ANTIBIOTIC-RESISTANT GENE PROFILING OF *Vibrio* AND *Salmonella* species ISOLATED FROM STOOLS OF DIARRHOEA PATIENTS IN SOME HOSPITALS IN BENIN METROPOLIS

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, FACULTY OF SCIENCE, IN PARTIAL FULFILLMENT OF THE AWARD OF MASTER OF SCIENCE (M.Sc) DEGREE IN MEDICAL MICROBIOLOGY, BENSON IDAHOSA UNIVERSITY, BENIN CITY, EDO STATE, NIGERIA.

JUNE, 2026.

CERTIFICATION

This is to certify that this dissertation report on the topic "ANTIBIOTIC RESISTANT GENE PROFILING OF *Vibrio* AND *Salmonella* species ISOLATED FROM STOOLS OF DIARRHOEA PATIENTS IN SOME HOSPITALS IN BENIN METROPOLIS" was completed and is being submitted by OBANOR, OSARUGUE ESTHER with matriculation number PG/MCB/180622 carried out under the supervision of PROF. STEPHEN. A. ENABULELE in the Department of Biological Sciences (Microbiology Option), Faculty of Science, Benson Idahosa University, Edo State, Nigeria.

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DECLARATION

I, Obanor, Osarugue Esther, do hereby declare that this project is entirely my own work and composition. The work embodied in this project has not been submitted in a candidature or any degree and is not currently being submitted for any other degree. All references to works of other persons has been duly acknowledged.

_____ _____

OBANOR. O. ESTHER DATE

DEDICATION

This report is dedicated to the almighty God, for his guidance, protection, love and grace

ACKNOWLEDGEMENT

First and foremost, I give all glory and gratitude to Almighty God for His grace, strength, and guidance throughout my academic journey and for the successful completion of this research work.

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ABSTRACT

Diarrheal diseases remain a major public health burden in Nigeria, with *Vibrio* and *Salmonella* species frequently implicated in outbreaks. Poor sanitation, limited diagnostic capacity, and widespread antibiotic misuse are drivers of the persistence of these pathogens and the emergence of multidrug resistance. Despite this, molecular data on the carriage of resistance genes among enteric bacteria in Nigeria remain limited. This study investigated the prevalence, antimicrobial susceptibility profiles, and resistance gene determinants of *Vibrio* and *Salmonella* spp. isolated from stool samples of diarrheal patients attending three hospitals in Benin Metropolis, Nigeria, namely Benin Medical Care (BMC), Edo Specialist Hospital (ESH) and City of Hope Hospital (COH). Thirty (30) Stool samples were collected and analyzed for the presence of diarrhoeagenic *Vibrio* spp. and *Salmonella* spp., using standard microbiological assay techniques and Molecular analysis. Antibiotic susceptibility testing was performed using the Kirby–Bauer disc diffusion method, while resistance gene profiling was conducted using Polymerase chain reaction (PCR) and 16S rRNA. Multiple Antibiotic Resistance (MAR) indices were calculated to assess the extent of resistance. ESH consistently recorded the highest microbial loads, with average heterotrophic counts of 16.79×10^4 *CFU/g*, *Salmonella* counts of 20.05×10^4 *CFU/g*, and *Vibrio* counts of 9.97×10^4 *CFU/g*. The percentage frequency distribution revealed that ESH accounted for 45% of *Salmonella* isolates and 47.62% of *Vibrio* isolates, compared to lower proportions at BMC and ESH hospitals. Susceptibility testing showed a high resistance to β-lactams and cephalosporins, with complete resistance to imipenem and amoxicillin-clavulanate. Fluoroquinolones such as ofloxacin and levofloxacin retained comparatively higher activity, though resistance remained substantial. MAR index ranged from 0.25 to 1.0 for *Vibrio* and 0.33 to 1.0 for *Salmonella*, with many isolates scoring ≥0.75, indicating exposure to high-risk environments with intense antibiotic use. Molecular assays confirmed the presence of multiple resistance genes, including *blaTEM* and *Sul2*, with several isolates harboring multiple genes simultaneously. These findings highlight the widespread multidrug resistance and genetic determinants of resistance among enteric pathogens in Benin Metropolis. The results underscore the urgent need for strengthened antimicrobial stewardship, routine molecular surveillance, and improved infection prevention strategies to mitigate the spread of resistant *Vibrio* and *Salmonella* strains in Nigeria.

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND OF STUDY

Diarrhea is a common problem worldwide and is strongly associated with the gut microbiota, including bacteria, fungi, and viruses. Generally, diarrheal patients or animals are characterized by gut microbiota dysbiosis, and pathogen infections may lead to diarrheal phenotypes (Li *et al.,* 2021). It consists of the passage of watery and loose stools multiple times a day (usually more than 3), though specific definitions may vary when applied to distinct studies or populations

According to the World Health Organization (WHO), Diarrheal disease is the third leading cause of death in children under 5 years old and is responsible for killing around 443,832 children every year. Diarrhea can last several days and leave the body without the water and salts necessary for survival. In the past, for most people, severe dehydration and fluid loss were the main causes of diarrhea-associated deaths.

A number of diseases and conditions can cause diarrhea, from cases like specific food intolerances, intake of artificial sweeteners, medicines e.g. laxatives, antibiotics, antacids etc., and microorganisms e.g. bacteria, parasites, and viruses.

There are several microorganisms that can cause diarrhea as this study focuses on the bacteria causes, some bacteria that cause this condition are *Escherichia coli, Helicobacter pylori, Salmonella, Vibrio* spp*, Bacillus* spp*, Klebsiella* spp*, Clostridium difficile* along with other myriads of microorganisms.

Mammals' digestive tracts are home to a wide variety of microorganisms, such as viruses, bacteria, fungi, and protozoa (Li *et al.,* 2021). Microorganisms fulfil their activities in various areas of the gastrointestinal system and vary in density and composition. Symbiotic relationships and homeostasis encourage a beneficial relationship between the microbiota and the host, which further

prevents the majority of foreign pathogens from colonizing and aids in physiological processes and nutritional absorption (Hillman *et al.,* 2017; Fan and Pedersen, 2020). For instance, following non-typhoidal *Salmonella* diarrhea, a healthy gut microbiota can enhance pathogen clearance from the gut lumen and guard against damage to epithelial cells. Furthermore, it has been documented how microbial balance contributes to the integrity of the intestinal barrier as well as the growth and maturation of the mucosal immune system (Li *et al.,* 2021). However, a number of factors (such as medications, environmental factors, infections, and dietary choices) can readily alter the variety and composition of gut microbiota, which in turn impacts human health (Li *et al.,* 2021).

Tests are advised if the symptoms persist after a couple of days, even though the majority of people with diarrhoea may not need any laboratory assessments. Testing might be necessary if the illness is severe, refractory, or has the potential to affect high-risk individuals (Akhondi and Simonsen, 2024).

Stool is undigested food substances and other internal wastes produced in the digestion process and removed from the digestive tract through the anus. A stool test is an indicative process involving analyzing fecal matter to check for signs of infection, inflammation, or disease as stool samples can provide critical information about a person's gastrointestinal system.

Antibiotic treatment for diarrhea brought on by infectious agents like bacteria should only be initiated, when necessary, as demonstrated by the results of sensitivity and culture tests. Oral rehydration solutions and zinc supplements are additional adjuvants or supportive therapies used to treat diarrhea (Gamela *et al.,* 2024).

Unfortunately, the overuse and abuse of antibiotics in the treatment of various illnesses, including diarrhea, has led to a steady rise in antibiotic resistance in recent years. Antimicrobial resistance (AMR) has been associated to the observational treatment of diarrhea with antibiotics (Gamela *et*

al., 2024), also the emergence of AMR in enteric bacterial pathogens is a serious public health issue that has an adverse effect on the management of diarrhea. There have been reports of high levels of bacterial resistance to common and easily accessible antibiotics like ampicillin, tetracyclines, and trimethoprim-sulphamethoxazole (co-trimoxazole). Additionally, the patterns of antibiotic susceptibility of *Shigella*, *Salmonella*, and *E. coli* have evolved over time due to the emergence of AMR and multi-drug resistance (MDR).

1.1 STATEMENT OF THE PROBLEM

Diarrheal diseases are increasingly contributing to hospital visits in Benin Metropolis, yet the underlying causes remain poorly understood due to a lack of experimental data. While multiple bacterial pathogens may be responsible as diarrhoea causal agents, species of the *Vibrio* and *Salmonella* genera are of particular concern, given their role in severe gastrointestinal infections and their growing antimicrobial resistance. In particular, *Vibrio cholerae* may be a major source and importance to the observed rise in diarrheal cases, though other *Vibrio* spp. and *Salmonella* spp. cannot be excluded. The limited data on the prevalence of these pathogens locally and their resistance patterns, obstruct effective public health interventions and clinical management, highlighting the urgent need for targeted research in Benin Metropolis.

The Nigeria Centre for Disease Control and Prevention (NCDC) reported that as of 11 June 2024, 1,141 suspected cholera cases had been recorded across 30 states in Nigeria since 1 January 2024 (UNICEF, 2024). This study is to determine how far and the definitive occurrence of cholera in Benin City and also to determine if the ongoing epidemic causing diarrhea is really cholera or is occurring as a result of another cause.

1.2 AIM AND OBJECTIVES

The aim of this study is to determine the prevalence of *Salmonella* and *Vibrio* spp in stool samples of patients in Benin City and profile the antibiotic-resistant genes of the isolated microorganisms.

The specific objectives include to;

- isolate and presumptively identify *Salmonella* and *Vibrio* spp, from stools sampled from hospitals in Benin City using cultural, morphological and biochemical properties
- identify the isolated organisms genotypically using the 16S rRNA sequence analysis
- determine the antimicrobial resistance profile of the bacteria isolates
- genetically profile the antibiotic-resistant genes of the isolates

CHAPTER TWO

LITERATURE REVIEW

2.0 INTRODUCTION

Diarrhea is a major contributor to morbidity and mortality worldwide and a frequent cause of clinic visits in low and middle-income countries (Theodore *et al.,* 2006). Worldwide control is made challenging by diverse etiologies, overlapping transmission routes, and rising antimicrobial resistance that undermines therapy considerations (Casmir *et al.,* 2009: Ballal, 2016)

Evidence has shown that diarrhea continues to cause substantial damage, leading to illnesses and even death, most especially in sub-Saharan Africa, where incidence and severe outcomes remain high (Ballal, 2016). Etiological factors of recurrent hardship like poor water, sanitation and hygiene, malnutrition, limited vaccine coverage, and constrained diagnostic capacity, together sustain transmission and delayed or inappropriate treatment (Casmir *et al.,* 2009: Ballal, 2016). In the southern region of Nigeria, diarrheal illnesses are often linked to bacterial entero-pathogens such as *Vibrio* species and *Salmonella* species, both of which are highly associated with severe gastroenteritis, enteric fever, and cholera-like symptoms (Akinnibosun *et al.,* 2022).

In recent years, the emergence and dissemination of antibiotic-resistant bacteria have complicated the treatment of infectious diarrhea. Overuse and improper use of antibiotics in clinical, veterinary, and agricultural situations have vastly increased the dissemination of multidrug resistance (MDR) genes among enteric pathogens (Olayinka *et al.,* 2020). Genetic profiling of antibiotic-resistant bacteria, particularly of the *Vibrio* and *Salmonella* spp from stool samples, provides important views into the molecular basis of resistance and its epidemiological patterns. Such profiling is critical in regions such as Benin Metropolis, where hospital-based diarrheal cases serve as an important indicator of community antimicrobial resistance (Osahon *et al.,* 2023).

In history, the problem of antibiotic resistance was first noted not long after the discovery of penicillin in the 1940s, when resistant *Staphylococcus aureus* strains emerged (Davies and Davies, 2010). Ever since, the discovery of new antibiotics has been gradually overtaken by the emergence of resistant strains, thus suggesting a natural evolutionary race between antimicrobial agents and bacterial adaptation, granting resistance. The emergence of high thorough output sequencing tech and bioinformatic tools has increased our monitoring ability of this resistance at the genomic level. By profiling resistance genes, researchers can determine not only which antibiotics are most likely to fail in treatment but also understand the underlying genetic mechanisms prompting resistance evolution and spread (Liu *et al.,* 2020).

In aquatic environments, there has been a major spread of antibiotic resistance genes (AGR's) which consequently has attracted more notice to the role of aquatic environments as a cause of resistance determinants. AGR's spread amongst bacterial populations is significantly facilitated using aquatic channels as a means for this spread through aquatic farming practices, and wastes and effluents from hospitals and the pharmaceutical industries disposed of in these water bodies (Zhang *et al.,* 2021). Many bacteria attain their resistance through these means but, *Vibrio* and *Salmonella* are of importance due to their ability of infecting and affecting animals and humans, their ubiquitousness, their constant presence in the environment capacity to acquire and distribute ARGs via horizontal gene transfer. Both genera are significant pathogens in humans and animals, causing diseases ranging from gastroenteritis to systemic infections, and their resistance patterns are of immense concern in both clinical and food safety contexts (Cabello *et al.,* 2020).

Antibiotic gene profiling plays an important role helping us understand this resistance at the molecular level. It enables identification and determination of certain resistance genes like genes which encode for β-lactamases resistance, aminoglycoside-modifying enzymes, and efflux pumps, which together contribute to multidrug resistance (MDR) phenotypes (Kumar *et al.,* 2022).

Furthermore, profiling enables the detection of mobile genetic elements (MGEs), such as plasmids, transposons, and integrons, which are mediators of horizontal gene transfer of ARGs. The detection and depiction of such elements are essential for predicting the potential spread of resistance within microbial communities and between ecosystems.

The application of antibiotic gene profiling goes far past clinical diagnostics and includes surveillance and policy development. National and international health agencies now integrate molecular profiling data into antimicrobial resistance (AMR) surveillance frameworks. Initiatives such as the Global Antimicrobial Resistance Surveillance System (GLASS) by WHO and various national AMR monitoring programs rely on genomic data to inform antibiotic stewardship and containment strategies (WHO, 2022). These programs underscore the need for continuous monitoring of ARGs not only in hospitals but also in environmental and foodborne bacterial isolates.

Antibiotic resistance in enteric pathogens poses a significant threat to global public health, particularly in hospital settings where vulnerable populations are treated. *Salmonella* spp. and *Vibrio* spp. are major causes of diarrheal disease worldwide, and understanding their resistance gene profiles is crucial for effective treatment and infection-control strategies.

2.1 MICROBIOLOGICAL CAUSES OF DIARRHOEA WORLDWIDE

Diarrhea has numerous causes worldwide, which could be parasitic, bacterial and viral, and their transmission could also be influenced by seasonal tendencies. Table 2.1 shows this epidemiology and transmission as well as the pathogenic groups of the etiological agents.

2.2 BACTERIAL DIVERSITY AND DYNAMICS

Clinic-based surveillance in Africa found *Salmonella, Shigella, Vibrio* spp, *Campylobacter* and diarrhoeagenic *E. coli* (DEC) among the most frequently recovered bacterial pathogens, with age-related patterns with *Campylobacter* and DEC more in young children, while *Shigella* is more frequent with increasing age (Aminshahidi *et al.,* 2017). For Viruses, rotavirus remains a dominant viral cause of severe pediatric gastroenteritis, where vaccination is incomplete; co-infection with bacteria is common (Omotade *et al.,* 2023). Bacterial pathogens often cluster with poor sanitation and antibiotic exposure; rotavirus and some bacterial syndromes have been found to show distinct seasonal peaks that can differ in tropical regions (Omotade *et al.,* 2023; Aminshahidi *et al.,* 2017).

2.3 MICROORGANISMS OF FOCUS

2.3.0 *Vibrio* spp.

The genus *Vibrio* comprises Gram-negative, curved, rod-shaped bacteria that are widely distributed in aquatic environments. Species of *Vibrio* like *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, are recognized as important human pathogens (Igbinosa *et al.,* 2019). *V. cholerae*, is the microbe responsible for diarrheagenic disease cholera and the organism releases highly potente enterotoxins which cause the body to massively secrete fluids through vomiting and persistent stooling which in turn leads to severe dehydration and eventually death is left untreated. The important virulence factors contributing to this are *ctxA* and *ctxB* genes encoding cholera toxin, as well as the *tcpA* gene encoding the toxin-coregulated pilus which is essential for colonization (Adekanmbi *et al.,* 2021).

Table 2.1: Main Diarrheal-Causing Microbial Pathogens

Pathogen group	**Key agents**	**Typical epidemiology and transmission**	**Pathogen group**
Bacteria	*Escherichia coli (diarrhoeagenic types), Shigella* spp*., Salmonella* spp*., Campylobacter* spp*., Vibrio cholerae*	Predominantly fecal-oral transmission via contaminated water, food, person-to-person spread; many cases self-limited but some require antibiotics (Casmir *et al.,* 2009: Ballal, 2016)	Bacteria
Viruses	Rotavirus, Norovirus, Adenovirus	Major cause of severe childhood diarrhea (rotavirus highest burden pre-vaccine); highly contagious via fecal-oral route and fomites (Casmir *et al.,* 2009).	Viruses
Parasites	*Giardia lamblia, Cryptosporidium* spp*., Entamoeba histolytica*	Waterborne and person-to-person transmission; cause of persistent or protracted diarrhea in children and immunocompromised hosts (Ballal, 2016)	Parasites

Cholera is endemic to Nigeria and as such there has been irregular outbreaks occurring in riverine areas and in some inland states with southern Nigeria experiencing the worse of the outbreaks which s likely due to contaminated water sources (Eze *et al.,* 2020). In Benin City, *Vibrio* species have been cultured and isolated from stool samples, wastewater, and ready-to-eat foods, suggesting it has both environmental and human reservoirs (Akinnibosun *et al.,* 2022). *Vibrio* spp ability to adapt to environmental stress and its ease of obtaining resistance determinance by HGT add to their persistence in the environment (Adebayo-Tayo *et al.,* 2019).

2.3.1 *Salmonella* spp.

Salmonella species are motile Gram-negative bacilli belonging to the family *Enterobacteriaceae*. *Salmonella* has only two species which are: *Salmonella enterica* and *Salmonella bongori*, with the former responsible for most human infections (Chukwu *et al.,* 2019). *S. enterica* species can further be divided into numerous serotypes such as *S. Typhimurium* and *S. enteritidis*, which are common causes of gastroenteritis, typhoid fever, and bacteremia in Nigeria (Okonko *et al.,* 2020). The major virulence factors of *Salmonella* include the *invA* gene responsible for epithelial invasion, *stn* gene encoding enterotoxin, and *fimA* gene associated with fimbria adhesion (Umeh *et al.,* 2021).

Recent studies diarrheagenic infections in Nigeria has identified an incline in cases of these infections caused by *Salmonella* which are more often caused by unhygienic food and water sources and poor hygiene practices (Afolabi *et al.,* 2021). In Edo State, *Salmonella* isolates from hospitals have ascertained to have widespread resistance to commonly prescribed antibiotics such as ampicillin, chloramphenicol, and ciprofloxacin (Iroha *et al.,* 2020). Its persistence in the environment (medical or otherwise) poses challenges in treatment administration and thus highlights the need for molecular surveillance of resistance determinants (Igbinosa *et al.,* 2020).

2.4 DIARRHEA EPIDEMIOLOGY IN NIGERIA

Diarrheal diseases in Nigeria are caused by a large number of pathogenic microbes, including bacteria, viruses, and protozoa. The spread and distribution of this illness varies in severeness across different regions in Nigeria with southern riverine regions facing the worst of it because of the regions geography, water pollution, and poor or improper waste management systems (Umeh *et al.,* 2021). According to NCDC surveillance data, Edo, Delta, and Rivers States frequently report diarrheal outbreaks associated with bacterial entero-pathogens, especially during the rainy season (NCDC, 2022).

Many clinical studies in southern Nigeria have determined that *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Salmonella enterica*, *Shigella flexneri*, and *Escherichia coli* are the most predominant bacterial pathogens isolated from patients with symptoms associated with acute watery diarrhea (Eze *et al.,* 2021; Akinnibosun *et al.,* 2022). In Benin Metropolis, the reoccurring incidences of diarrhea has been significantly increased because of factors like inadequate public water systems, consumption of unhygienically prepared street foods and misuse and overuse of antibiotics (Igbinosa *et al.,* 2020). Studies from hospitals in Edo State have also shown an upsurge in the rates of multidrug resistance among stool isolates, reflecting poor antibiotic distribution management and unregulated access to antimicrobials (Odetoyin *et al.,* 2019; Osahon *et al.,* 2023).

Observational studies in Nigeria have prompted a realization in the elevated rate of diarrhea causing pathogens in children. The vulnerability of infants (still developing immune systems), house hold factors and environmental risk factors has been identified as the major sources and causes of this increased illness rates (Akinlabi *et al.,* 2023). Antimicrobial resistance among bacterial isolates is very often reported across multiple Nigerian areas (Pawlowski *et al.,* 2009; Akinwumi *et al.,* 2021).

There has not been a lot of studies that have addressed antibiotic resistant gene profiling of enteric bacteria in Benin Metropolis. Akinnibosun *et al*. (2022) investigated stool samples from diarrhoeal patients in tertiary hospitals and found a high prevalence of *Vibrio* and *Salmonella* species exhibiting multidrug resistance. Similarly, Osahon *et al*. (2023) reported detection of resistance genes including *blaTEM* and *tetA* in isolates from both clinical and environmental sources in Benin City. These findings highlight the interconnectedness of environmental and clinical reservoirs of resistance genes.

The presence of MDR *Vibrio* and *Salmonella* in hospital and community settings poses great risks for failure of treatment available and prolonged infection duration. Also, the detection of resistance genes in environmental isolates shows a potential for widespread dissemination through water systems and food chains (Igbinosa *et al.,* 2020). Despite these findings, comprehensive molecular characterization of resistance genes among stool isolates in Benin Metropolis remains scarce, underscoring the need for the present study.

2.5 PREVALENCE AND AGE PATTERNS

1. **Pathogen detection rates:** Studies in hospital environments and rural areas depict a high pathogen detection of diarrhea in children and infants: pathogen detection in a case series had ranged from ~40% to >60% depending on methods used and setting of the population sampled (Ogbu *et al.,* 2007; Omotade *et al.,* 2023; Akinlabi *et al.,* 2023).
2. **Age distribution:** Children under 12 months old and toddlers have been shown to continuously have the highest exposure to enteric pathogenic and more specifically DEC (Ogbu *et al.,* 2007; Pandey *et al.,* 2011; Olayinka *et al.,* 2024).
3. **Risk Factors and Exposure Pathways**
    - Socioeconomic and Caregiver factors: A care giver with lower education, shorter duration of breastfeeding of infants and low household earnings leading to a lower nutritional status

have been associated with a higher risk of diarrhoea in many Nigerian divisions (Omotade *et al.,* 2023; Olayinka *et al.,* 2024).

- Environmental Exposures: Unsafe well water, consumption of street-vended foods, and contaminated household and community water supply were repeatedly implicated; a genomic study in Ibadan found non-potable well water a common means of DEC carriage across households (Akinlabi *et al.,* 2023; Ogunbiyi *et al.,* 2023).

4. **Morbidity and mortality**

- Clinical burden: According to Theodore *et al.,* ((2006), diarrhea is a major focus as a contributor to hospital visits and its linkage with malnutrition indicators and hospitalization risk among young children.
- Urban vs Rural: A series of investigation on local clinics showed the presence of diahorreal pathogens in both urban and peri-urban, rural and semi urban clinics and that environmental contamination (wells, street foods) drives risk across, though specific national urban-rural comparative prevalence metrics were not available in the supplied papers (Akinlabi *et al.,* 2023; Ogunbiyi *et al.,* 2023).

5. **Seasonal patterns in Nigeria**

Variable seasonality by Etiology: Examination in Ile-Ife and Osun state, conducted by Omotade *et al.*, (2023); Olayinka *et al*., (2024), reported rotavirus predominating in the dry season while some bacterial DEC pathotypes peaked in the wet season, suggesting seasonal etiology of the pathogenic microorganisms which could guide diagnostics and resource allocation.

2.6 ANTIBIOTIC RESISTANCE PATTERNS

Multiple Nigerian regional surveillance studies have documented a high rate of antibiotic resistance amongst enteric bacterial pathogens, frequent multidrug resistance (MDR), transferable

resistance plasmids, and resistance profiles that compromise commonly used first-line treatment agents (Aminshahidi *et al.,* 2017; Alhaji *et al.,* 2022).

2.6.1 Resistance Patterns in Major Pathogens

1. Diarrhoeagenic *E. coli* has a high resistance to numerous antibiotics including: sulfonamides, trimethoprim, ampicillin and first-line penicillins as reported in multiple Nigerian studies; isolates often remained susceptible to fluoroquinolones and carbapenems in several series, although local variability existed (Pawlowski *et al.,* 2009; Olayinka *et al.,* 2024).
2. *Salmonella* and *Shigella:* Isolates in Nigeria have shown very high resistance to ampicillin and trimethoprim-sulfamethoxazole, with a bit of limited susceptibility to some quinolones in certain local areas. However, increasing quinolone resistance elsewhere (Ogbu *et al.,* 2007; Aminshahidi *et al.,* 2017).
3. Plasmid-mediated and transferable resistance: Plasmid profiling in isolates cultured from samples collected from children demonstrated diverse, highly transferable resistance plasmids, supporting horizontal gene transfer amongst enteric bacteria in Nigeria (Alhaji *et al.,* 2022).
    a. Mechanisms and drivers: DEC isolates have been shown to include plasmid-borne resistance determinants and indications of efflux-pump overexpression, which is consistent with transferable beta-lactamase and quinolone-associated alterations (Alhaji *et al.,* 2022; Ogunbiyi *et al.,* 2023). The selection and spread of resistant strains are facilitated by the widespread use of antibiotics, a lack of diagnostic testing, environmental contamination, poverty, and poor stewardship (Ballal, 2016; Aminshahidi *et al.,* 2017).

2.7 MECHANISM OF RESISTANCE

Antibiotic resistance in bacteria is a phenomenon having many sides which could in turn be driven by genetic, biochemical, and ecological factors. Basically, resistance arises through the attainment or through transformation of genetic elements that reduce the effectiveness and strength of antimicrobial agents. These mechanisms can be broadly categorized into four major classes: enzymatic degradation or modification of antibiotics, alteration of target sites, decreased permeability or increased efflux of drugs, and adaptive resistance through regulatory network modulation (Blair *et al.,* 2015; Munita and Arias, 2016). Understanding these molecular mechanisms is extremely important in understanding and profiling antibiotic genes, as each resistance determinant corresponds to a specific genetic signature that can be detected and monitored by molecular assays.

1. Enzymatic Inactivation of Antibiotics

Antibiotic breakdown or enzymatic alteration is the most prevalent mechanism of bacterial resistance. Among the most popular examples are β-lactamases, which hydrolyse the β-lactam ring of carbapenems, cephalosporins, and penicillins, making them useless. Genes encoding β-lactamases such as *blaTEM*, *blaSHV*, *blaCTX-M*, and *blaOXA* have been detected spread across numerous Gram-negative bacteria, including but not limited to the *Vibrio* and *Salmonella* species (Bush and Jacoby, 2010; Xia *et al.,* 2021). In addition to β-lactamases, aminoglycoside-modifying enzymes (AMEs) such as acetyltransferases, phosphotransferases, and nucleotidyl transferases modify aminoglycosides at specific sites, thus stopping them from freely attaching to their ribosomal targets. Genes like *aac(3)-IIa* and *aph(3')-Ia* are normal of this class and are frequently linked with mobile genetic elements that help with horizontal transfer across various bacterial populations (Zhou *et al.,* 2023).

2. Alteration of Antibiotic Targets

Resistance also has a probability of developing due to structural alterations at antibiotic target sites. Antibiotic binding can be inhibited by point mutations in chromosomal genes encoding ribosomal RNA, DNA gyrase, or topoisomerase IV. For example, fluoroquinolone resistance in *Salmonella enterica* and *Vibrio cholerae* often results from transformations at the *gyrA* and *parC* genes, which alter the quinolone-resistance determining regions (QRDRs) (Le and Lei, 2019). Also, mutations in penicillin-binding proteins (PBPs) lower the binding tendency of β-lactams, this mechanism is more frequently related with extended-spectrum β-lactamase (ESBL) producers and carbapenem-resistant isolates (Cheng *et al.,* 2022). By methylating 23S rRNA, ribosomal methyltransferases like erm genes induce resistance to macrolides by blocking antibiotic interaction with the ribosomal binding site (Roberts, 2020). Sequencing important genomic regions during resistance gene profiling can help in identifying the changes occurring at the target site, which are frequently persistent and chromosomally encoded.

3. Efflux Pumps and Reduced Permeability

For Multidrug Resistance phenotypes, efflux systems are very necessary contributors, these transmembrane protein transporters/complexes actively pumps out antibiotics and other toxic compounds from bacterial cells, hence maintaining intracellular concentrations below inhibitory levels. In microorganisms like *Vibrio* and *Salmonella*, the Resistance-Nodulation-Division (RND) family of efflux pumps, including *AcrAB-TolC* and *VexAB*, plays a central role in resistance to tetracyclines, quinolones, and chloramphenicol (Yamasaki *et al.,* 2019; Kim *et al.,* 2023). Moreover, the reduced permeability through modification or loss of outer membrane porins (e.g., OmpF and OmpC) further limits antibiotic entry. Low-level but broad-spectrum resistance is more often conferred by the synergistic impact of efflux and permeability alterations, which can thereafter be enhanced by additional mechanisms.

4. Horizontal Gene Transfer and Mobile Genetic Elements

There are many defining features of bacterial resistance one of which is the ability of these microbes to share their genetic material inter and intra species and genera through Horizontal Gene Transfer (HGT). Mobile genetic elements (MGEs) like plasmids, integrons, transposons, and bacteriophages, help in the promotion of rapid distribution of ARGs within microbial communities (Partridge *et al.,* 2018). In both *Vibrio* and *Salmonella*, conjugative plasmids and class 1 integrons are more often identified as carriers of resistance determinants such as *sul1*, *dfrA*, *aadA*, and *blaCTX-M*. These MGEs do not only encode resistance genes in the bacteria but they also carry insertion sequences and recombination sites which allow them integrate into diverse genomic contexts (Liu *et al.,* 2020). The profiling these mobile genetic elements provides an important view into the potential for interspecies gene flow and the emergence of novel resistance combinations.

5. Adaptive and Regulatory Resistance Mechanisms

Other than a bacteria acquiring resistance trough the attainment of genetic material, this resistance may also be gotten by regulatory network changes. Processes like stress response pathways, quorum sensing, and biofilm formation can modulate antibiotic susceptibility by altering gene expression and physiological states. For example, when examining the structure that is biofilms, we see that it can create a physical barrier which can limit an antibiotics ability to penetrate into an organism while also promoting HGT within the bacterial populations presently existing within the biofilm (Flemming and Wingender, 2019). In *Vibrio* species, the control of biofilm formation through quorum-sensing has been connected to an increased tolerance to multiple antibiotics (Lee *et al.,* 2020). Similarly, in *Salmonella*, the activation of the *marA* and *ramA* regulatory networks

enhances efflux pump expression while reducing porin production, collectively contributing to multidrug resistance phenotypes (Abraham *et al.,* 2021).

6. The Resistome and Co-selection

All resistance genes found in a bacterial genome or microbiome is referred to as the resistome. Both functional ARGs and cryptic genes that could provide resistance under selective pressure are included in this. The co-location of resistance genes with heavy metal or biocide tolerance genes on the same plasmids or integrons promotes co-selection, whereby non-antibiotic agents can indirectly be selected for ARG maintenance (Pal *et al.,* 2017). Environmental reservoirs, particularly aquatic systems, are known to facilitate such co-selection processes. *Vibrio* species in marine environments often carry plasmids containing both antibiotic and metal resistance determinants, showing their strong survivability by adapting to such polluted waters (Zhang *et al.,* 2021). The profiling of this multi-resistance provides insights into environmental factors driving the evolution of antimicrobial resistance.

7. Molecular basis and gene interactions

The incorporation of AGR's and its manifestation are strictly controlled by gene promoters, gene copy number and hosts genome at the molecular level. Some of these genes conferring resistance are expressed naturally by the organism while others are activated or induced in response to external exposure to the antibiotic. An analysis into the genomic structures of microorganisms has revealed resistance determinants often form clusters to create what is known as genomic islands which serve as focal points for recombination and gene exchange (Kumar *et al.,* 2022).

2.7.0 MECHANISM OF RESISTANCE IN *Vibrio* spp AND *Salmonella* spp

Vibrio spp.

In *Vibrio* spp, mechanisms like efflux pumps, acquisitions through plasmids and integrons, enzymatic degradation and target modification are the means through which an organism can gain resistance. *tetA* and *tetB* genes confer resistance to tetracyclines, *sul1* and *sul2* cause sulfonamide resistance, and *qnrA* and *qnrS* are for quinolone resistance (Adekanmbi *et al.,* 2021). *Vibrio cholerae* strains from Nigeria have also been found to carry *blaCTX-M* and *blaTEM* genes encoding extended-spectrum β-lactamases (ESBLs), which render them resistant to third-generation cephalosporins (Eze *et al.,* 2020).

Mobile genetic elements such as integrons and transposons play a major role in spreading resistance among *Vibrio* populations (Adebayo-Tayo *et al.,* 2019). Class 1 integrons, in particular, are frequently detected in multidrug-resistant *Vibrio* isolates, contributing to the rapid dissemination of resistance determinants across environmental and clinical niches (Igbinosa *et al.,* 2020).

COMMON RESISTANT GENES IN *Vibrio* spp

Vibrio species isolated from diarrheal patients demonstrate diverse resistance gene profiles with concerning trends toward mobile colistin resistance:

1. **Tetracycline Resistance Genes**

Tetracycline determinants are ubiquitous in clinical *Vibrio* isolates. In a large genomic surveillance study of *V. parahaemolyticus* from Zhejiang Province hospitals (2016-2023), *tet(B)* was nearly universal among clinical isolates. Similarly, *tetA* was detected in 76.9% of *V. cholerae* isolates from a Peshawar outbreak (Ahmad *et al.,* 2025; Huang *et al.,* 2025). The arrival of resistant *Vibrio* strains in aquatic habitats has been associated with both man-made causes and the organism's

ability to facilitate natural gene exchanges. Tetracyclines, sulfonamides, fluoroquinolones, and β-lactams which are antibiotics frequently used in fish farming tends to create selective pressure on the microbes which hastens their evolution and acquisition of his resistance in order to prevent their dying. *Vibrio* spp interact with other organisms (enteric) in these aquatic environments (which serve as mixing grounds) which facilitate horizontal gene transfer via plasmids, integrons, and transposons (Eze *et al.,*2020). As such, the environmental resistome associated with *Vibrio* is becoming increasingly recognized globally as an important part of the dissemination of antimicrobial resistance (Huang *et al.,* 2025). Tetracycline resistance is conferred by efflux pump genes (*tetA*, *tetB*, *tetE*, *tetR*) and ribosomal protection proteins such as *tetM* and *tetO* (Eze *et al.,* 2020).

In clinical contexts *V. cholerae* and *V. parahaemolyticus* are more often than other species studied for antibiotic resistance capabilities because of its significance as causal agents for diarrheagenic diseases outbreaks. Studies generated reports from different countries like from Nigeria, China, and India have indicated a more rampant spread of multidrug resistance among clinical isolates, with resistance against common over-the-counter antibiotics e.g. ampicillin, erythromycin, ciprofloxacin, and trimethoprim-sulfamethoxazole (Adebayo-Tayo *et al.,* 2019; Chen *et al.,* 2022). Understanding geographical differences in resistance dynamics is made easier by profiling the resistance genes in these isolates, which offers genetic evidence to validate phenotypic observations.

2. Beta-Lactamase Genes

Multiple beta-lactamase gene families are prevalent in *Vibrio* isolates. In the Peshawar *V. cholerae* outbreak study, blaTEM was detected in 84.6% of isolates, blaCTX-M in 69.2%, blaOXA in 61.5%, and blaSHV in 38.5% (Ahmad *et al.,* 2025). These high prevalence rates indicate that β-lactamase genes such as *blaTEM*, *blaSHV*, and *blaCTX-M* are widespread and contribute to

resistance against penicillins and cephalosporins. Moreover, *Vibrio* strains harboring *blaNDM-1* or *blaOXA-48*-like carbapenemase genes have recently been detected, raising concern over resistance to last-resort β-lactam antibiotics (Zhou *et al.,* 2024)**.**

3. Sulfonamide and Trimethoprim Resistance Genes

Sulfonamide and trimethoprim resistance genes are highly prevalent in *V. cholerae* outbreak isolates. The Peshawar study (2023-2024 cholera outbreak in Peshawar, Pakistan) found *sul1* in 92.3%, *sul2* in 76.9%, and *dfrA1* in 92.3% of molecularly profiled isolates (Ahmad *et al.,* 2025). Sulfonamide and trimethoprim resistance genes (*sul1*, *sul2*, *dfrA*) are also prevalent, often linked with class 1 integrons, which facilitate gene cassettes' horizontal mobility (Eze *et al.,* 2020).

4. Quinolone Resistance Genes

Quinolone resistance in *Vibrio* species is presented by plasmid-mediated genes (*qnrA*, *qnrB*, *qnrS*) and chromosomal mutations in *gyrA* and *parC*. *aadA1*, *aac(3)-IIa*, and *aph(3')-Ia* which are drivers of aminoglycoside resistancehave been discovered in organisms isolated from the environment, further demonstrating the genetic variety of the *Vibrio* resistome (Ahmad *et al.,* 2025). The occurrence of multiple AGR's at the same time in a single isolate may suggest intense elective pressure and examines *Vibrio*'s role as both a pathogen and a reservoir in the aquatic AMR system.

5. Mobile Colistin Resistance

The most alarming discovery is that the mobile colistin resistance gene *mcr*-1 was present in 53.8% of MDR *V. cholerae* isolates from the Peshawar outbreak (Ahmad *et al*., 2025). Serious concerns concerning colistin resistance pools and their transmission to other illnesses are raised by this finding.

***Salmonella* spp.**

Salmonella spp. exhibit diverse resistance mechanisms, including β-lactamase production, efflux systems, and modifications in target sites. ESBL-producing *Salmonella* isolates have been more progressively reported in Nigerian hospitals, with most possesing genes such as *blaSHV*, *blaTEM*, and *blaCTX-M* (Iroha *et al.,* 2020). Quinolone resistance has been linked to plasmid-mediated *qnr* genes and mutations in the *gyrA* and *parC* genes of the quinolone resistance-determining regions (QRDRs) (Chukwu *et al.,* 2019).

Research recently conducted in molecular biology has also identified multidrug resistance genes, including *aadA*, *catA*, and *sul2*, in *Salmonella* isolates from diarrheal patients in southern Nigeria (Afolabi *et al.,* 2021). These genes code resistance to aminoglycosides, chloramphenicol, and sulfonamides.

COMMON RESISTANT GENES IN *Salmonella* spp

Salmonella isolates from hospital stool samples have demonstrated high prevalence of multiple antibiotic resistance genes as determined from multiple studies.

1. **Tetracycline Resistance Genes**

The factors involved in tetracycline resistance (genes), particularly *tet(A)*, are among the most frequently detected resistance genes in clinical *Salmonella* isolates. In a whole-genome sequencing study of 87 non-typhoidal *Salmonella* isolates from outpatients in Shaoxing, China, *tet(A)* was present in 52.9% of isolates (Chen *et al.,* 2022). These genes are commonly in the microbes plasmid thus making them more readily disseminated amongst bacterial populations through horizontal gene transfer.

2. Beta-Lactamase Genes

blaTEM gene variants confer resistance to beta-lactamases and it show rems a remarkably high ubiquity. The Shaoxing study discussed priorly also reported *blaTEM-1B* in 65.5% of isolates, while *aac(6')-Iaa* was detected in 100% of the sequenced strains (Chen *et al.,* 2022). These genes grant resistance to commonly used beta-lactam antibiotics, thereby severely limiting treatment options

3. Macrolide Resistance Genes

The resistance of antibiotic azithromycin is enabled due to the presence of the *mphA* gene, which has emerged as of significant concern in pediatric patients. Another study from Shenzhen Children's Hospital found that among 487 *Salmonella* isolates, 15 (3.08%) were azithromycin-resistant with MICs ranging from 32 to >256 μg/ml. It was noted later in the study that all azithromycin-resistant isolates carried plasmid-encoded *mphA* genes on diverse types of plasmids (Wang *et al.,* 2023). This plasmid-mediated resistance poses a considerable risk for horizontal spread.

4. Quinolone Resistance Determinants

Plasmid-mediated quinolone resistance (PMQR) genes have been prevalently on a rise in children recently and has been more commonly associated with pediatric *Salmonella* isolates. An Iranian study of children with diarrhea found high nalidixic acid resistance (71.4%) and detected multiple PMQR determinants: *qnrS* in 60% of PMQR-positive isolates, *qnrA* in 40%, and *qnrB* in 20%. In addition to the above, class 1 integrons was found to be present in 71.4% of isolates, indicating potential for accumulation of additional resistance genes (Abbasi and Ghaznavi-Rad, 2021). Widespread PMQR determinants and integrons complicate empiric quinolone therapy. The high

prevalence of these genes favors a treatment plan involving a more localized AST-guided treatment rather than empiric quinolone use (Abbasi and Ghaznavi-Rad, 2021).

2.8 DETECTION METHODS FOR ANTIBIOTIC RESISTANCE

2.8.0 PHENOTYPIC BASED DETECTION METHODS

1. Disk Diffusion Method

The foundational test majorly used in antibiotic susceptibility testing (AST) is the Kirby-Bauer disk diffusion method of testing, particularly in laboratories with limited resources (CLSI, 2021). This testing method involves placing disks infused with a defined gram of antibiotic onto agar plates inoculated with the test organism (usually with spread plate or swabbed) and measuring zones of inhibition after incubation (Jorgensen and Ferraro, 2020). While inexpensive and standardized, it has notable drawbacks, which includes a lengthy incubation period (16–24 hours) and limited quantitative accuracy (Hombach *et al.,* 2020).

In an attempt to maximize disc diffusion, recent research has integrated digital image analysis to automate zone diameter interpretation, improving reproducibility and lowering human error. (Hombach *et al.,* 2013).

2. Broth Microdilution and E-test

This method of susceptibility testing is the model standard for identifying the lowest concentration of antibiotic that visibly inhibits bacterial growth (minimum inhibitory concentrations (MICs) (EUCAST, 2023). The E-test of microdilution employs a gradient strip which allows semi-quantitative MIC determination. Automation of microdilution using systems like VITEK 2 and BD Phoenix has improved throughput and standardization (Van Belkum *et al.,* 2020). However, their high-cost limits accessibility for low-income laboratories.

As stated in a comparative study by Chishti *et al*. (2022), automated MIC technologies struggled with polymicrobial cultures but obtained 95% concordance with manual assays, highlighting the necessity of confirmatory molecular identification.

2.8.1 MOLECULAR-BASED DETECTION METHODS

1. Polymerase Chain Reaction (PCR) and Quantitative PCR (qPCR)

Techniques involving polymerase chain reactions have modified antibiotic resistance detection for the better by identifying specific genetic markers associated with resistance, like: *bla*, *mecA*, or *vanA* genes (Choi *et al.,* 2019). Quantitative PCR (qPCR) stretches this functionality by measuring the total amount of genetic material present in a sample thus offering a more rapid and sensitive detection of the specific resistance genes within hours (Mouftah *et al.,* 2020).

Recently, there have been advancements in this technique include multiplex PCR assays that acts as a more improved version of the qPCR by detecting multiple resistance genes at the same time thereby improving diagnostic efficiency (Lima *et al.,* 2021). Despite these strengths, PCR requires existing comprehension of target genes and cannot detect unknown or new resistance mechanisms (Yousefi *et al.,* 2023).

2. Loop-Mediated Isothermal Amplification (LAMP)

LAMP has been regarded for its care site applications due to its rapid amplification under isothermal conditions (Notomi *et al.,* 2020). Since 2019, several studies have validated LAMP assays for detecting methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenemase-producing *Enterobacterales* (Li *et al.,* 2021). Its applicability in field settings is because of the technique's strength and its minimality in equipment, although its high sensitivity to contamination and non-specific amplification remain concerns (Park *et al.,* 2022).

3. Whole-Genome Sequencing (WGS)

Whole-genome sequencing provides broader views into bacterial resistance determinants and phylogeny (Köser *et al.,* 2020). The decreasing cost of sequencing and the development of user-friendly bioinformatics pipelines, such as ResFinder and CARD, have expanded WGS applications in routine surveillance (Hendriksen *et al.,* 2019).

WGS has become the well-known exemplary standard for a more diversified approach in the profiling of resistant genes. Studies employed both Illumina and Nanopore sequencing platforms (often in hybrid approaches) to achieve complete genome assembly and accurate characterization of plasmids. In-silico tools were used to identify antimicrobial resistance genes (ARGs), plasmid replicon types, and mobile genetic elements (Chen *et al.,* 2022; Wang *et al.,* 2023; Liyanage, 2023). However, difficulties include the intricacy of data interpretation, the requirement for infrastructure, and moral issues with sharing genomic data (Balloux *et al.,* 2022).

2.8.2 MASS SPECTROMETRY AND SPECTROSCOPIC APPROACHES

1. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) MS

Bacterial identification has been transformed by MALDI-TOF MS, which is rapidly being modified for AMR detection (Clark *et al*., 2021). By examining the proteomic fingerprint of bacterial cells, resistance can be deduced from distinctive changes in mass spectra linked to enzyme-mediated breakdown, like β-lactamase activity (Camara *et al.,* 2022).

Novel methods, including the MALDIxin assay, allow rapid identification of colistin resistance by detecting lipid A modifications (Furniss *et al.,* 2020). While this assay is truly efficient and rapid (under 30 minutes), it requires skilled interpretation of the result data, also the equipment used in preforming the assay is expensive, limiting their widespread use outside tertiary centers.

2. Raman and Infrared Spectroscopy

The Raman and Fourier-transform infrared (FTIR) spectroscopy which is a type of vibrational spectroscopic method is being researched for a more non-destructive approach into resistance determination and gene profiling (Cheng *et al.,* 2020). These methods utilize the organism's response to external stimuli (in this case the antibiotic administered) and the resulting biochemical alterations in response to the exposure.

A study carried out by Lin *et al*. (2023) showed its potential in ultra-rapid phenotypic AST by using surface-enhanced Raman scattering (SERS) to differentiate between susceptible and resistant *E. coli* strains in less than an hour. Reproducibility and standardization, however, continue to cause substantial constraints (Singh *et al.,* 2022).

2.8.3 BIOSENSOR-BASED DETECTION TECHNOLOGIES

The use of biosensors presents for a quicker, reactive and a lighter weight means of antibiotic resistance detection. They work by coupling biological recognition elements (enzymes, antibodies, or nucleic acids) with signal transducers (electrochemical, optical, or piezoelectric).

1. Electrochemical Biosensors

Electrochemical biosensors have been widely developed for AMR detection due to their high sensitivity and miniaturisation potential (Nisar *et al.,* 2021). For example, a graphene-based biosensor developed by Zhang *et al.* (2022) found *blaNDM-1* gene in less than 30 minutes with a high specificity.

Integrating microfluidic chips enabled care site diagnostics, however, challenges remain regarding stability, mass production, and sample matrix interference (Abdelhamid *et al.,* 2023).

2. Optical Biosensors

Light-based transduction uses optical biosensors in order to identify biomolecular interactions. Systems based on plasmonics and fluorescence have become popular for identifying antibiotic residues and resistance genes (Qian *et al.,* 2020). A study conducted by Luo *et al*. (2024) introduced a dual-mode surface plasmon resonance (SPR) biosensor capable of quantifying resistance gene copies at femtomolar concentrations.

The future of biosensors lies in multiplexed, smartphone-integrated systems capable of providing real-time data analytics for surveillance (Rahman *et al.,* 2022).

2.8.4 CRISPR-BASED DIAGNOSTIC METHODS

The CRISPR-Cas system has emerged as a powerful tool for gene editing and diagnostics. CRISPR-based detection systems such as SHERLOCK and DETECTR utilize Cas nucleases to recognize specific resistance gene sequences and generate detectable signals (Kellner *et al.,* 2021).

In 2022, Aman *et al*. demonstrated a CRISPR-Cas12a assay capable of detecting *mcr-1* colistin resistance gene in under 30 minutes, outperforming PCR in sensitivity. Recent integration of CRISPR assays with lateral flow devices has enabled instrument-free field testing (Liang *et al.,* 2024). However, CRISPR diagnostics still face technical challenges such as off-target effects, reagent stability, and limited multiplexing capabilities (Abudayyeh and Gootenberg, 2023).

2.8.5 ARTIFICIAL INTELLIGENCE (AI) AND COMPUTATIONAL METHODS

Artificial intelligence and machine learning (ML) are help in determining resistance by analyzing probable resistance based on already occurring resistance patterns and through the analysis of

genomic and phenotypic data (Pesesky *et al.,* 2021). Deep learning models trained on large genomic datasets can predict resistance phenotypes with high accuracy, even for untested drugs (Dimitriu *et al.,* 2022).

DeepARG and AMRNet have been integrated with multi-omics data in order to comprehensively predict resistance (Arango-Argoty *et al.,* 2023). Moreover, AI-enhanced image analysis in automated microscopy and disk diffusion assays has reduced human bias (Chang *et al.,* 2021). However, AI models require extensive validation and standardized datasets to ensure generalizability across bacterial species and geographies (Sewell *et al.,* 2024).

2.9 IMPACT ON TREATMENT OUTCOMES

1. Therapeutic failure risk: The increased rate of isolates resistant to widely used first-line antimicrobials increases the likelihood of treatment failure and prolonged illness, especially where diagnostics and alternative treatment agents are limited (Ballal, 2016; Aminshahidi *et al.,* 2017). Resistant infections often require longer hospital stays, repeated medical interventions, and the use of more toxic, expensive or experimental alternative drug (Balloux *et al.,* 2022).
2. Public-health implications: The combined effect of multidrug resistance in enteric pathogens and the transmission of this resistance environmentally significantly increases the probability of outbreaks and complicates the management of this infection ren (Alhaji *et al.,* 2022; Olayinka *et al.,* 2024). Antibiotic resistance not only affects the outcomes of the patients involved but also imposes significant economic burdens on healthcare systems (costs arising from prolonged hospitalizations, additional diagnostic testing, and the need for advanced antibiotics) (O'Neill, 2021). The World Health Organization (2023) estimates that AMR-related infections could cause 10 million deaths annually by 2050 if current trends persist. In low- and middle-income

countries, the economic burden is worsened by restricted access to newer antibiotics and diagnostic technologies (Nguyen *et al.,* 2023).

Also, antibiotic resistance compromises infection prevention and control in medical procedures such as surgery, organ transplantation, and chemotherapy. Patients undergoing these treatments are at increased risk of developing untreatable infections due to compromised immunity and exposure to resistant pathogens (Van Belkum *et al.,* 2020).

3. Impact on disease transmission: Infections with their causal agents being resistant to antibiotics tend to spread very quickly within a hospital and in a community. Organisms like the methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), and carbapenem-resistant *Enterobacteriaceae* (CRE) can remain on the surfaces of objects for long periods without dying out and can be contracted and spread by the healthcare workers (Hendriksen *et al.,* 2019). Factors like bad sanitation practices, wrong and improper use of antibiotics and improper waste management systems in agriculture contribute to the dissemination of resistance genes through the food chain and environment (Rahman *et al.,* 2022). Consequently, resistance undermines global infection control programs, limiting the effectiveness of antibiotics for both common and severe infections.
4. Loss of last resort antibiotics: Detection of *mcr-1* in clinical *V. cholerae* is of particular concern as it indicates potential colistin resistance reservoirs. Colistin is often considered a last-resort antibiotic for severe, refractory infections, and its compromise through mobile resistance genes poses a significant therapeutic challenge (Ahmad *et al.,* 2025).
5. Narrowing therapeutic options: While some studies report that fluoroquinolones and third-generation cephalosporins remain effective in certain settings, the overall trend indicates narrowing therapeutic options. The Beijing *V. furnissii* study noted 100% cefazolin resistance and emphasized the need for sustained surveillance (Zhou *et al.,* 2024).

CHAPTER THREE

MATERIALS AND METHODS

3.0 ETHICAL APPROVAL

Before beginning the study an application for ethical approval was submitted to the Review Committee for Ethical Approval of Human Studies at Benson Idahosa University and was granted.

3.1 SAMPLE COLLECTION

A total of 30 stool samples were collected from diarrhoeal patients from three hospitals in the Benin Metropolis namely, Benin Medical Care (BMC), Edo Specialist Hospital (ESH) and City of Hope Hospital (COH). Each sample was collected aseptically using sterile sample containers and were preserved with ice packs in a sample cooler after which were transferred to the laboratory for microbiological analysis within 3 hours of collection. The samples were collected irrespective of the patient's age and sex.

3.2 BACTERIOLOGICAL ANALYSIS

3.2.0 Sample Preparation

A gram of stool sample was placed in a test tube containing 9 mL of sterile peptone water. Afterwards, the test tube was left to rest for approximately 45 minutes. Serial dilutions were made up to five-fold**.** For *Vibrio* spp alkaline peptone water was used and normal peptone water was used for *Salmonella* spp. All the dilutions were used in plating, 1ml of dilutions was plated on Thiosulphate bile salts agar (TCBS), *Salmonella Shigella* agar (SS) and Nutrient agar media using the pour plate method

3.2.1 Enumeration of Total Heterotrophic Count

Colony count was carried out using a counting chamber. The counting chamber creates a volumetric grid divided into differently sized cubes for accuracy in counting the number of colonies in a cube and in calculating the concentration of the entire sample. The colony was reported as Colony Forming Units per gram of sample (cfu/g).

3.2.2 Isolation of *Vibrio* spp

About 1ml of the sample was dispensed into a test tube containing sterile alkaline peptone water at 37 °C for 5 hours. For selective isolation of *Vibrio* spp, surface growth was then streaked onto Thiosulfate citrate bile salt sucrose agar (TCBS) plates. The agar plates were incubated at 37° C for 24 hrs. After incubation, *Vibrio* colonies on TCBS agar were distinguished by bright yellow or green colonies, depending on whether they were able to ferment sucrose.

3.2.3 Isolation of *Salmonella* spp

About 1 ml of each of the dilutions was plated on the differential medium, *Salmonella* Shigella (SS) Agar using pour plate method. The media was allowed to solidify and then incubated at 37°C for 24 hrs. The colonies were represented as mucoid transparent colonies with black centers.

3.2.4 Cultural Characteristics of Isolates

After incubation, the cultural and morphological characteristics on the agar plates were carefully observed and recorded. Morphological observed characteristics include: Margin (curled, entire, undulate, serrated or lobate), Colour, Elevation (raised, flat, or convex), Texture (slimy, moist, viscous, transparent, shiny or brittle), Shape (irregular, spindle, rhizoid, round or filamentous) and Size.

3.2.5 Subculture of isolates

Subculture was carried out on nutrient media through streak plate method. Colonies on the selective media were streaked onto the nutrient agar and incubated at 37°C overnight. After which the pure cultures were transferred to McCartney bottles containing nutrient agar for storage for further analysis.

3.3 GRAM STAINING

The isolated cells were smeared on a clean glass slide; it was left to air dry and then heat fixed to the slide. The primary dye, crystal violet, was then poured and kept for about 30 seconds to 1 minute and rinsed with water. Iodine (mordant that fixes the primary dye to the bacteria cell wall) was used to flood the slide for a minute and was then rinsed with water for 3 seconds under a gentle stream. This was followed by rinsing off with acetone for about 10-20 seconds and rinsing with water. Safranin was added for about 1 minute and washed with water. The slide was left to air dry. Results were viewed with the aid of a microscope. Gram-positive bacteria shows purple colouration, and Gram-negative shows a pink to red colouration

3.4 BIOCHEMICAL IDENTIFICATION OF ISOLATES

The obtained isolates were presumptively identified using their biochemical characteristics.

3.4.0 Catalase test

A sterile inoculating loop was used to transfer a colony to a dry sterile glass slide. A drop of 3% H,02 was dropped on the glass slide, the appearance of oxygen bubbles signifies a catalase positive result.

3.4.1 Citrate Utilization Test

Simmon citrate agar was used for this test. The agar was prepared according to the manufacturer's instructions at half strength. The prepared agar was then dispensed (4-5ml) into test tubes. The tubes were then autoclaved at 121°C for 15 mins. The agar was then cooled in a slanted position. The medium was inoculated with the isolates and incubated at 37°C. A colour change from green to blue along the slant was taken as a positive result.

3.4.2 Indole Test

This test demonstrates the ability of bacteria to utilize amino acid tryptophan to produce indole. Test tubes containing 4ml of tryptophan broth were inoculated with the isolate and then incubated at 37°C for 24-28 hours. 0.5ml of Kovac's reagent was added 3 drops to the broth culture. Formation of pink to red colour in the reagent layer on top of the medium within seconds of adding, the medium indicated a positive result. No color change was taken as a negative result.

3.4.3 Oxidase Test

Oxidase test determines the presence of cytochrome oxidase C. This test is used to assist in the identification of *Pseudomonas, Neisseria, Alcaligenes, Aeromonas, Campylobacter*, *Vibrio*, *Brucella* and *Pasteurella,* all of which produce the enzyme cytochrome oxidase. A strip of the filter paper was soaked with a little freshly made 1% solution of the reagent. A speck of culture was smeared on the filter paper using a wooden applicator stick. A positive result was shown by the appearance of a strong, deep purple color within 5–10 seconds. A delayed positive result was marked by color development between 10 and 60 seconds. A negative result was indicated when no color appeared after 60 seconds.

3.4.4 Motility Test

Motility is the ability of an organism to move by itself by means of propeller-like flagella unique to bacteria or by special fibrils that produce a gliding form of movement. It is used for the differentiation of microorganisms based on motility. Motility by a bacterium is mostly demonstrated in a semisolid agar medium. In semi-solid agar media, motile bacteria "swarm' and give a diffuse spreading growth that is easily recognized by the naked eye. Nutrient agar in half strength was used for this test. Inoculum was collected from a 24hr. pure culture and was stabbed into the medium to a depth of only ½ to ½ inch in the middle of the tube.

The tubes were then incubated at 37°C for 24 hrs. They were observed for a diffused zone of growth flaring out from the line of inoculation. Diffused, hazy growths that spread throughout the medium, rendering it slightly opaque was a positive result, while growth that is confined to the stab-line, with sharply defined margins and leaving the surrounding medium clearly transparent, was negative.

3.4.5 Urease Test

A pure culture of the isolate suspected to be *Salmonella* was inoculated onto a sterile urea agar slant using a sterile inoculating loop. The inoculated medium was then incubated at 35–37°C for 18 to 24 hours. During incubation, organisms capable of producing the enzyme urease hydrolyzed urea into ammonia and carbon dioxide, resulting in an alkaline reaction that caused the phenol red indicator in the medium to change from yellow to bright pink. A positive urease reaction (pink color) indicated urease production.

3.5 STANDARDIZATION OF THE INOCULUM AND MULTI-SENSITIVITY DISK TEST

3.5.1 Standardization of the Isolates

Isolates obtained from a 24-hour pure culture were inoculated into test tubes containing 5 ml of sterile peptone water broth. The media was incubated at 37 °C for 24 hrs. After incubation, the broth was diluted using sterile peptone water to obtain a density of 0.05-0.1 absorbance units at a wavelength of 625nm, using a spectrophotometer.

3.5.1 Antibiotic Susceptibility of Isolates

This test was carried out using the Disk Diffusion Method to detect possible antimicrobial resistance and susceptibility. Disk diffusion method is classified as an agar diffusion method (ADM) because the extract to be tested diffuses from its reservoir through the agar medium seeded with the test microorganism. Generally, the reservoir is a filter paper disk that is placed on top of an agar surface. Muller-Hinton agar was used for the culture.

Sterile swab sticks were used to streak the inoculum on the Miller Hinton agar plates. The disk was placed on the inoculated plate with the aid of sterile forceps and incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured in millimetres using a metric rule. The Clinical and Laboratory Standard Institute (CLSI) protocol was used to interpret the readings of the susceptibility test.

3.6 MOLECULAR IDENTIFICATION OF ISOLATES

3.6.0 DNA Extraction

Genomic DNA was extracted from the received samples using the *Quick-DNA Fungal/Bacterial Miniprep Kit* (Zymo Research, Catalogue No. D6005) according to the manufacturer's

instructions, with slight modifications. Approximately 50–100 mg (wet weight) of bacterial cells was transferred into a ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm), after which 750 µL of BashingBead™ Buffer was added. The tube was then secured in a bead beater (Disruptor Genie) and processed for 20 minutes to ensure complete cell lysis. Following disruption, the lysate was centrifuged at 10,000 × g for 1 minute to pellet cellular debris.

From the resulting supernatant, 400 µL was carefully transferred into a Zymo-Spin™ III-F Filter placed in a collection tube and centrifuged at 8,000 × g for 1 minute. The filter was subsequently discarded, and 1,200 µL of Genomic Lysis Buffer was added to the filtrate and mixed thoroughly. To bind the DNA, 800 µL of the mixture was transferred to a Zymo-Spin™ IICR Column in a collection tube and centrifuged at 10,000 × g for 1 minute. The flow-through was discarded, and this binding step was repeated to maximize DNA recovery.

The column was then transferred to a new collection tube, and 200 µL of DNA Pre-Wash Buffer was added, followed by centrifugation at 10,000 × g for 1 minute. The flow-through was discarded, and 500 µL of g-DNA Wash Buffer was added to the column, which was centrifuged again at 10,000 × g for 1 minute to remove residual contaminants. Finally, the Zymo-Spin™ IICR Column was placed into a clean 1.5 mL microcentrifuge tube, and 50 µL of DNA Elution Buffer was added directly to the column matrix. The assembly was centrifuged at 10,000 × g for 30 seconds to elute the purified genomic DNA, which was subsequently stored at −20°C until further use

3.6.1 DNA Quantification

The purity and concentration of the extracted DNA were assessed using a *Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer*. The instrument was first blanked with 1 µL of DNA Elution Buffer. Subsequently, 1 µL of each DNA sample was placed on the pedestal and analyzed. The DNA concentration (in ng/µL), A260/280 ratio, and A260/230 ratio were

recorded for each sample to evaluate DNA yield and purity. Samples with acceptable purity ratios were used for downstream applications.

3.6.2 PCR Amplification

The 16S rRNA gene region was amplified using *OneTaq® Quick-Load® 2X Master Mix* (New England Biolabs, Catalogue No. M0486). Each PCR reaction was carried out in a total volume of 25 µL, consisting of 12.5 µL of OneTaq® Quick-Load® 2X Master Mix, 0.5 µL each of 10 µM forward and reverse primers, 2–3 µL of template DNA, and nuclease-free water to make up the final volume. The universal bacterial primers used were 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (CGGTTACCTTGTTACGACTT), which target the conserved regions of the 16S rRNA gene.

The amplification reactions were performed using an *Eppendorf Mastercycler Nexus gradient 230*. The thermal cycling conditions consisted of an initial denaturation step, followed by a series of denaturation, annealing, and extension cycles, optimised to ensure efficient amplification of the target region. The resulting PCR products were then stored at 4°C before electrophoretic analysis and sequencing.

3.6.3 Sequencing

The fragments were sequenced using the Nimagen, Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to manufacturer's instructions:

The labelled products were then cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053). The cleaned products were injected on the Applied Biosystems ABI 3500XL Genetic Analyser with a 50cm array, using POP7: thermofisher product catalog and sequence data collected.

3.7 MOLECULAR DETECTION OF ANTIBIOTIC-RESISTANT GENES

Genes coding for beta-lactamase resistance was carried out using PCR: PCR was performed with the following primers; Primers for antibiotic resistant gene *blaTEM* F-5'-ATAAAATTCTTGAAGACGAAA -3'and R-5'-GACAGTTACCAATGCTTAATC-3', Primers for gene *sul2* F-5'-GCGCTCAAGGCAGATGGCAT-3' R-5'- GCGTTTGATACCGGCACCCT-3' and *blaIMP* F: GAATAGAGTGGATTAATTCTC, R: GGTTTAAYAAAACAACCACC.

The reaction mixture contained inqaba PCR Premix (Inqaba, South Africa), which is premixed ready-to-use solution containing Tag DNA polymerase, dNTP, and MgClz. The reaction mixture was prepared in 0.2 ml PCR tubes with 25 ul reaction volumes (12.5 ul Premix, 8.5 ul nuclease free water, 0.5 ul forward primer, 0.5 ul reverse primer and 3.0 ul template DNA) and done under the following thermocycling conditions in a Gene Amp PCR system (Gene amp, Singapore) 94 °C for 4 min for initial denaturation then 31 cycles, each at 94 °C for 45 seconds, 55 °C for 1 min for CTX-M and TEM, 68 °C for 1 minute and final extension at 68 °C for 8 minutes after which PCR products were separated in 1.5% agarose gel which was stained with ethidium bromide. PCR products on gel were visualized under UV trans-illuminator (Geneix biotech, Asia).

3.7.0 Agarose gel preparation

Two drops of ethidium bromide were added to a 0.8 percent Agarose gel, which was then allowed to gel. Each well was loaded with ten microlitres of the isolated plasmid and the loading dye (NO70225 Bio lab England). The last well was loaded with 10l of the 1kb DNA ladder (NO4685 Bio lab England). The electrophoresis was run at 100v for 40min after which bands were visualized with UV trans illuminator

3.7 STATISTICAL ANALYSIS

3.8.0 ANALYSIS OF VARIANCE (ANOVA)

Analysis of Variance (ANOVA) was used to analyse the laboratory data collected. This was done using the Statistical Package for the Social Sciences (SPSS) and Microsoft Office Excel package.

CHAPTER FOUR

RESULTS

The microbial analysis of stool samples collected from hospitals within the Benin Metropolis revealed marked variations in bacterial loads across the three facilities. These variations were evident in the heterotrophic bacterial counts, *Salmonella* counts, and *Vibrio* counts obtained from the samples. The observed differences in the prevalence variability of microbial growth among the hospitals may reflect differences in patient populations/ socioeconomic statuses, sanitation practices, environmental exposure, or other epidemiological and environmental factors.

For the heterotrophic counts, Benin Medical Care Hospital recorded values ranging between 2.65 ± 0.2 × 10^4 CFU/g and 28.65 ± 0.4 × 10^4 CFU/g, with an average of 9.81 ± 0.2 × 10^4 CFU/g. Edo Specialist Hospital consistently exhibited higher values, ranging from 4.50 ± 0.2 × 10^4 CFU/g to 29.65 ± 0.2 × 10^4 CFU/g, and an average of 16.79 ± 0.32 × 10^4 CFU/g. City of Hope Hospital, on the other hand, presented comparatively lower counts, ranging from 2.55 ± 0.3 × 10^4 CFU/g to 13.25 ± 0.5 × 10^4 CFU/g, with an average of 6.39 ± 0.41 × 10^4 CFU/g. The total mean heterotrophic count across all hospitals was 10.99 ± 0.9 × 10^4 CFU/g, placing Edo Specialist Hospital as the site with the greatest microbial burden in this category, as depicted in Table 4.1.

Similarly, for the *Salmonella* counts (Table 4.2) BMC Hospital, values ranged from 2.95 ± 0.3 × 10^4 CFU/g to 20.00 ± 0.2 × 10^4 CFU/g, with an average of 7.51 ± 0.33 × 10^4 CFU/g. Edo Specialist Hospital again recorded the highest values, ranging between 12.00 ± 0.4 × 10^4 CFU/g and 28.95 ± 0.2 × 10^4 CFU/g, with an average of 20.05 ± 0.4 × 10^4 CFU/g. City of Hope Hospital showed lower counts, ranging from 1.01 ± 0.6 × 10^4 CFU/g to 20.25 ± 0.6 × 10^4 CFU/g, with an average of 6.13 ± 0.4 × 10^4 CFU/g. The overall mean *Salmonella* count across the hospitals was 11.23 ± 0.4 × 10^4 CFU/g.

For the *Vibrio* counts (Table 4.3), BMC Hospital recorded values between 2.80 ± 0.1 × 10^4 CFU/g and 17.85 ± 0.6 × 10^4 CFU/g, with an average of 6.23 ± 0.42 × 10^4 CFU/g. Edo Specialist Hospital again demonstrated the highest microbial load, with counts ranging from 2.65 ± 0.07 × 10^4 CFU/g to 29.55 ± 0.5 × 10^4 CFU/g, and an average of 9.97 ± 0.48 × 10^4 CFU/g. City of Hope Hospital recorded values between 2.50 ± 0.2 × 10^4 CFU/g and 29.25 ± 0.7 × 10^4 CFU/g, with an average of 6.04 ± 0.39 × 10^4 CFU/g. The total mean *Vibrio* count across the three hospitals was 7.41 ± 0.43 × 10^4 CFU/g.

The combined analysis of these results show a clear pattern across the three hospitals with ESH showing the highest average counts amongst the three including heterotrophic bacteria, *Salmonella* species, and *Vibrio* species. These results may infer that samples retrieved from patients attending this hospital contained comparatively higher microbial loads than those obtained from the other facilities included in the study. This consistence in the higher counts across all categories may be as a result of several environmental and socio-economic factors as well as the accessibility of the hospital to individuals or variations in sanitation and hygiene conditions of the patients of the hospital.

In comparison, Benin Medical Care (BMC) Hospital generally displayed intermediate microbial counts across the different bacterial groups examined. While the values recorded at this facility were lower than those observed at Edo Specialist Hospital, they were still higher than those documented for City of Hope Hospital in some instances. This pattern indicates that although microbial presence was evident in samples from BMC Hospital, the overall burden was relatively moderate when compared with the high values observed totally.

City of Hope Hospital, on the other hand, consistently recorded the lowest average microbial counts for most of the bacterial groups. It is possible that variations in patient demographics, such

as socioeconomic status, living conditions, or access to clean water and sanitation, may contribute to the comparatively reduced microbial levels observed in stool samples from this hospital.

Overall, the differences observed among the three hospitals highlight the presence of noticeable variability in microbial prevalence within healthcare facilities located in the same metropolitan area. These findings therefore, underscore the importance of continuous microbiological surveillance within healthcare settings. From a wider stand point, the results attained from this study provide useful baseline information for public health authorities and hospital administrators involved in disease monitoring and management within the Benin Metropolis.

Table 4.1 Mean Heterotrophic Counts from Stools Sampled from Hospitals at Varying Locations in Benin Metropolis.

BENIN MEDICAL CARE		EDO SPECIALIST		CITY OF HOPE		
Samples	**Mean Heterotrophic counts (NA) ($\times 10^4$ cfu/g) ± S. D**	**Samples**	**Mean Heterotrophic counts (NA) ($\times 10^4$ cfu/g) ± S. D**	**Samples**	**Mean Heterotrophic counts (NA) ($\times 10^4$ cfu/g) ± S. D**	**TOTAL Average counts ($\times 10^4$ cfu/g) ± S. D**
1	5.8 ± 0.1	1	14.1 ± 0.2	1	13.25 ± 0.5	
2	3.0 ± 0.3	2	14.9 ± 0.4	2	3.15 ± 0.07	
3	2.65 ± 0.2	3	5.1 ± 0.2	3	2.55 ± 0.3	
4	28.65 ± 0.4	4	4.5 ± 0.2	4	3.0 ± 0.7	
5	13.95 ± 0.2	5	26.05± 0.2	5	7.8 ± 0.3	
6	10.0 ± 0.1	6	19.4 ± 0.7	6	10.0 ± 0.3	
7	5.85 ± 0.2	7	23.7 ± 0.5	7	2.65 ± 0.07	
8	12.15 ± 0.3	8	29.65 ± 0.2	8	10.1 ± 0.5	
9	6.3 ± 0.1	9	18.5 ± 0.5	9	8.7 ± 1	
10	9.7 ± 0.1	10	11.95± 0.07	10	2.7 ± 0.4	
TOTAL	98.05 ± 2.0		167.85 ± 3.17		63.9 ± 4.14	329.8 ± 9.31
AVERAGE	9.81± 0.2		16.79 ± 0.32		6.39 ± 0.41	10.99 ±0.93

KEY:

S.D = Standard Deviation

Table 4.2. Total *Salmonella* counts on *Salmonella Shigella* Agar (SSA)from Stool Samples Obtained from Varying Hospitals in the Benin Metropolis

BENIN MEDICAL CARE		**EDO SPECIALIST**		**CITY OF HOPE**		
Samples	**Mean Heterotrophic counts (SSA) (×10⁴ cfu/g) ± S. D**	**Samples**	**Mean Heterotrophic counts (SSA) (×10⁴ cfu/g) ± S. D**	**Samples**	**Mean Heterotrophic counts (SSA) (×10⁴ cfu/g) ± S. D**	**TOTAL Average counts (×10⁴ cfu/g) ± S. D**
1	7.65 ± 0.4	1	28.95 ± 0.2	1	20.25 ± 0.6	
2	4.0 ± 0.1	2	21.8 ± 0.3	2	2.8 ± 0.4	
3	3.6 ± 0.5	3	18.7 ± 0.4	3	2.55 ± 0.4	
4	20.0 ± 0.2	4	15.85 ± 0.4	4	2.85 ± 0.5	
5	7.85 ± 0.3	5	19.45 ± 0.4	5	7.8 ± 0.3	
6	3.1 ± 0.1	6	27.55± 0.7	6	9.85 ± 0.4	
7	2.95 ± 0.3	7	12.0 ± 0.4	7	2.65 ± 0.07	
8	10.85 ± 0.2	8	17.4 ± 0.2	8	1.01 ± 0.6	
9	4.45 ± 0.2	9	19.1 ± 0.2	9	8.65 ± 1	
10	10.6 ± 1	10	19.75 ± 0.4	10	2.85 ± 0.2	
TOTAL	75.05 ± 3.3		200.55 ± 3.8		61.26 ± 4.47	336.86 ± 11.57
AVERAGE	7.51 ± 0.33		20.05 ± 0.38		6.13 ± 0.45	11.23 ± 0.39

KEY:

S.D = Standard Deviation

Table 4.3 Total *Vibrio* Count on Thiosulphate Bile Salts Agar (TCBS) from Stool Samples Obtained from Hospitals in the Benin Metropolis

BENIN MEDICAL CARE		**EDO SPECIALIST**		**CITY OF HOPE**		
Samples	**Mean Heterotrophic counts (TCBS) (×10⁴ cfu/g) ± S.D**	**Samples**	**Mean Heterotrophic counts (TCBS) (×10⁴ cfu/g) ± S.D**	**Samples**	**Mean Heterotrophic counts (TCBS) (×10⁴ cfu/g) ± S.D**	**TOTAL Average counts (×10⁴ cfu/g) ± S. D**
1	5.60 ± 0.6	1	3.35 ± 0.4	1	29.25 ± 0.7	
2	Nil	2	4.35 ± 0.6	2	3.65 ± 0.5	
3	Nil	3	5.65 ± 0.5	3	3.0 ± 0.1	
4	13.0 ± 0.3	4	2.65 ± 0.07	4	2.5 ± 0.2	
5	5.2 ± 1	5	20.25 ± 0.5	5	3.05 ± 0.8	
6	2.80 ± 0.1	6	6.8 ± 0.4	6	4.15 ± 0.2	
7	6.55 ± 0.6	7	2.85 ± 0.7	7	4.6 ± 0.3	
8	3.7 ± 0.4	8	29.55 ± 0.5	8	2.9 ± 0.7	
9	7.6 ± 0.6	9	11.65 ± 0.5	9	2.9 ± 0.3	
10	17.85 ± 0.6	10	12.6 ± 0.6	10	4.35 ± 0.07	
TOTAL	62.3 ± 4.2		99.7 ± 4.77		60.35 ± 3.87	222.35 ± 12.84
AVERAGE	6.23 ± 0.42		9.97 ± 0.48		6.04 ±0.39	7.41 ± 0.43

KEY:

S.D = Standard Deviation

The analysis of the prevalence of isolates obtained from stool samples across the three hospitals (Benin Medical Care (BMC), City of Hope Hospital, and Edo Specialist Hospital) revealed notable variations in the distribution of both *Vibrio* and *Salmonella* species as depicted in Table 4.4.

For *Vibrio* isolates, a total of 42 isolates were recovered from all sampling locations. The distribution showed that Edo Specialist Hospital recorded the highest number of isolates (20), corresponding to a percentage frequency of 47.62%. This indicates that nearly half of all *Vibrio* isolates identified in this study originated from this location. City of Hope Hospital followed with 12 isolates (28.57%), representing a moderate level of occurrence. In contrast, Benin Medical Care (BMC) recorded the lowest number, with 10 isolates accounting for 23.81% of the total.

The distribution of *Salmonella* isolates also showed variation across the three hospitals. Out of a total of 40 *Salmonella* isolates, Edo Specialist Hospital again recorded the highest number (18), corresponding to a percentage frequency of 45%. Followed by Benin Medical Care, which had 12 isolates (30%), while City of Hope Hospital recorded the lowest occurrence with 10 isolates (25%). Although the differences among the hospitals were less pronounced than those observed for *Vibrio*, the trend still indicates a higher burden of *Salmonella* isolates in Edo Specialist Hospital.

When both organisms are considered collectively, Edo Specialist Hospital consistently had the highest frequency of occurrence for both *Vibrio* and *Salmonella* isolates, suggesting a relatively greater prevalence of enteric bacterial pathogens in stool samples obtained from this hospital. On the other hand, COH generally showed lower frequencies, particularly for *Salmonella*, while Benin Medical Care demonstrated intermediate values, with relatively lower *Vibrio* occurrence but higher *Salmonella* frequency compared to City of Hope Hospital.

A comparative examination of Figure 4.1 therefore highlights a clear pattern in the distribution of the microbial isolates. Edo Specialist Hospital consistently showed the highest contribution to the

overall microbial prevalence, particularly in relation to *Salmonella* species, where it accounted for more than half of the isolates recovered in the study. On the other hand, Benin Medical care Hospital demonstrated the lowest representation of *Salmonella* isolates, indicating a comparatively lower occurrence in the stool samples obtained. Despite this difference, the distribution of *Vibrio* isolates between City of Hope and BMC Hospital was relatively similar, suggesting that the prevalence of *Vibrio* species may be more evenly spread between these two hospitals.

Table 4.4. Prevalence of Isolates from Stool Sample

Bacterial Isolate	Benin Medical Care (BMC)	City of Hope	Edo Specialist Hospital	Total Isolates	Prevalence (%)
***Vibrio* spp.**	10	12	20	42	51.22 %
***Salmonella* spp.**	12	10	18	40	48.78 %
Total	22	22	38	82	100 %

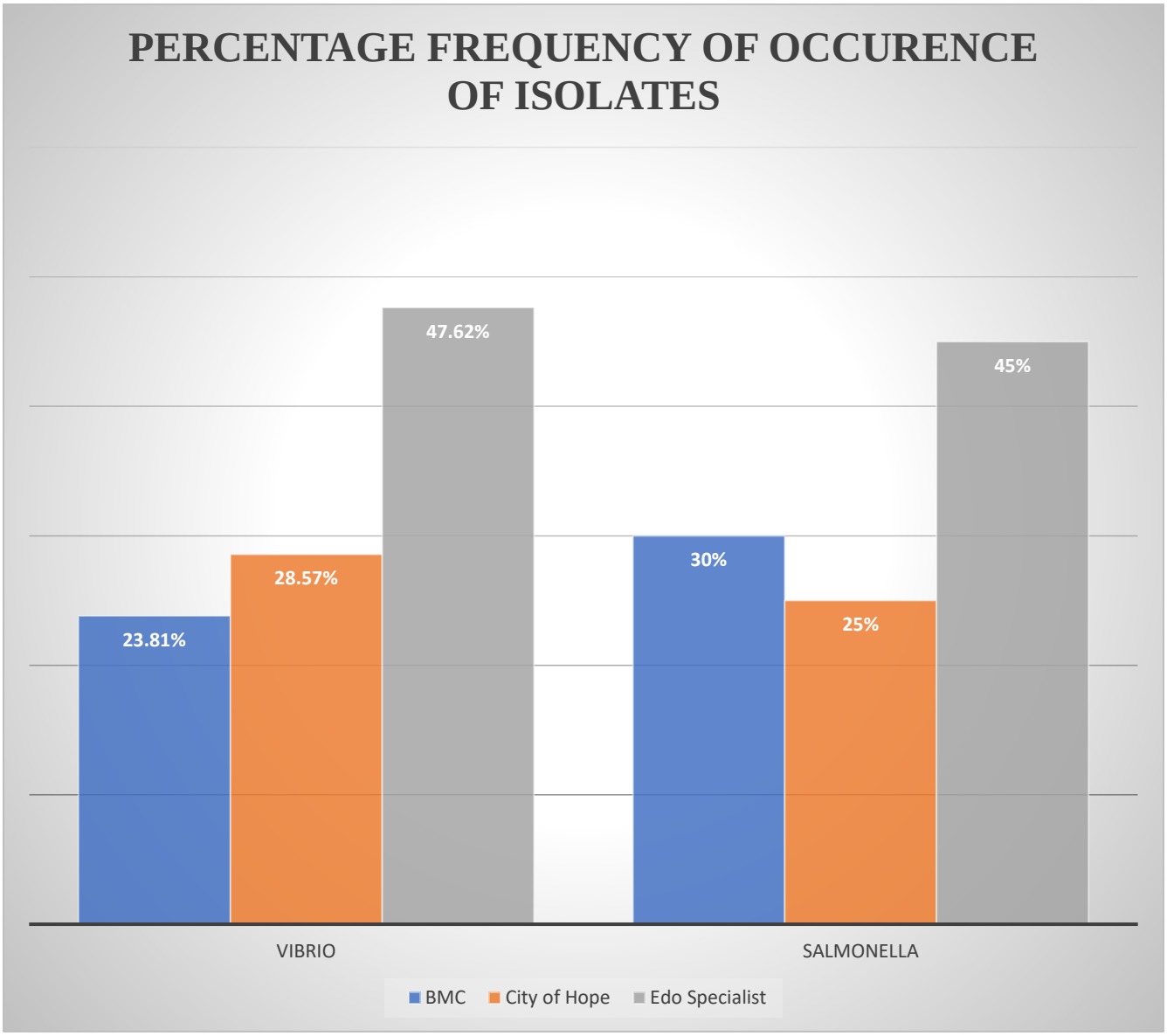


Fig 4.1. Bar chart showing the percentage frequency of occurrence of Isolates from Hospitals

The antibiotic resistance patterns observed in *Vibrio spp.* and *Salmonella spp.* isolates from stool samples revealed a concerning prevalence of multidrug resistance.

For *Vibrio spp.* Table 4.5 shows resistance was high against several commonly used antibiotics. Complete resistance (100%) was observed with Imipenem/Cilastatin (IMP), while near-total resistance was recorded for Cefuroxime (CXM), Ampliclox (ACX), Cefexime (ZEM), and Amoxicillin-Clavulanate (AUG), each showing resistance rates above 94%. Moderate resistance was seen with Cefotaxime (CTX) at 44.12%, and Nalidixic Acid (NA) at 58.82%. However, *Vibrio spp.* showed relatively higher sensitivity to Ofloxacin (OFX) and Levofloxacin (LBC), with sensitivity rates of 73.53% and 58.82% respectively, suggesting these fluoroquinolones may still be an effective treatment options. Gentamycin (GN) showed mixed results, with only 26.47% sensitivity and a high intermediate response (29.41%).

The *Salmonella spp.* isolates exhibited even broader resistance. In Table 4.6, it was depicted that all isolates were resistant to Imipenem/Cilastatin (IMP) and Amoxicillin-Clavulanate (AUG), with an almost complete resistance to Cefuroxime (CXM), Ampliclox (ACX), and Cefexime (ZEM) (≥97.5%). Resistance to Cefotaxime (CTX) and Nalidixic Acid (NA) was also high, at 72.5% and 85% respectively. Sensitivity was highest with Ofloxacin (42.5%) and Levofloxacin (32.5%), though both still showed substantial resistance and intermediate responses. Gentamycin (GN) had a sensitivity rate of 20%, with a notable 27.5% intermediate response, indicating variable effect on several bacteria populations.

In total, the derived data from the research suggest that both *Vibrio* and *Salmonella* isolates from the sampled population exhibit extensive resistance to β-lactam antibiotics and cephalosporins, with antibiotic fluoroquinolones like Ofloxacin and Levofloxacin showing comparatively better performance.

Table 4.5. Antibiotic Resistance Pattern of *Vibrio* spp Isolates from Stool Samples (n=34)

Antibiotics	Sensitive (%)	Resistant (%)	Intermediate (%)
CXM	0 (0%)	33 (97.06%)	1(2.94%)
ACX	0 (0%)	33 (97.06%)	1 (2.94%)
CTX	13 (38.23%)	15 (44.12%)	6 (17.65%)
IMP	0 (0%)	34 (100%)	0 (0%)
OFX	25 (73.53%)	8 (23.53%)	1 (2.94%)
ZEM	0 (0%)	32 (94.12%)	2 (5.88%)
NA	11 (32.35%)	20 (58.82%)	3 (8.82%)
CRO	5 (14.71%)	24 (70.59%)	5 (14.71%)
NF	0 (0%)	29 (85.29)	5 (14.71%)
LBC	20 (58.82%)	10 (29.41%)	4 (11.76%)
GN	9 (26.47%)	15 (44.12%)	10 (29.41%)
AUG	1 (2.94%)	33 (97.06%)	0 (0%)

KEYS:

CXM - CEFUROXIME

ACX - AMPLICOX

CTX – CEFOTAXIME

IMP – IMIPENEM/CILASTATIN

OFX – OFLOXACIN

ZEM – CEFEXIME

NA – NALIDIXIC ACID

CRO – CEFTRIAXONE SULBACTAM

NF – NITROFURANTONIN

LBC – LEVOFLOXACIN

GN – GENTAMYCIN

AUG – AMOXICILLIN CLAVULANATE

Table 4.6. Antibiotic Percentage of Sensitivity and Resistance of *Salmonella* spp Isolates from Stool Samples. (n=40)

Antibiotics	Sensitive (%)	Resistant (%)	Intermediate (%)
CXM	0 (0%)	39 (97.5%)	1 (2.5%)
ACX	1 (2.5%)	39 (97.5%)	0 (0%)
CTX	8 (20%)	29 (72.5%)	3 (7.5%)
IMP	0 (0%)	40 (100%)	0 (0%)
OFX	17 (42.5%)	20 (50%)	3 (7.5%)
ZEM	0 (0%)	39 (97.5%)	1 (2.5%)
NA	5 (12.5%)	34 (85%)	1 (2.5%)
CRO	4 (10%)	36 (90%)	0 (0%)
NF	1 (2.5%)	33 (82.5%)	6 (15%)
LBC	13 (32.5%)	20 (50%)	7 (17.5%)
GN	8 (20%)	21 (52.5%)	11 (27.5%)
AUG	0 (0%)	40 (100%)	0 (0%)

KEYS:

CXM - CEFUROXIME

ACX - AMPLICOX

CTX – CEFOTAXIME

IMP – IMIPENEM/CILASTATIN

OFX – OFLOXACIN

ZEM – CEFEXIME

NA – NALIDIXIC ACID

CRO – CEFTRIAXONE SULBACTAM

NF – NITROFURANTONIN

LBC – LEVOFLOXACIN

GN – GENTAMYCIN

AUG – AMOXICILLIN CLAVULANATE

Table 4.7 shows the *Vibrio spp.* isolates MAR index which ranged from 0.25 to 1.0, with several isolates (31TI, 18T, 16T, 19T) recording the maximum value of 1.0, indicating resistance to all 12 antibiotics tested. A significant proportion of isolates clustered between 0.5 and 0.92, indicating resistance to 6–11 antibiotics. Only one isolate (1T) showed a relatively low MAR index of 0.25, suggesting limited resistance. Overall, most *Vibrio* isolates exhibited MAR indices well above the critical threshold of 0.2, indicating a high-risk environment with heavy antibiotic exposure.

For the *Salmonella spp.* isolates, the MAR indexes similarly ranged from 0.33 to 1.0, with many isolates (e.g., 7S, 12S, 20S, 9SII, 31SI, 19S, 32SI, 32SII, 3S, 16SII, 26SII, 27SI, 31SI again, etc.) recording 1.0, showing resistance to all antibiotics tested. Several isolates fell within the 0.75–0.92 range, resistant to 9–11 antibiotics, while a few isolates (e.g., 14S at 0.33 and 8SI at 0.5) showed comparatively lower resistance. Nonetheless, the overwhelming majority of *Salmonella* isolates had MAR indexes far above 0.2, again indicating exposure to environments with intense antibiotic use, as portrayed in Table 4.8.

Antibiotic-resistant gene patterns on the gel image are illustrated in Figure 4.2, which shows that several isolates carry resistance genes, as evidenced by the visible DNA bands at expected positions. The variation in band intensity reflects differences in gene copy number or amplification efficiency.

The evolutionary history was inferred using the Neighbour-Joining method. The optimal tree with the sum of branch length = 0.125 is shown in Fig. 4 .2. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analytical procedure encompassed 6 nucleotide sequences. The pairwise deletion option was applied to all ambiguous positions for each sequence pair resulting in a final data set comprising 1,540 positions. Evolutionary analyses were conducted in MEGA12 .

Table 4.7. Multiple Antibiotic Resistance (MAR) Index of *Vibrio* Isolates from Stool Samples

Isolates	Number of Resistant Organisms/ Antibiotic (n=12)	**MAR Index**
12T	8	0.67
28T	7	0.58
25TI	9	0.75
31TI	12	1
1T	3	0.25
7T	8	0.67
3TII	7	0.58
30TII	8	0.67
11T	10	0.83
21T	9	0.75
18T	12	1
22TI	7	0.58
32TI	6	0.5
30TII	8	0.67
32TII	6	0.5
16T	12	1
23TI	9	0.75
1T	8	0.67
17T	6	0.5
26T	6	0.5
19T	12	1
27T	8	0.67
28T	6	0.5
22T	7	0.58
11T	8	0.67
24TI	11	0.92
24TII	11	0.92
27TII	6	0.5
26TII	9	0.75
3TI	10	0.83
25TII	8	0.67
8T	11	0.92
2T	10	0.83
9T	8	0.67

Table 4.8 Multiple Antibiotic Resistance (MAR) Index of *Salmonella* Isolates from Stool Samples

Isolates	Number of Resistant Organisms/ Antibiotic (n=12)	**MAR Index**
23SI	7	0.58
31SII	8	0.67
9S	7	0.58
7S	12	1
12S	12	1
14S	6	0.5
20S	12	1
9SII	12	1
12SII	9	0.75
31SI	12	1
16SI	11	0.92
8SI	6	0.5
30SI	10	0.83
7SII	9	0.75
23SII	6	0.5
14S	4	0.33
19S	12	1
20S	11	0.92
8SII	8	0.67
15SI	7	0.58
32SI	12	1
31SI	12	1
32SII	12	1
3S	12	1
30SII	11	0.92
26SI	10	0.83
22SI	8	0.67
13S	9	0.75
1S	10	0.83
16SII	12	1
23S	8	0.67
15SII	8	0.67
18S	11	0.92
2SI	10	0.83
26SII	12	1
27SI	12	1
22SII	9	0.75
24S	11	0.92
27SII	11	0.92
2SII	8	0.67

Table 4.9 Molecular identification of the isolates

Isolate Code	Identified Organism	E. Value	Accession Number
S9SI	***Salmonella enterica* (99.93%)**	**0.0**	**CP000026.1**
14SII	***Salmonella enterica* (99.87%)**	**0.0**	**CP008927.1**
S32TII	***Proteus mirabilis* (99.87%)**	**0.0**	**CP110673.1**
32SI	***Escherichia coli* (99.93%)**	**0.0**	**CP029579.1**
9SII	***Escherichia coli* (99.87%)**	**0.0**	**CP065203.1**
S31TI	***Vibrio parahaemolyticus* (99.80%)**	**0.0**	**CP000526.1**

The phylogenetic tree in Fig. 4 .2 shows the evolutionary relationships among the bacterial isolates based on their gene sequences. From the tree, the two *Salmonella enterica* sequences (CP54556.1 and CP000026.1) cluster closely together, indicating that they are highly similar genetically. The bootstrap value of 86% supporting this branch suggests a reliable relationship between these two isolates.

These *Salmonella* sequences are also closely related to the *Escherichia coli* isolates (CP029579.1 and CP065203.1). The clustering of *Salmonella* and *E. coli* with high bootstrap values (98% and 97%) reflects their close evolutionary relationship, which is expected because both organisms belong to the same bacterial family, Enterobacteriaceae.

Proteus mirabilis (CP110673.1) appears on a separate branch that is more distant from the *Salmonella* and *E. coli* cluster. Although it still belongs to the Enterobacteriaceae family, its position on the tree suggests that it is genetically more divergent compared to the other members of the group.

Vibrio parahaemolyticus (CP000526.1) forms a distant branch in the tree. This separation indicates a greater evolutionary distance from the other organisms, which is expected because *Vibrio* belongs to a different bacterial family, Vibrionaceae.

Overall, the tree clearly shows that *Salmonella* and *Escherichia coli* are closely related, *Proteus mirabilis* is moderately related, while *Vibrio parahaemolyticus* is the most distantly related organism among the isolates studied. The scale bar (0.01) represents the genetic distance between the sequences, where shorter branch lengths indicate greater similarity between organisms.

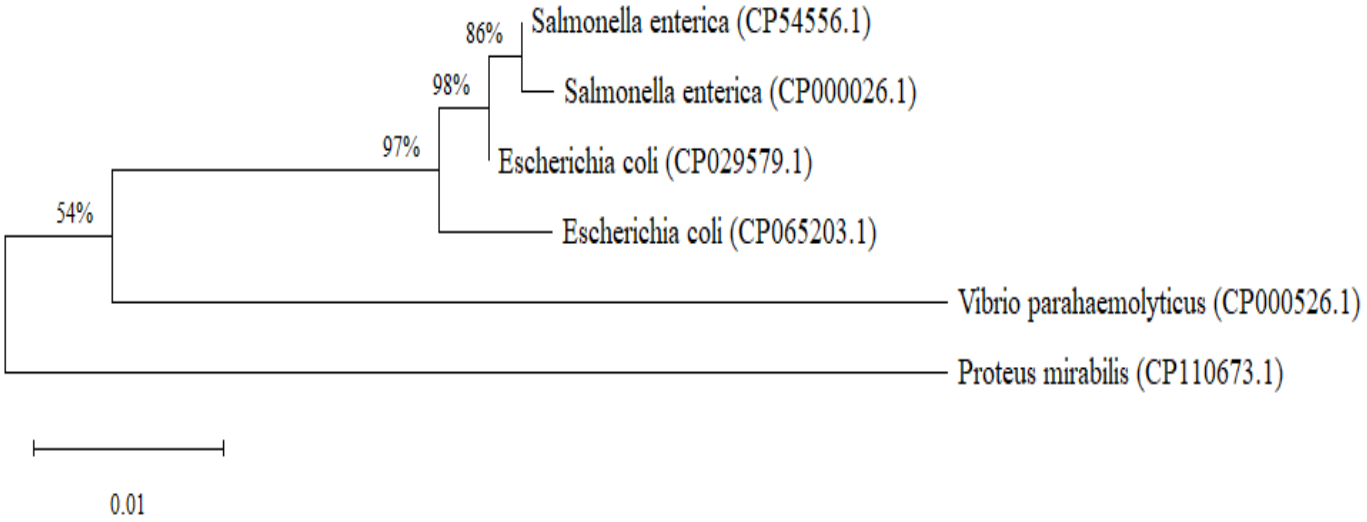


Fig 4.2 Neighbour-Joining Phylogenetic tree of Bacteria Isolates based on 16S rRNA sequencing.



Fig 4.3. Identification of multidrug resistance genes in isolates

Table 4.10. Identified Antibiotic Resistant Genes in Isolates

Isolates	blaTEM	BlaIMP	SuL2
9SI (*Salmonella enterica)*	+		+
14S (*Salmonella enterica)*	+		+
32T (*Proteus mirabilis)*	+		
31T *(Vibrio parahaemolyticus)*	+		

CHAPTER FIVE

DISCUSSION

The frequency of diarrheagenic case**s** reached an unprecedented level, causing an influx of patients with complaints of diarrhea to hospitals and clinical centers which compelled the onset of this investigation to ascertain if the diarrheagenic illnesses were indeed caused by the ongoing cholera endemic or by other notable causes as well as to determine the resistance profile of the causal agent. From the results obtained, Table 4.1 shows the total heterotrophic count of the microorganisms, within which samples from Edo Specialist hospital had the highest microbial load, with a range of 4.5 ± 0.2 to 29.65 ± 0.2 and a total average heterotrophic count across all hospitals was 10.99 ± 0.9 × 10^4 CFU/g, underscoring Edo Specialist Hospital as the site with the greatest microbial burden in this category**.** However, for the *Salmonella* count, the Edo specialist hospital also seems to have a significantly higher load than the others, with a range of 12.0 ± 0.4 to 28.95 ± 0.2. The total *Vibrio* count also has the highest value, with the specialist hospital 2.65 ± 0.07 to 29.55 ± 0.5 as shown in Tables 4.2 and 4.3, respectively.

These differences in population bio-load could be owing to the geographical location of the hospitals as well as the socioeconomic status (SES) of the patients with access to the hospitals, which could imply that patients of higher socioeconomic statuses could have less exposure to the etiologic agents than those of lower socioeconomic statuses (Barakat and Konstantinidis, 2023). The hospitals from which samples were collected two (2) were Private institutions, hence the lower microbial counts as shown in Tables 4.1, 4.2 and 4.3 while the last hospital is more of a public-private partnership hospital and it has the highest microbial load of the three (3) hospitals investigated, proving the aforementioned statement. It is well documented by Gautam *et al.,* 2023 that children and adolescents from high parental SES backgrounds have more access to education,

housing, good and hygienically significant water and food sources, clothing, proper health care services, and social services, hence improving overall health than those of lower SES. It could also be as an effect of sample storage conditions and the time between sample collection and culturing.

There were marked variations in the antibiogram profile of the respective bacterial strains, with most of the isolates exhibiting a high resistance pattern to most of the antibiotics used in this study, as seen in Tables 4.5 and 4.6. It can also be noted from tables that antibiotics levofloxacin and ofloxacin of 20% and 25% respectively, had the highest percentage of sensitivity, followed by cefotaxime and nalidixic acid, to which only 13% and 11% respectively of the isolates were sensitive to. The isolates from the SSA media showed a comparatively greater overall resistance to the antibiotics used compared to the *Vibrio* spp isolates Table 4.6 depicts that only 17% of the *Salmonella* spp isolates were sensitive to ofloxacin which had the greater sensitivity rate of all the antibiotics utilized.

This Multi-Drug Resistance (MDR) pattern of *Salmonella* spp was similar to that shown in Shen *et al.* (2020), where it can be associated with the coexistence of resistance-related genes and their transmission, making it difficult for treatment. Genes *blaTEM* and *Sul2* were found to be present in some isolates. These genes confer resistance to beta-lactams and sulfonamides respectively (Jia-Jin *et al.,* 2024). This could also have resulted from inappropriate prescribing practices by healthcare professionals, such as the excessive use of broad-spectrum antibiotics, which contribute to the development of resistance. The absence of stringent regulations and enforcement mechanisms enables the persistence of these practises, thereby complicating efforts to control antibiotic overuse effectively (Mohakud *et al.,* 2022).

MAR index ranged from which had a range of 0.25 to 1 for the *Vibrio* isolates and 0.5 to 1 for the *Salmonella* isolates, as shown in Tables 4.7 and 4.8, respectively, which further highlights the alarmingly high use of antibiotics which can be similarly compared to the work of Ogefere *et al.,*

(2023) where *Salmonella* and *Proteus mirabilis* and *E.coli* isolated from clinical samples had similar index ranges of ($p<0.05$- $p< 0.01$)

From molecular identification of some isolates, it was shown that not only *Vibrio* and *Salmonella* spp. were causative agents of the diarrhea, but also *Proteus mirabilis* as well as *Escherichia coli* in Table 4.9. While *Proteus mirabilis* are considered commensals of the human intestine, they are more often isolated from the stools of patients with diarrhea than from those of healthy patients (Zhang *et al.,* 2021). Selective methods were used to suppress the growth of non-target organisms while allowing the proliferation of specific pathogens of interest. However, when these selective methods yield *Escherichia coli* and *Proteus mirabilis* instead of its intended *Vibrio* and *Salmonella,* it could be indicative of several molecular reasons which brings to focus the complexity of microbial ecology and also the limits of culture based diagnostic methods. Selective media utilize reactions of chemicals, bile salts or dyes in order to repress the growth of other unwanted microbes, according to this, it could be inferred that *E. coli* and *P. mirabilis* may possess molecular mechanisms that gives them resistance to the inhibitory agents. For *Proteus* and *E. coli,* certain adaptations have enabled them to bypass these selectivism. For example, *Proteus* spp often harbor efflux pumps and enzymes that neutralize toxic compounds and *E. coli* strains most often carry plasmid encoded resistance genes (Li *et al.,* 2016). Differential media on the other hand tends to exploit the metabolic differences between species so as to differentiate them such as lactose fermenting ability and hydrogen sulfide production. *E. coli* and *P. mirabilis* tend to share similarities in metabolic traits with *Salmonella* spp, some *E. coli* strains ferment lactose variably, while some *Proteus* strains tend to produce hydrogen sulfide, a well-known reaction associated with *Salmonella* spp. This overlap in their metabolic similarities can lead to a false positive result thereby causing a mess in the organism's identification (Pathiranage *et al.,* 2021). In summary, the selective and differential overlaying similarities between *E. coli, Proteus* and the organisms of

research interest increase the risk of false positives, their rapid growth may also suppress or hide the presence of the *Vibrio* and *Salmonella* (Li *et al.,* 2016: Pathiranage *et al.,* 2021). Secondly, it emphasizes the limitations of relying solely on culture-based methods (Sousa *et al.,* 2024).

The main causes of the spread of antimicrobial resistance in Nigeria are majorly due to the high rate of antibiotic consumption resulting from illiteracy and the absence of appropriate drug control laws (which make prescription-grade antibiotics widely accessible to the ignorant populace) (Olagunju, *et al.,* 2025).

5.0 CONCLUSION

This study provides useful information into the antibiotic-resistant gene profiles of *Vibrio* and *Salmonella* species isolated from the stools of diarrheal patients in hospitals within Benin Metropolis. The findings revealed that certain enteric pathogens remain major contributors to diarrheal infections in the area and high levels of resistance to commonly used antibiotics. The molecular analysis showed the presence of genes coding for β-lactamase production and sulfonamide resistance, highlighting the persistence of these resistance mechanisms among circulating strains. The coexistence of such resistance determinants highlights the growing concern over the spread of multidrug-resistant pathogens capable of undermining the effectiveness of first-line antimicrobial treatments.

The high detection rates of *Vibrio* spp. and *Salmonella* spp. in patient stool samples by morphological and cultural identification methods may infer the importance and significance of these microbes in diarrhea induced hospital visits across the Benin Metropolis. The ability of these organisms to be resistant to multiple antibiotics used in this study which are also common treatment antibiotics as well as the presence of the β-lactam and sulfonamide antibiotic resistance

genes poses a serious clinical challenge, as these drug classes are frequently used in the treatment and management of gastrointestinal infections. The persistence of these resistant strains may result in longer illness durations, recurring infections, and increased treatment expenses, all of which have devastating effects on public health.

These resistance patterns across the microorganisms also reflect another issue of significance which is antibiotic abuse, misuse and overuse and the over-the-counter accessibility to antibiotics in the community. The uncontrolled use of sulfonamide and β-lactam antibiotics in both human and animal contexts may have added to the selection pressure causing resistance genes to persist. The transfer of factors of resistance between clinical and environmental bacterial populations is aggravated by environmental pollution caused by inappropriate waste disposal and insufficient sewage treatment. This means that the city's environmental, clinical, and molecular surveillance systems need to be better developed and properly incorporated into communities.

Furthermore, the limited availability of experimental and molecular data on diarrheagenic pathogens in Benin Metropolis has limited public health agencies' capacity to carry out data-driven initiatives. This study lays the groundwork for more thorough surveillance and policy creation by demonstrating the connection between incidences of diarrheal illness and antibiotic-resistant *Vibrio* and *Salmonella* species. The results points out the need for more stringent antibiotic prescribing guidelines, regular antimicrobial susceptibility testing in hospitals, and the creation of antimicrobial stewardship initiatives.

5.1 RECOMMENDATION

The results of this study make known the extreme importance of continuous surveillance and molecular monitoring of β-lactamase and sulfonamide resistance among diarrheagenic pathogens

in Benin. In addressing the issue of antibiotic resistance as highlighted by this study, improved laboratory diagnostic capabilities and public awareness campaigns encouraging careful antibiotic use are required. Measures should be taken by the city in order to prevent the spread of multidrug resistance by implementing regulations focusing on better waste management and sanitation. All of these actions will help lower the incidence of resistant *Vibrio* and *Salmonella* infections and improving diarrheal disease management in Benin Metropolis.

5.2 CONTRIBUTION TO KNOWLEDGE

1. This study makes substantial and multidimensional contributions to knowledge in medical microbiology, infectious disease epidemiology, and antimicrobial resistance research in Nigeria as it offers up-to-date hospital-based epidemiological data on the prevalence and distribution of *Vibrio* and *Salmonella* species among diarrheal patients in Benin Metropolis. Such localized data are still hard to come by in Nigeria, particularly in studies that use molecular confirmation. By documenting pathogen incidence within particular healthcare facilities, this study contributes to the regional evidence base needed for targeted public health planning and intervention.
2. Furthermore, the study provides useful data on the patterns of antibiotic susceptibility of intestinal infections that are therapeutically significant. The study produces locally relevant data that can guide empirical treatment recommendations by methodically assessing resistance to frequently given antibiotics. Such localized surveillance data are crucial for improving therapeutic decision-making and lowering treatment failure since antimicrobial resistance is dynamic and context-specific.
3. The molecular detection of resistance determinants such as *blaTEM* and *sul2* gives more information on the genetic processes responsible for the phenotypsic resistance that was seen.

The identification of these genes brings to point the possibility of horizontal gene transfer and additional resistance spread by indicating the circulation of transferable resistance elements throughout the research population. This molecular evidence helps us better understand the evolutionary processes of antibiotic resistance in Nigerian gut bacteria. By demonstrating linkages between genotypic markers and phenotypic resistance patterns, the study also emphasizes the importance of incorporating molecular surveillance into antimicrobial resistance monitoring systems. This contributes to the broader discussion of connecting regional research projects with global monitoring systems such as the WHO Global Antimicrobial Resistance Monitoring System (GLASS) and improving national antimicrobial resistance action plans.

4. This study has improved our understanding of the public health implications of multidrug-resistant enteric pathogens in the management of diarrheal diseases. As a result, it can serve as an empirical basis for promoting a more robust antimicrobial stewardship program, better infection prevention and control procedures, and improved laboratory capacity building within Nigerian healthcare institutions.
5. Finally, this study creates a baseline dataset for upcoming multicenter and longitudinal research examining the transmission dynamics, resistance gene diversity, and temporal trends of enteric infections in Nigeria. It provides a thorough method that significantly advances scientific knowledge of antimicrobial-resistant diarrheal illnesses and aids in the development of evidence-based policy by combining epidemiological, phenotypic, and molecular data into a single study framework.

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APPENDIX I

A. Composition of Media *(Salmonella Shigella* Agar and Thiosulfate citrate bile salts Agar)

INGREDIENTS	*Salmonella Shigella* AGAR (G/L)	THIOSULFATE CITRATE BILE SALT (TCBS) AGAR (G/L)
Beef Extract	5.00	–
Enzymatic Digest of Casein	2.50	–
Enzymatic Digest of Animal Tissue	2.50	–
Proteose Peptone	–	10.0
Yeast Extract	–	5.0
Lactose	10.0	–
Sucrose	–	20.0
Bile Salts / Bile	8.50	8.0
Sodium Citrate	8.50	10.0
Sodium Thiosulfate / Sodium Thiosulphate	8.50	10.0
Sodium Chloride	–	10.0
Ferric Citrate	1.00	1.0
Brilliant Green	0.00033	–
Neutral Red	0.025	–
Bromothymol Blue	–	0.04
Thymol Blue	–	0.04
Agar	13.50	15.0
Distilled Water	1000 ml	–
pH (at 25°C)	7.0 ± 0.2	8.6 ± 0.2

B. Composition of Pre-enrichment media

Ingredients	Normal Peptone	Alkaline Peptone
Peptone	10.0	10.0
Sodium Chloride	5.0	5.0
Sodium Hydrochloride	-	Added to Adjust Alkalinity
Final PH	7.2 ± 0.2	8.4 -8.6

C. Composition of Nutrient Agar

Component	Amount (g/L)
Peptone	5.0
Beef extract	3.0
Sodium chloride (NaCl)	5.0
Agar	15.0
Distilled water	Up to 1 L
Final pH	7.3 ± 0.2

APPENDIX II

A. Statistical analysis of Mean *Vibrio* spp count from Thio-Sulphate Bile Salts Agar from BMC Hospital

Samples	1	2	SUM	MEAN	STANDARD DEVIATION
1	60	52	112	56	5.656854
2	0	0	0	0	0
3	0	0	0	0	0
4	128	132	260	130	2.828427
5	59	45	104	52	9.899495
6	29	27	56	28	1.414214
7	61	70	131	65.5	6.363961
8	34	40	74	37	4.242641
9	72	80	152	76	5.656854
10	174	183	357	178.5	6.363961

B. Statistical analysis of Mean *Vibrio* spp count from Thio-Sulphate Bile Salts Agar from Edo Specialist Hospital

Samples	1	2	SUM	MEAN	STANDARD DEVIATION
1	31	36	67	33.5	3.535534
2	48	39	87	43.5	6.363961
3	53	60	113	56.5	4.949747
4	26	27	53	26.5	0.707107
5	206	199	405	202.5	4.949747
6	65	71	136	68	4.242641
7	29	28	57	28.5	0.707107
8	292	299	591	295.5	4.949747
9	120	113	233	116.5	4.949747
10	122	130	252	126	5.656854

C. Statistical Analysis of Mean *Vibrio* spp counts from Thio-Sulphate Bile Salts Agar from City of Hope Medical Center

Samples	1	2	SUM	MEAN	STANDARD DEVIATION

1	298	287	585	292.5	7.778175
2	33	40	73	36.5	4.949747
3	29	31	60	30	1.414214
4	27	23	50	25	2.828427
5	25	36	61	30.5	7.778175
6	40	43	83	41.5	2.12132
7	44	48	92	46	2.828427
8	24	34	58	29	7.071068
9	27	31	58	29	2.828427
10	43	44	87	43.5	0.707107

D. Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
BMC	10	623	62.3	3112.067
ESH	10	997	99.7	7857.678
COH	10	603.5	60.35	6703.892

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9836.617	2	4918.308	0.834855	0.444832	3.354131
Within Groups	159062.7	27	5891.212			
Total	168899.3	29				

E. Statistical analysis of Mean *Salmonella* spp count from BMC

Samples	1	2	SUM	MEAN	STANDARD DEVIATION
1	73	80	153	76.5	4.949747
2	39	41	80	40	1.414214
3	32	40	72	36	5.656854
4	198	202	400	200	2.828427
5	76	81	157	78.5	3.535534
6	32	30	62	31	1.414214
7	32	27	59	29.5	3.535534
8	107	110	217	108.5	2.12132
9	43	46	89	44.5	2.12132
10	113	99	212	106	9.899495

F. Statistical analysis of Mean *Salmonella* spp count from Edo Specialist Hospital

Samples	1	2	SUM	MEAN	STANDARD DEVIATION
1	291	288	579	289.5	2.12132
2	220	216	436	218	2.828427
3	184	190	374	187	4.242641
4	162	155	317	158.5	4.949747
5	197	192	389	194.5	3.535534
6	270	281	551	275.5	7.778175
7	123	117	240	120	4.242641
8	176	172	348	174	2.828427
9	189	193	382	191	2.828427
10	200	195	395	197.5	3.535534

G. Statistical analysis of Mean *Salmonella* spp count from

Samples					STANDARD
	1	2	SUM	MEAN	DEVIATION
1	207	198	405	202.5	6.363961
2	25	31	56	28	4.242641
3	28	23	51	25.5	3.535534
4	25	32	57	28.5	4.949747
5	80	76	156	78	2.828427
6	102	95	197	98.5	4.949747
7	26	27	53	26.5	0.707107
8	97	105	202	101	5.656854
9	94	79	173	86.5	10.6066
10	30	27	57	28.5	2.12132

H. Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
BMC	10	750.5	75.05	2822.803
ESH	10	2005.5	200.55	2568.469
COH	10	703.5	70.35	3193.947

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	109081.3	2	54540.63	19.05856	6.89E-06	3.354131
Within Groups	77266.98	27	2861.74			
Total	186348.2	29				

I. Statistical Analysis of Total heterotrophic Count from BMC

Samples	1	2	SUM	MEAN	STANDARD DEVIATION
1	57	59	116	58	1.414214
2	32	28	60	30	2.828427
3	28	25	53	26.5	2.12132
4	283	290	573	286.5	4.949747
5	138	141	279	139.5	2.12132
6	101	99	200	100	1.414214
7	60	57	117	58.5	2.12132
8	124	119	243	121.5	3.535534
9	64	62	126	63	1.414214
10	98	96	194	97	1.414214

J. Statistical Analysis of Total heterotrophic Count from Edo Specialist Hospital

Samples	1	2	SUM	MEAN	STANDARD DEVIATION
1	143	139	282	141	2.828427
2	146	152	298	149	4.242641
3	49	53	102	51	2.828427
4	43	47	90	45	2.828427
5	262	259	521	260.5	2.12132
6	189	200	389	194.5	7.778175
7	233	241	474	237	5.656854
8	298	295	593	296.5	2.12132
9	189	181	370	185	5.656854
10					

K. Statistical Analysis of Total heterotrophic Count from City of Hope Medical Center

Samples	1	2	SUM	MEAN	STANDARD DEVIATION
1	136	129	265	132.5	4.949747
2	32	31	63	31.5	0.707107
3	28	23	51	25.5	3.535534
4	25	35	60	30	7.071068
5	80	76	156	78	2.828427
6	102	98	200	100	2.828427
7	26	27	53	26.5	0.707107
8	97	105	202	101	5.656854
9	94	80	174	87	9.899495
10	30	24	54	27	4.242641

L. Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
BMC	10	980.5	98.05	5770.358
ESH	10	1679	167.9	7008.989
COH	10	639	63.9	1616.878

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	56204.15	2	28102.08	5.856134	0.007717	3.354131
Within Groups	129566	27	4798.742			
Total	185770.2	29				

APPENDIX III

A. Gram Staining and Biochemical Test Results for SSA Isolates

Isolates	Gram Stain	Catalase Test	Motility	Oxidase	Indole	Citrate	Urease
1S	**- ROD**	+	+	-	+	-	-
14SI	**- ROD**	+	+	-	-	-	-
15SI	**- ROD**	+	+	-	-	+	-
15SII	**- ROD**	+	+	-	-	+	-
3S	**- ROD**	+	+	-	-	+	-
13S	**- ROD**	+	+	-	-	+	-
7S	**- ROD**	+	+	-	-	+	-
8SI	**- ROD**	+	+	-	-	-	-
14SII	**- ROD**	+	+	-	-	-	-
12S	**- ROD**	+	+	+	-	+	-
9SI	**- ROD**	+	+	-	-	+	-
27SI	**- ROD**	+	+	-	+	+	-
27SII	**- ROD**	+	+	-	-	+	-
24SI	**- ROD**	+	+	-	+	-	-
24SII	**- ROD**	+	+	-	-	-	-
25SI	**- ROD**	+	-	-	-	+	-
25SII	**- ROD**	+	+	-	-	+	-
26SI	**- ROD**	+	+	-	-	-	-
26SII	**- ROD**	+	+	-	-	-	-

22SI	**- ROD**	+	+	-	-	-	-
22SII	**- ROD**	+	+	-	-	-	-
23SI	**- ROD**	+	+	+	-	+	-
23SII	**- ROD**	+	+	-	+	-	-
30SI	**- ROD**	+	+	-	-	+	-
30SII	**- ROD**	+	+	-	-	+	-
32SII	**- ROD**	+	+	-	-	-	-
31SI	**- ROD**	+	+	-	-	-	-
32SI	**- ROD**	+	-	-	-	-	-
31SII	**- ROD**	+	+	-	-	+	-
16S	**- ROD**	+	+	-	-	+	-
2SI	**- ROD**	+	+	-	-	+	-
2SII	**- ROD**	+	+	-	+	+	-
7SI	**- ROD**	+	+	-	+	+	-
8SII	**- ROD**	+	+	+	-	+	-
9SII	**- ROD**	+	+	-	-	+	-
11S	**- ROD**	+	+	-	-	-	-
12SII	**- ROD**	+	+	-	-	+	-
17S	**- ROD**	+	+	-	+	+	-
18S	**- ROD**	+	+	-	-	-	-
19S	**- ROD**	+	+	-	-	-	-
20S	**- ROD**	+	+	-	-	-	-
21S	**- ROD**	+	+	-	-	+	-

KEYS:

1S- SAMPLE I FROM SSA

II-SECOND ISOLATE FROM SAME SAMPLE

1S11- SECOND ISOLATED OBTAINED FROM SSA AGAR CULTURE OF SAMPLE 1

B. **Gram Stain and Biochemical Test Results for *Vibrio* spp**

Isolate	Gram Stain	Catalase	Citrate	Motility	Oxidase	Urease	Indole
23TI	**- ROD**	+	+	+	-	-	+
22TI	**- ROD**	+	-	+	+	-	+
23TII	**- ROD**	+	-	+	+	-	-
11TI	**- ROD**	+	-	+	+	-	+
12TI	**- ROD**	+	-	+	-	-	-
28TI	**- ROD**	+	+	+	+	+	+
28TII	**- ROD**	+	+	+	+	+	-
26TI	**- ROD**	+	+	+	+	-	+
1T	**- ROD**	+	+	+	-	-	-
3TI	**- ROD**	+	+	+	+	-	-
15TI	**- ROD**	+	+	+	+	-	+
15TII	**- ROD**	+	-	+	-	-	-
22TII	**- ROD**	+	+	+	+	-	+
3TII	**- ROD**	+	+	+	+	-	-
27TI	**- ROD**	+	+	+	+	-	-
27TII	**- ROD**	+	+	+	-	-	-
24TI	**- ROD**	+	-	+	-	-	+
8TI	**- ROD**	+	+	+	+	+	-
2TI	**- ROD**	+	+	+	+	-	-

7TI	**- ROD**	+	+	+	+	+	+
25TI	**- ROD**	+	+	+	+	-	+
26TII	**- ROD**	+	+	+	-	-	+
25TI	**- ROD**	+	-	+	+	-	+
24TII	**- ROD**	+	+	+	+	-	+
19T	**- ROD**	+	-	-	-	-	+
18T	**- ROD**	+	-	+	+	-	+
20T	**- ROD**	+	-	+	+	-	+
17T	**- ROD**	+	+	+	+	-	+
21T	**- ROD**	+	+	+	-	-	+
32TI	**- ROD**	+	-	+	+	-	+
31TII	**- ROD**	+	+	+	+	-	-
32TII	**- ROD**	+	+	+	+	-	+
30TI	**- ROD**	+	+	+	+	+	+
30TII	**- ROD**	+	+	+	+	-	+
31TI	**- ROD**	+	+	+	+	-	-
16T	**- ROD**	+	+	+	-	-	+
1TII	**- ROD**	+	+	+	-	-	+
11TII	**- ROD**	+	+	+	+	-	-

APPENDIX IV

Percentage Frequency of Distribution of Isolates from Stool Samples

Organism	BMC	Edo Specialist	City of Hope
Vibrio	23.81%	28.57%	47.62%
Salmonella	30%	25%	45%

APPENDIX V

A. Antibiotic Susceptibility Pattern of *Vibrio* isolates from Stool Samples

Isolates	CXM	ACX	CTX	IMP	OFX	ZEM	NA	CRO	NF	LBC	GN	AUG
12T	R	R	S	R	S	R	R	R	R	S	I	R
28T	R	R	R	R	S	I	R	I	R	S	I	R
25TI	R	R	I	R	R	R	R	I	R	R	S	R
31TI	R	R	R	R	R	R	R	R	R	R	R	R
1T	R	R	S	R	S	I	S	S	I	I	S	S
7T	R	R	S	R	S	R	I	R	R	S	R	R
3TII	R	R	I	R	S	R	R	I	R	S	S	R
30TII	R	R	R	R	S	R	S	R	I	S	R	R
11T	R	R	S	R	S	R	R	R	R	R	R	R
21T	R	R	R	R	S	R	S	R	R	S	R	R
18T	R	R	R	R	R	R	R	R	R	R	R	R
22TI	R	R	S	R	S	R	R	I	R	S	I	R
32TI	R	R	I	R	S	R	S	R	I	S	I	R
30TII	R	R	R	R	S	R	S	R	I	S	R	R
32TII	R	I	S	R	S	R	S	S	R	I	R	R
16T	R	R	R	R	R	R	R	R	R	R	R	R
23TI	R	R	S	R	S	R	R	R	R	S	R	R
1T	R	R	R	R	S	R	R	R	I	S	S	R
17T	I	R	S	R	S	R	I	R	R	S	I	R
26T	R	R	I	R	S	R	S	S	R	S	S	R
Dt	R	R	R	R	R	R	R	R	R	R	R	R
27T	R	R	S	R	S	R	S	R	R	I	R	R
28T	R	R	S	R	S	R	I	S	R	S	I	R
22T	R	R	S	R	S	R	R	I	R	S	I	R
11T	R	R	S	R	S	R	R	R	R	S	S	R
24TI	R	R	R	R	R	R	R	R	R	R	I	R
24TII	R	R	R	R	R	R	R	R	R	R	I	R
27TII	R	R	S	R	S	R	S	S	R	S	S	R
26TII	R	R	I	R	S	R	R	R	R	R	S	R
3TI	R	R	R	R	S	R	R	R	R	S	R	R
25TII	R	R	R	R	S	R	S	R	R	S	S	R
8T	R	R	R	R	I	R	R	R	R	R	R	R
2T	R	R	R	R	R	R	S	R	R	I	R	R
9T	R	R	I	R	S	R	R	R	R	S	I	R

B. Antibiotic Susceptibility Pattern of *Salmonella* Isolates from Stool Samples

Isolates	CXM	ACX	CTX	IMP	OFX	ZEM	NA	CRO	NF	LBC	GN	AUG
23SI	R	R	I	R	S	R	R	R	I	S	I	R
31SII	R	R	S	R	S	R	R	R	I	R	S	R
9S	R	R	S	R	S	R	R	R	I	I	I	R
7S	R	R	R	R	R	R	R	R	R	R	R	R
12S	R	R	R	R	R	R	R	R	R	R	R	R
14S	R	R	I	R	S	R	S	S	R	S	I	R
20SI	R	R	R	R	R	R	R	R	R	R	R	R
9SII	R	R	R	R	R	R	R	R	R	R	R	R
12SII	R	R	R	R	I	R	R	R	R	S	I	R
31SI	R	R	R	R	R	R	R	R	R	R	R	R
16S	R	R	R	R	R	R	R	R	R	I	R	R
8SI	R	S	S	R	S	R	R	R	I	S	I	R
30SI	R	R	R	R	I	R	R	R	R	I	R	R
7SII	R	R	S	R	R	R	R	S	R	R	I	R
23SII	R	R	I	R	S	R	S	S	R	I	S	R
14S	I	R	S	R	S	R	S	S	I	S	I	R
19S	R	R	R	R	R	R	R	R	R	R	R	R
20SII	R	R	R	R	I	R	R	R	R	R	R	R
8SII	R	R	S	R	S	R	R	R	R	S	I	R
15SI	R	R	R	R	S	R	S	R	S	I	I	R
32SI	R	R	R	R	R	R	R	R	R	R	R	R
31SI	R	R	R	R	R	R	R	R	R	R	R	R
32SII	R	R	R	R	R	R	R	R	R	R	R	R
3S	R	R	R	R	R	R	R	R	R	R	R	R
30SII	R	R	R	R	R	R	R	R	R	R	S	R
26SI	R	R	R	R	R	R	R	R	I	R	I	R
22SI	R	R	R	R	S	I	R	R	R	S	S	R
13S	R	R	R	R	S	R	R	R	R	S	I	R
1S	R	R	R	R	S	R	R	R	R	S	R	R
16S	R	R	R	R	R	R	R	R	R	R	R	R
23S	R	R	S	R	S	R	R	R	R	S	S	R
15SII	R	R	S	R	S	R	I	R	R	S	R	R
18S	R	R	R	R	R	R	R	R	R	I	R	R
2SI	R	R	R	R	S	R	R	R	R	S	R	R
26SII	R	R	R	R	R	R	R	R	R	R	R	R
27SI	R	R	R	R	R	R	R	R	R	R	R	R
22SII	R	R	R	R	S	R	R	R	R	S	S	R
24S	R	R	R	R	R	R	R	R	R	R	S	R
27SII	R	R	R	R	R	R	R	R	R	I	R	R
2SII	R	R	R	R	S	R	S	R	R	S	S	R

APPENDIX VI

A. S9SII—

Escherichia coli (99.93%)
CP065203.1
AGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAA
GTCGAACGGTAACAGGAAGCAGCTTGCTGTTTCGCTGACGAGTGGCGGACGG
GTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACG
GTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCT
CTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTC
ACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAA
CTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA
ATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGT
TGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCAT
TGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGT
AATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCA
GGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCAT
CTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGC
GGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG
GACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGAT
ACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAG
GCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCG
CAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
GTGGTTTAATTCGATGCAACGCGAAGAACCTTACCAGGTCTTGACATCCACAG
AACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCAT
GGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCC
AGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACG
ACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGC
GAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAA
CTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGT
GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTT
GCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATT
CATGACTGGGGTGAAGTCGTAACAAGGTAACCGTA

B. **S32SI**

Escherichia coli
CP029579.1

TCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACG
GTAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAA
TGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAAT
ACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATC
GGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCG
ACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACAC
GGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCA
AGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTA
CTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTAC
CCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAG
GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGT
TAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGG
CAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATG
CGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGAC
TGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTA
GTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTT
CCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAA
AACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAAT
TCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAG
AGATGAGAATGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTC
AGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATC
CTTTGTTGCCAGCGGTCCGTGCGGGAACTCAAAGGAGACTGCCAGTGATAAA
CTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGC
TACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAA
GCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCC
ATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTT
CCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGA
AGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTG
GGGTGAAGTCGTAACAAGGTAACCGTA

C. **S14Sii**

Salmonella enterica

CP054556.1

AGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAA
GTCGAACGGTAACAGGAAGCAGCTTGCTGTTTCGCTGACGAGTGGCGGACGG
GTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACG
GTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCT
CTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTC
ACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAA
CTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA
ATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGT

TGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCAT
TGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGT
AATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCA
GGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCAT
CTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGC
GGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG
GACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGAT
ACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAG
GCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCG
CAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
GTGGTTTAATTCGATGCAACGCGAAGAACCTTACCAGGTCTTGACATCCACAG
AACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCAT
GGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCC
AGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACG
ACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGC
GAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAA
CTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGT
GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTT
GCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATT
CATGACTGGGGTGAAGTCGTAACAAGGTAACCGTA

D. **S9SI**

Salmonella enterica

CP000026.1

AGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAA
GTCGAACGGTAACAGGAAGCAGCTTGCTGTTTCGCTGACGAGTGGCGGACGG
GTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACG
GTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCT
CTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTC
ACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAA
CTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA
ATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGT
TGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCAT
TGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGT
AATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCA
GGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCAT
CTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGC
GGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG
GACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGAT

ACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAG
GCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCG
CAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
GTGGTTTAATTCGATGCAACGCGAAGAACCTTACCAGGTCTTGACATCCACAG
AACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCAT
GGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCC
AGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACG
ACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGC
GAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAA
CTCGACTCCA

E. **S31TI**

Proteus mirabilis

CP110673.1

AGAGTTTAATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAA
GTCGAGCGGTAACAGGAGAAAGCTTGCTTTCTTGCTGACGAGCGGCGGACGG
GTGAGTAATGTATGGGGATCTGCCCGATAGAGGGGGATAACTACTGGAAACGG
TGGCTAATACCGCATAATGTCTACGGACCAAAGCAGGGGCTCTTCGGACCTTG
CACTATCGGATGAACCCATATGGGATTAGCTAGTAGGTGGGGTAAAGGCTCAC
CTAGGCGACGATCTCTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTG
AGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATG
GGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTAGGGTTGT
AAAGTACTTTCAGCGGGGAGGAAGGTGATAAGGTTAATACCCTTGTCAATTGA
CGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAAT
ACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGC
GGTCAATTAAGTCAGATGTGAAAGCCCCGAGCTTAACTTGGGAATTGCATCTG
AAACTGGTTGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCATGTGTAGCGGT
GAAATGCGTAGAGATGTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGAC
AAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC
CTGGTAGTCCACGCTGTAAACGATGTCGATTTAGAGGTTGTGGTCTTGAACCG
TGGCTTCTGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAA
GGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGCGAATC
CTTTGAGATAGAGGAGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCT
GTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACC
CTTATCCTTTGTTGCCAGCACGTAATGGTGGGAACTCAAAGGAGACTGCCGGT
GATAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAG
TAGGGCTACACACGTGCTACAATGGCAGATACAAAGAGAAGCGACCTCGCGA
GAGCAAGCGGAACTCATAAAGTCTGTCGTAGTCCGGATTGGAGTCTGCAACTC
GACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAA

TACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCA
AAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCAT
GACTGGGGTGAAGTCGTAACAAGGTAACCGTA

F. **S32TI**

Vibrio parahaemolyticus

CP000526.1

AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAATACATGCAA
GTCGAGCGGTAGAGAGAGCTTGCTCTTGCTGACGAGTGGCGGACGGGTGAGT
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CATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAG
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APPENDIX VII

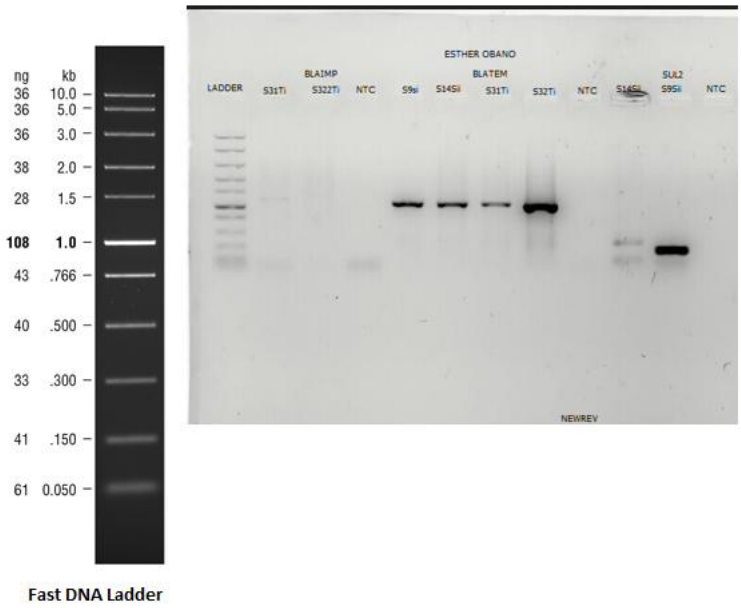


Fig 1. Identification of multidrug resistance genes in isolates

APPENDIX VIII



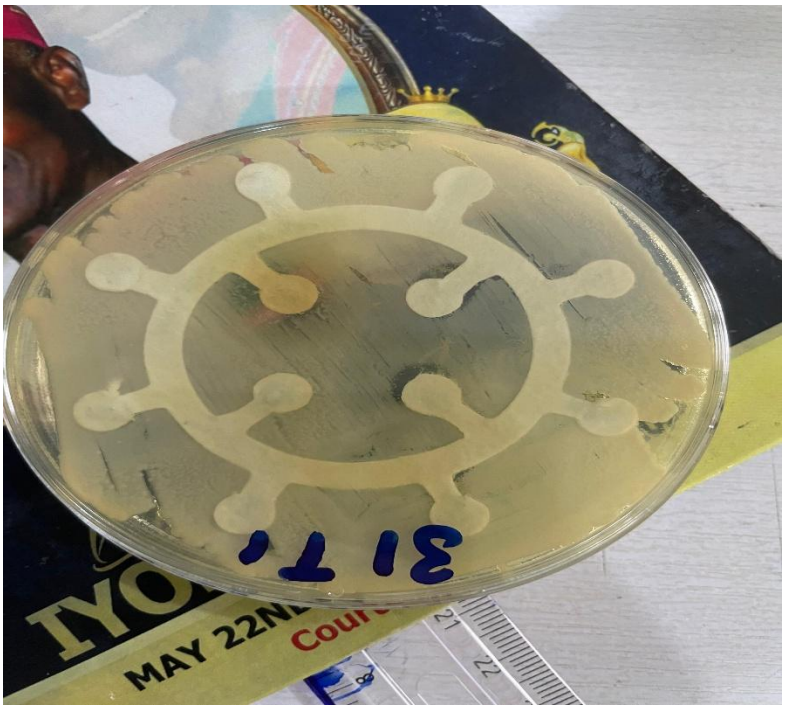


Plate 1. Antibiotic disk





Plate 2. TCBS Agar plates

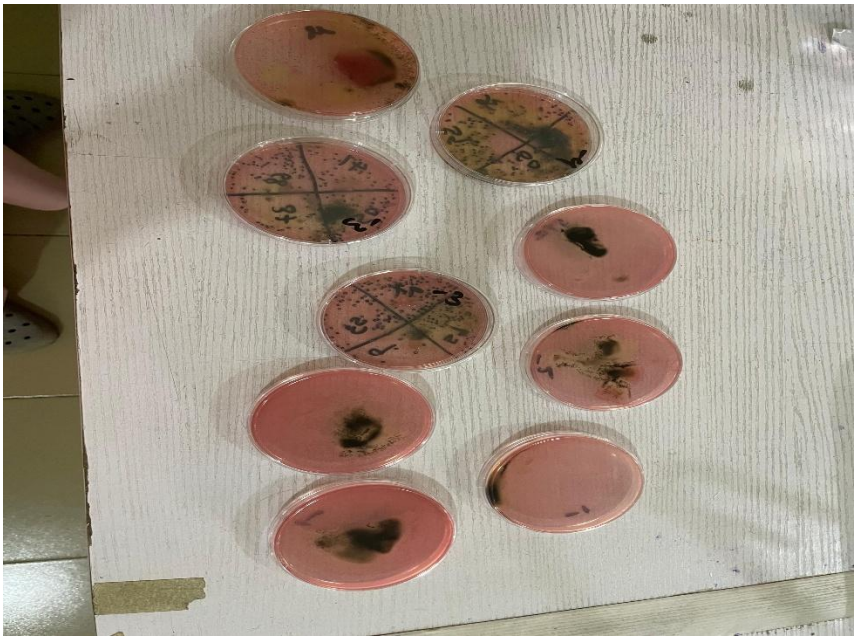




Plate 3. SSA Agar plates