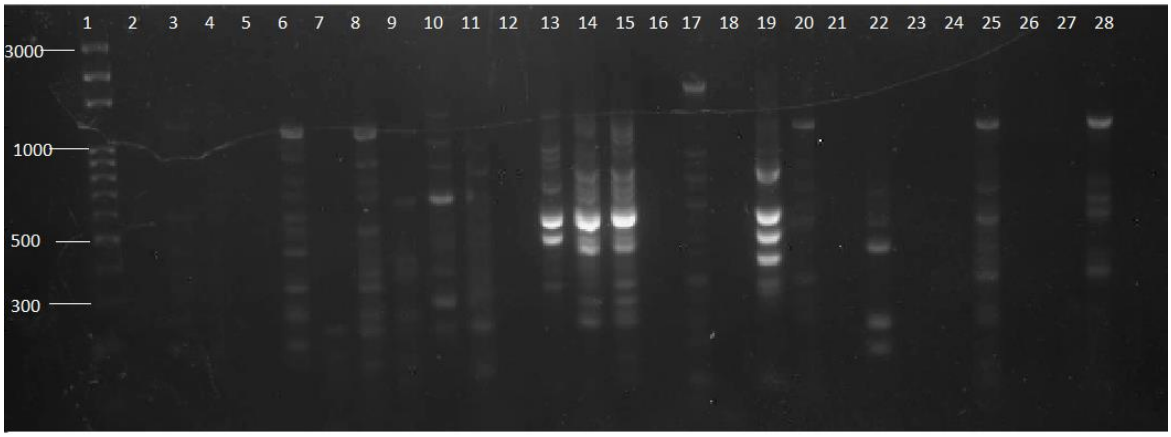
Antibiotics for Gram-Positive Bacteria

Code	Antibiotics	Concentration (µg)	Resistance Value ()
CTR	Ceftriaxone	30	<24
ERY	Erythromycin	5	<13
CXC	Cloxacillin	5	<15
OFL	Ofloxacin	5	<12
AUG	Augumentin	30	<13
CAZ	Ceftazidime	30	<14
CRX	Cefuroxime	30	<14
GEN	Gentamicin	10	<12

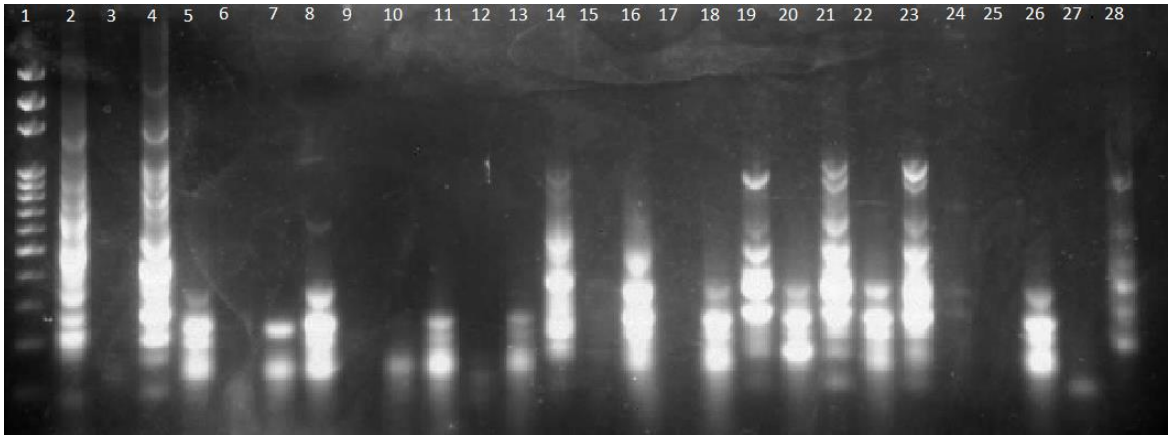
APPENDIX 9: GEL PICTURES



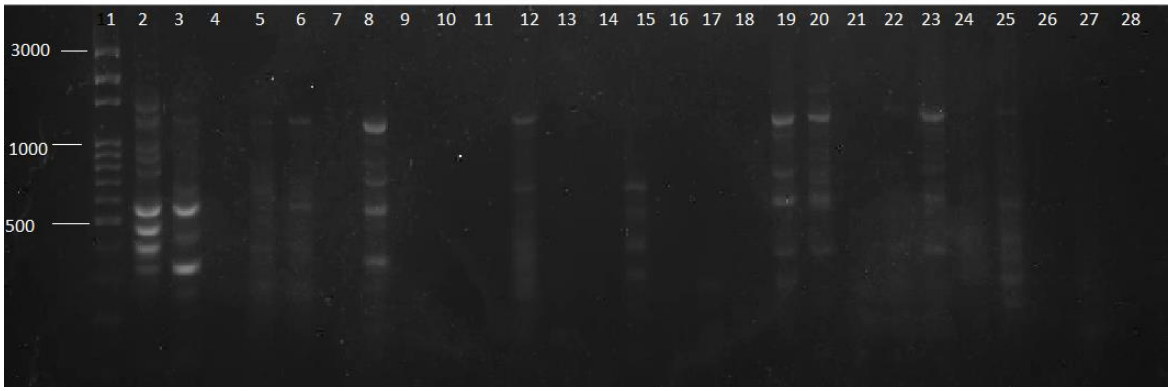
The representative gel image for samples 1-26 of RAPD-PCR (with primer OPA-11) showing presence of different bands corresponding to various DNA bands from the bacteria isolated from boarding schools' FSEs as visualised on 1% agarose gel



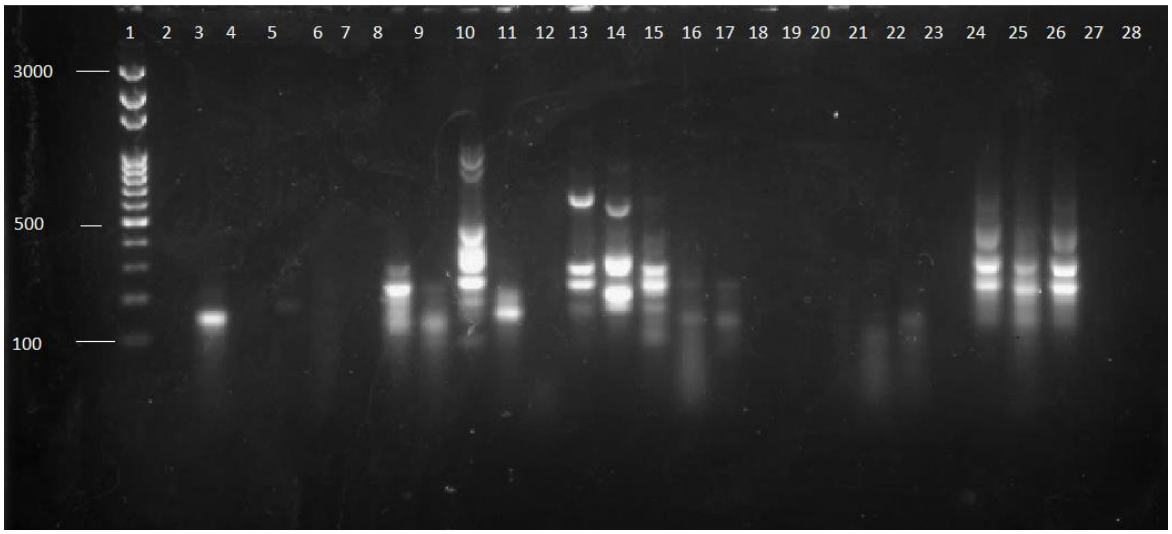
The representative gel image for samples 1-26 of RAPD-PCR (with primer OPA-06) showing presence of different bands corresponding to various DNA bands from the bacteria isolated from boarding schools' FSEs as visualised on 1% agarose gel.



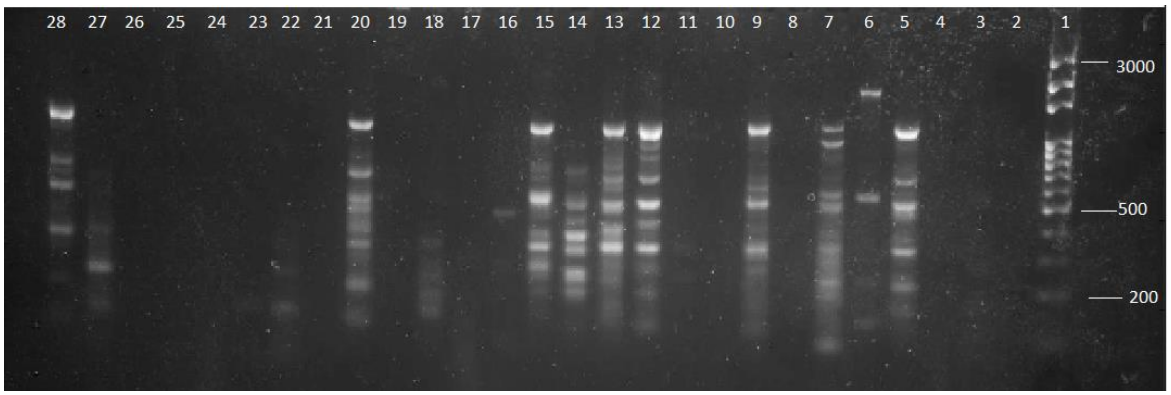
The representative gel image for samples 27-53 of RAPD-PCR (with primer OPA-11) showing presence of different bands corresponding to various DNA bands from the bacteria isolated from boarding schools' FSEs as visualised on 1% agarose gel.



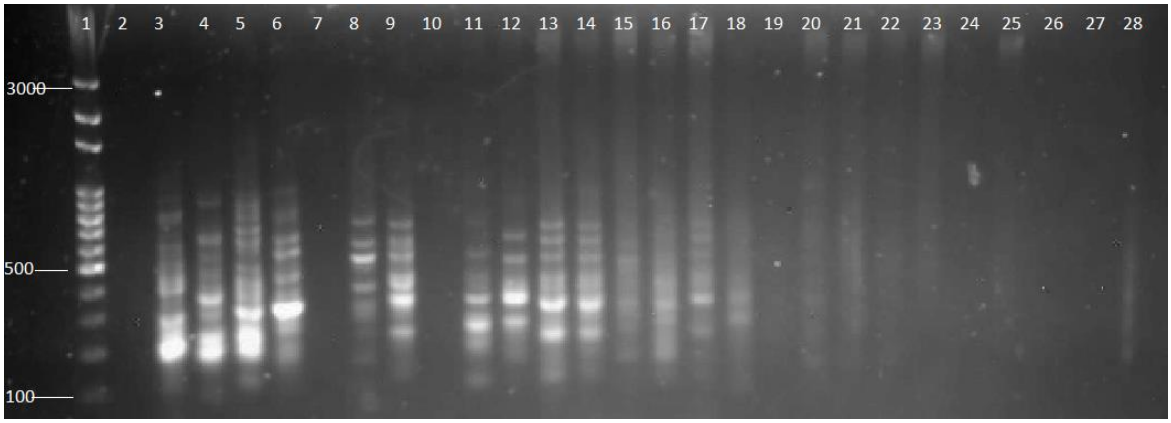
The representative gel image for samples 27-53 of RAPD-PCR (with primer OPA-06) showing presence of different bands corresponding to various DNA bands from the bacteria isolated from boarding schools' FSEs as visualised on 1% agarose gel.



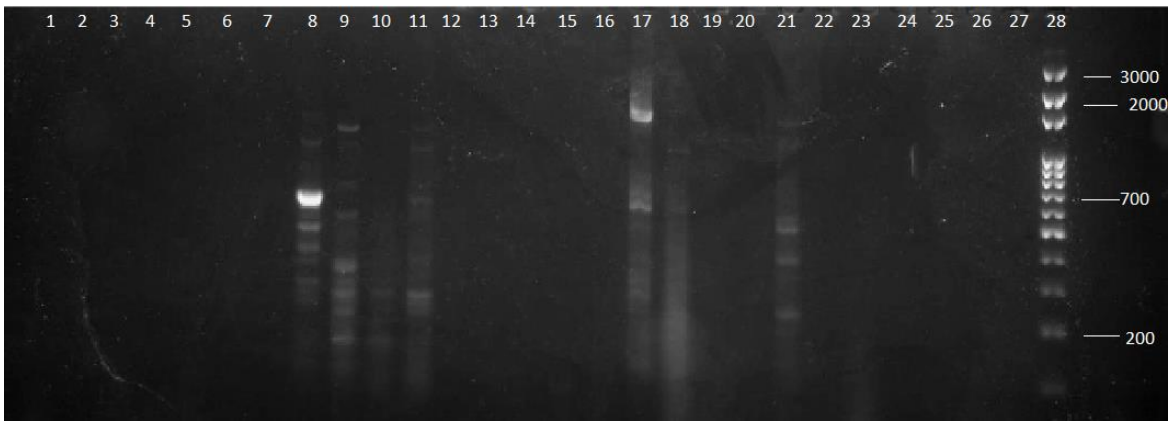
The representative gel image for samples 54- 78 of RAPD-PCR (with primer OPA-11) showing presence of different bands corresponding to various DNA bands from the bacteria isolated from boarding schools' FSEs as visualised on 1% agarose gel



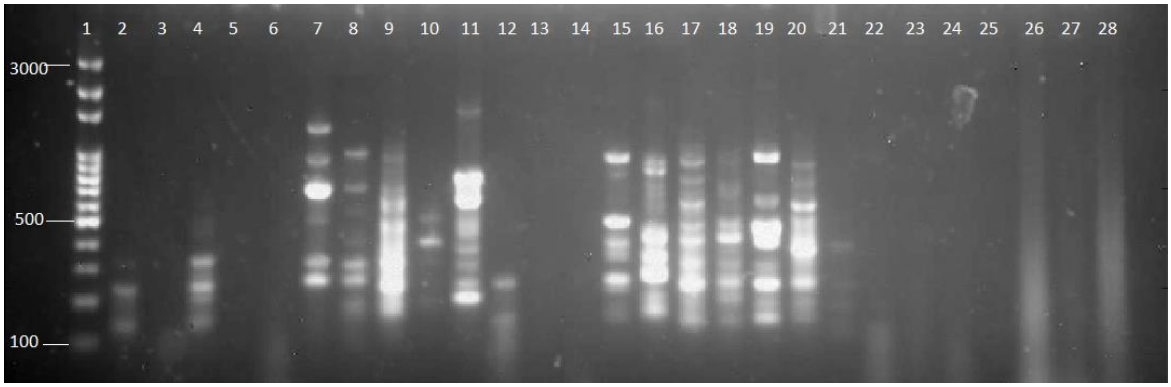
The representative gel image for samples 54- 78 of RAPD-PCR (with primer OPA-06) showing presence of different bands corresponding to various DNA bands from the bacteria isolated from boarding schools' FSEs as visualised on 1% agarose gel



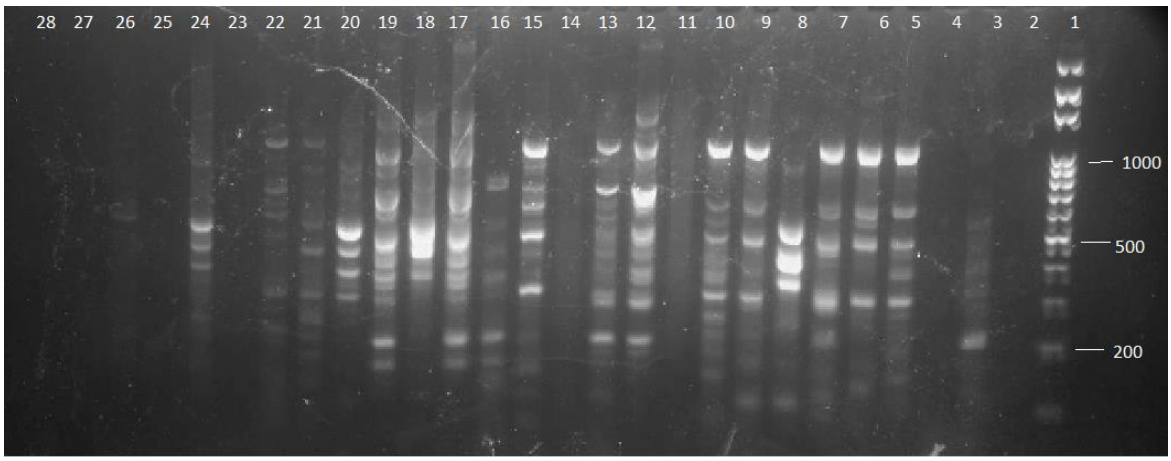
The representative gel image for samples 79- 104 of RAPD-PCR (with primer OPA-11) showing presence of different bands corresponding to various DNA bands from the bacteria isolated from boarding schools' FSEs as visualised on 1% agarose gel



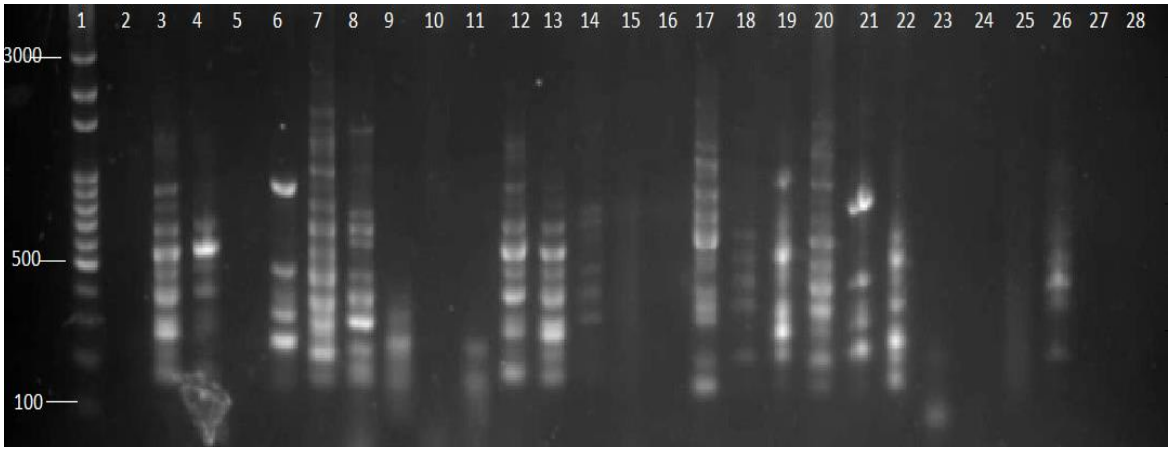
The representative gel image for samples 80- 105 of RAPD-PCR (with primer OPA-06) showing presence of different bands corresponding to various DNA bands from the bacteria isolated from boarding schools' FSEs as visualised on 1% agarose gel



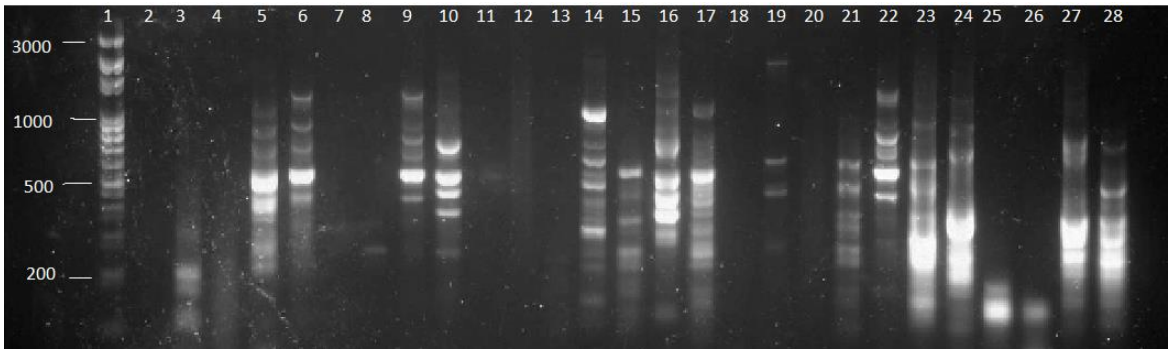
The representative gel image for samples 105- 131 of RAPD-PCR (with primer OPA-11) showing presence of different bands corresponding to various DNA bands from the bacteria isolated from boarding schools' FSEs as visualised on 1% agarose gel



The representative gel image for samples 106- 129 of RAPD-PCR (with primer OPA-06) showing presence of different bands corresponding to various DNA bands from the bacteria isolated from boarding schools' FSEs as visualised on 1% agarose gel



The representative gel image for samples 132- 155 of RAPD-PCR (with primer OPA-11) showing presence of different bands corresponding to various DNA bands from the bacteria isolated from boarding schools' FSEs as visualised on 1% agarose gel



The representative gel image for samples 130- 155 of RAPD-PCR (with primer OPA-06) showing presence of different bands corresponding to various DNA bands from the bacteria isolated from boarding schools' FSEs as visualised on 1% agarose gel

APPENDIX 10: RAPD-PCR CODES FOR CLUSTER ANALYSIS

Cluster	RAPD Code	Lab Code	Source	Cluster	RAPD Code	Lab Code	Source
1 (Lilac)	B11	MVFHC1	Food Handler	4 (Green)	B16	MVDW4	Drinking water
	B12	MVFHC2	Food Handler		B21	TCHB1	Chopping Board
	B18	MVCW2	Cooking water		B25	GFH3B	Food Handler
	B32	MVCHB3	Chopping Board		B29	PKNI	Knife
	B4	PFH4	Food Handler		B33	MCTT1	Countertop
	B5	MVBR1	Bread		B34	MCTT2	Countertop
	B6	MVBR2	Bread		B36	MKN2	Knife
	B7	MVSMLN1	Semolina		E1	MVFHA	Food Handler
	MD7	PGRM2	Grinder		MW11	TTRY	Tray
	MW18	PFEG2	Fried Egg		MW14	GKN1	Knife
					MW2	GFH2A	Food Handler
2 (Small Black)	B14	MVSLP2	Semolina		MW3	GKN2	Knife
	B20	TFS2	Fisf Stew		MW5	TFS2	Fisf Stew
	MW13	PAM2	Amala		S14	MVFHA3	Food Handler
	SA16	MVCHB3	Chopping Board		S18	MVCTT2	Countertop
	SA8	PFEG2	Fried Egg		S21	TFS	Fisf Stew
					S3	PCHB	Chopping Board
3 (Purple)	B13	MVTRY2	Tray		S5	PTRY3	Tray
	B17	MVFHD1	Food Handler		SA17	TCHB1	Chopping Board
	B19	MVDWT	Drinking water tap		SA18	PDNT	Dining Table
	B23	TFH4	Food Handler		SA2	MVCHB2	Chopping Board
	B24	GFH3A	Food Handler		SA29	TKN	Knife
	B28	PCHB	Chopping Board				
	B35	MKN1	Knife	5 (Blue)	E19	TCHB1	Chopping Board
	B8	MVCCT2	Countertop		E20	TCHB2	Chopping Board
	E15	TFS1	Fish Stew		E25	PAM2	Amala
	E22	PAM1	Amala		MD1	MTRY1	Tray
	MW1	PGRM1	Grinder		MD3	PCHB1	Chopping Board
	MW10	TKN	Knife		MD4	PCHB2	Chopping Board
	MW12	PAM1	Amala		MD5	PGRM1	Grinder
	MW4	TFS1	Fisf Stew		S2	PCHB1	Chopping Board
	MW6	TDNT1	Dining Table		S20	TCHB2	Chopping Board
	MW7	TDNT2	Dining Table		S8	GFH2A	Food Handler
	MW8	PAM1	Amala		SA10	MVCCT	Countertop
	MW9	TFH3	Food Handler		SA11	MVCHB	Chopping Board
	S7	PFH5B	Food Handler		SA18	PDNT	Dining Table
	SA1	MVCHB1	Chopping Board				
	SA3	MVFHC1	Food Handler				
	SA4	MVDW1	Drinking water				
	SA5	PAM	Amala				
	SA6	GBCW	Cooking water				

	SA7	TTRY	Tray				
5 (Red)	B1	PSPG/R	Rice	7(Large black)	B22	TCHB2	Chopping Board
	B10	MCW	Cooking water		B3	MVFHC2	Food Handler
	B2	PFH1A	Food Handler		E21	TCHB3	Chopping Board
	B26	GCHB2	Chopping Board		E23	PFEG	Fried Egg
	B27	TGRM	Grinder		E3	MVFHB1	Food Handler
	B30	MCHB1	Chopping Board		E9	MVDWT2	Drinking water tap
	B31	MCHB2	Chopping Board		MD18	GTRY1	Tray
	B37	MGRM	Grinder		MD19	GTRY2	Tray
	B38	PGRM	Grinder		MD2	MTRY2	Tray
	B9	MVCHB2	Chopping Board		MD20	MGRM2	Grinder
	E10	MVKN1	Knife		MD6	PGRM2	Grinder
	E11	MVFHB4	Food Handler		MD9	MKN1	Knife
	E12	MVFHD1	Food Handler		MW16	PCHB	Chopping Board
	E13	GFH2	Food Handler		S1	PCHB	Chopping Board
	E14	GFH4	Food Handler		S10	GKN1	Knife
	E16	TFS2	Fish Stew		S11	PBR2	Bread
	E17	TFS3	Fish Stew		S12	PFEG1	Fried Egg
	E18	TFS4	Fish Stew		S13	PFEG2	Fried Egg
	E19A	TCHB1	Chopping Board		S17	MVCW1	Cooking water
	E2	MFHA2	Food Handler		S22	GFH2B	Food Handler
	E24	PBR	Bread		S23	GKN1	Knife
	E26	PTRY3	Tray		S24	MVCTT1	Countertop
	E27	PTRY4	Tray		S4	PTRY2	Tray
	E4	MVFHB2	Food Handler		S6	PFH5A	Food Handler
	E5	MVCTT1	Countertop		SA12	MVGRM	Grinder
	E6	MVCTT2	Countertop		SA13	TGRM	Grinder
	MD10	TCTT1	Countertop		SA14	MVFHA5	Food Handler
	MD11	MCHB	Chopping Board		SA15	PKN1	Knife
	MD12	TCTT2	Countertop		SA20	MVCTT2	Countertop
	MD13	GTRY2B	Tray		SA21	TFH3	Food Handler
	MD14	GCHB2	Chopping Board		SA22	MVCHB3	Chopping Board
	MD16	PCHB	Chopping Board		SA23	MKN	Knife
	MD17	PDNT2	Dining Table		SA24	MKN2	Knife
	MD8	MKN1	Knife		SA25	MCHB	Chopping Board
	MW15	GKN2	Knife		SA26	PTRY2	Tray
	S15	MVFHC1	Food Handler		SA27	MVFHD1	Food Handler
	S16	MVFHC2	Food Handler		SA28	PGRM	Grinder
	S19	GFH2C	Food Handler		SA30	TDNT	Dining Table
	S9	PGRM4	Grinder		SA9	MVTRY2	Tray
	SA31	PFH5	Food Handler				