EVALUATION OF THE RELATIONSHIP BETWEEN BODY MASS INDEX (BMI) AND ANTIBIOTIC RESISTANCE OF COMMENSAL *Escherichia coli* ISOLATED FROM APPARENTLY HEALTHY UNIBEN STUDENTS.

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BEING A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELORS DEGREE IN MEDICAL LABORATORY SCIENCE (BMLS), UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

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CERTIFICATION

This is to certify that this research work reported in this project work was carried out by AWOMEJU ISRAEL OLUWAYOMI with Matriculation Number: BMS2005024 in the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin in partial fulfilment of the requirement for the award of Bachelor of Medical Laboratory Science (BMLS) degree.

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DEDICATION

I dedicate the success of this project work to the ALMIGHTY GOD, The Great Redeemer, who has been the origin of all my strength, wisdom and knowledge and also to my beloved parents for their support towards the success of this project work and to every guardians and friends, thank you for your support and being part of this journey.

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All praises and glory be unto God, the Alpha and Omega, the Beginning and the End, the Guardian of my soul. I am deeply grateful to Him for His Grace, Lovingkindness, Wisdom, Knowledge, Understanding, and Strength, He bestowed on me to successfully complete this project.

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ABSTRACT

Antibiotic resistance is one of the global health concern, as commensal bacteria such as *Escherichia coli* could serves as an important reservoirs of the spread of resistance genes. This study evaluated the relationship between Body Mass Index (BMI) and antibiotic resistance of commensal *Escherichia coli* isolated from the stool samples of apparently healthy students at the University of Benin (UNIBEN). A total of 70 stool samples were collected and processed using standard microbiological methods. The isolates were identified by biochemical tests, and antibiotic susceptibility testing was performed using the Kirby–Bauer disc diffusion method in line with CLSI guidelines. The Extended Spectrum β-Lactamase (ESBL) production was screened phenotypically. The overall prevalence of *Escherichia coli* detected across all BMI categories was 85.7%. The ESBL-producing *Escherichia coli* accounted for 16.7% of isolates, with a higher proportion among underweight participants (26.1%) compared to those of normal weight (11.8%), though this difference was not statistically significant ($p = 0.2984$). Antibiotic susceptibility testing revealed that Ofloxacin was the most effective antibiotic, while Cefotaxime showed the highest resistance rates. Statistical analysis demonstrated no significant association between BMI and antibiotic resistance patterns ($p > 0.05$), although a borderline association was observed with Cefotaxime ($p = 0.0523$). The findings indicate that while BMI may not be a major determinant of *Escherichia coli* resistance carriage, healthy students can harbor resistant and ESBL-producing strains that pose a potential public health risk. Regular surveillance and strict antibiotic stewardship are therefore recommended.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Antibiotic resistance (AR) has remained one of the most pressing global health challenges since its emergence in the 1940s, largely due to the inappropriate, unnecessary and over use of antibiotics and also natural bacterial genetic adaptability (Murray *et al.,* 2022; Habboush and Guzmen, 2023; Alabi *et al.,* 2025). The ability of bacteria to evade the inhibitory effects of antibiotic agents through mechanisms such as enzymatic degradation, alteration of target sites, efflux pumps, and horizontal gene transfer has led to prolonged hospital stays, increased treatment costs, limited therapeutic options, and heightened morbidity and mortality worldwide (Habboush and Guzmen, 2023; Onah and Umar, 2024; WHO, 2025).

Among Gram-negative bacteria, *Escherichia coli* (*E. coli*) has received significant attention in antibiotic resistance research due to its ubiquity, genetic flexibility, and dual role as both a harmless commensal and an opportunistic pathogen (Nji *et al.,* 2021; Basavaraju and Gunashree, 2022; Tawfick *et al.,* 2022). *Escherichia coli* normally inhabits the gastrointestinal tract of humans and other warm-blooded animals as part of the gut microbiota (Cheng *et al.,* 2022). However, commensal strains of *Escherichia coli* are recognized as reservoirs of antibiotic resistance genes (ARGs) that can be transferred to pathogenic counterparts through mobile genetic elements, thus facilitating the spread of resistance within the human population and the environment (Wollein Waldetoft *et al.,* 2023; Rana *et al.,* 2024). Consequently, the prevalence and resistance profile of commensal *Escherichia coli* serve as important indicators for monitoring community-level antibiotic resistance (Anjum *et al.,* 2021; Sali *et al.,* 2021; Méndez-Polonieski *et al.,* 2024).

In recent years, the human gut microbiota has been increasingly implicated in metabolic and physiological processes, including obesity and immune modulation (Enache *et al.,* 2024; McBurney and Cho, 2024). The composition and diversity of gut microbiota differs significantly across body weight categories, suggesting a link between the gut microbial ecosystem and Body Mass Index (BMI) (Aranaz *et al.,* 2021; Companys *et al.,* 2021). Moreso, researches have demonstrated that individuals with higher BMI tend to exhibit an altered Firmicutes/Bacteroidetes ratio and reduced microbial diversity and both of which were related with metabolic dysregulation and inflammatory responses (Duan *et al.,* 2021; Karačić *et al.,* 2024).

Furthermore, emerging evidence suggests that obesity-related gut dysbiosis may influence the gut resistome the collection of antibiotic resistance genes in the microbiome (Fri *et al.,* 2024). For example, *Escherichia coli* strains within the gut of obese individuals have been shown to exacerbate metabolic dysfunction and insulin resistance, indicating a potential relationship between body composition and microbial behaviour (Ju *et al.,* 2023; Takeuchi *et al.,* 2023). Therefore, BMI might play an indirect role in shaping the resistance patterns of commensal bacteria by altering gut microbial balance and selection pressures.

In Nigeria, antibiotic resistance has reached alarming levels due to widespread irrational antibiotic use, including self-medication, incomplete prescriptions, and over-the-counter access without professional supervision (Iheanacho and Eze, 2022; Alabi *et al.,* 2025). University students, who represent a key segment of the young adult population, have been identified as frequent participants in self-medication and inappropriate antibiotic practices (Popoola *et al.,* 2024; Onukansi *et al.,* 2025). These behaviors are often driven by factors such as academic stress, financial constraints and poor awareness of antibiotic resistance risks.

The combination of such practices with lifestyle factors influencing BMI may have compounding effects on the carriage and resistance of commensal *Escherichia coli* in apparently healthy individuals.

Despite the growing research on antibiotic resistance and gut microbiota globally, there remains a paucity of data linking BMI with their resistance profiles of commensal *Escherichia coli* among apparently healthy individuals in sub-Saharan Africa, including university populations. Evaluating this relationship among UNIBEN students will not only contribute to understanding local antibiotic resistance dynamics but may also provide insights into the role of host physiology and lifestyle in shaping bacterial ecology and resistance potential in the human gut.

1.2 Statement of the Problem

Antibiotic resistance (AR) continues to pose a critical global health threat, undermining the efficacy of antibiotics that have long been essential in the treatment of infectious diseases (Murray *et al.,* 2022; WHO, 2025). The World Health Organization (2025) has identified antibiotic resistance as one of the top ten global public health threats, with projections suggesting that resistance-related infections could cause millions of deaths annually if urgent action is not taken. While a significant number of studies have characterized antibiotic resistance patterns in clinical pathogens, relatively fewer have examined commensal bacteria such as *Escherichia coli* in healthy populations (Nji *et al.,* 2021; Wollein Waldetoft *et al.,* 2023). These commensal organisms often act as silent reservoirs of resistance genes that can be transferred to pathogenic strains through horizontal gene transfer, contributing to the

persistence and spread of resistance within communities (Tawfick *et al.,* 2022; Rana *et al.,* 2024).

In Nigeria and other low and middle income countries, the inappropriate and overuse of antibiotics remain widespread due to self-medication, inadequate prescription control, and limited public awareness (Onah and Umar, 2024; Alabi *et al.,* 2025). University students represent a particularly vulnerable demographic, as studies have revealed high levels of irrational antibiotic use in this group are often driven by factors such as academic stress, financial limitations and the easy accessibility of antibiotics without prescription (Popoola *et al.,* 2024; Onukansi *et al.,* 2025). This behavioral pattern increases the risk of antibiotic-resistant bacteria being maintained and transmitted among apparently healthy individuals, further complicating local antibiotic resistance surveillance efforts.

At the same time, recent advances in microbiome research have revealed a complex relationship between host physiology, gut microbial composition, and metabolic health (Enache *et al.,* 2024; McBurney and Cho, 2024). Variations in Body Mass Index (BMI) a key indicator of nutritional and metabolic status have been linked to significant differences in gut microbiota structure and diversity (Aranaz *et al.,* 2021; Duan *et al.,* 2021). Obese individuals, for example, often exhibit a higher Firmicutes/Bacteroidetes ratio and altered microbial metabolic activity that may promote inflammation and metabolic disorders (Cheng *et al.,* 2022; Karačić *et al.,* 2024). Moreover, obesity-associated gut dysbiosis has been implicated in influencing the gut resistome the collective pool of antibiotic resistance genes in the microbiota (Ju *et al.,* 2023; Fri *et al.,* 2024). This suggests that BMI may indirectly affect the abundance and resistance characteristics of commensal bacteria such as *Escherichia coli*.

Despite the growing global recognition of the interrelationship between gut microbiota, obesity, and antibiotic resistance, there is limited or no data in Nigeria exploring the potential link between BMI and antibiotic resistance in commensal *Escherichia coli* among apparently healthy individuals. This knowledge gap is particularly concerning in university environments, where lifestyle, dietary habits, and antibiotic use behaviors can collectively shape the gut microbiota and resistance patterns. Without evidence-based data, it becomes difficult to design targeted preventive strategies that address both antibiotic resistance and nutritional health in young adults.

Therefore, this study seeks to evaluate the relationship between Body Mass Index (BMI) and antibiotic resistance of commensal *Escherichia coli* isolated from apparently healthy students at the University of Benin (UNIBEN), Nigeria. By addressing this gap, the research aims to contribute to the better understanding of the multifactorial drivers of antibiotic resistance in the community and inform public health policies on antibiotic stewardship and lifestyle interventions among young adults.

1.3 Justification of the Study

1. Public Health Importance: Antibiotic resistant bacteria are a major public health threat, studying them in apparently healthy individuals could help to identify hidden reservoirs.
2. Nutritional and Microbiota relationship: Since BMI influences gut microbial ecology, understanding its relationship with antibiotic resistance could reveal new determinants of antibiotic resistance.

3. Local Relevance: In Nigeria, where both malnutrition and antibiotic misuse are prevalent, this study provides context-specific evidence to guide policy and interventions.
4. Preventive Approach: Findings may inform antibiotic stewardship programs and health education campaigns targeted at students, thereby reducing community transmission of resistant strains.

1.4 Aim of Study

This study was aimed to evaluate the relationship between Body Mass Index (BMI) and antibiotic resistance of commensal *Escherichia coli* isolated from the stool samples of apparently healthy students present at University of Benin (UNIBEN).

1.5 Specific Objectives

The specific objectives of this study was aimed to:

1. determine the BMI of apparently healthy students sampled in the study.
2. isolate and identify commensal *Escherichia coli* from stool samples of the participants.
3. determine the antibiotic resistance profile in the *Escherichia coli* isolates.
4. assess the relationship between BMI and antibiotic resistance patterns of the isolates organism.

1.6 Research Questions

1. What are the BMI categories of apparently healthy students in the study population?
2. What is the prevalence of antibiotic resistance of commensal *Escherichia coli* isolated from these students?

3. Is there an association between BMI and the resistance profile of commensal *Escherichia coli* ?

1.7 Hypotheses

1. Null Hypothesis (H_0): There is no significant relationship between BMI and antibiotic resistance of commensal *Escherichia coli* isolated from stool samples of apparently healthy students at UNIBEN.
2. Alternative Hypothesis (H_1): There is a significant relationship between BMI and antibiotic resistance of commensal *Escherichia coli* isolated from stool samples of apparently healthy students at UNIBEN.

1.8 Scope of the Study

The study will be conducted among apparently healthy undergraduate students of the University of Benin. Stool samples will be collected for the isolation of commensal *Escherichia coli* and their antibiotic resistance patterns will be determined. The BMI of each participant will be calculated and categorized according to WHO standards (underweight, normal weight, overweight, obese) and only commonly prescribed antibiotics in Nigeria will be used for the test.

1.9 Limitations of the Study

1. The study is limited to a single university population and may not be generalizable to other settings.
2. Body mass index (BMI) is a simple nutritional measure and may not reflect body fat distribution or other nutritional factors influencing gut microbiota.
3. Due to resource constraints, biochemical tests like indole, citrate and urease tests will be skipped and the use of cultural characteristics, motility and Gram biochemical

reactions will be employed to presumptive identification of *Escherichia coli* isolate and the laboratory analysis will focus only on the isolate phenotypic resistance; genotypic mechanisms of resistance of the isolate would not be investigated

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical and Concept of Body Mass Index (BMI)

The concept of Body Mass Index (BMI) has its roots in the 19th century when Lambert Adolphe Jacques Quetelet, a Belgian astronomer, mathematician, and statistician, introduced the *Quetelet Index* as part of his study on human physical characteristics. Between 1830 and 1850, Quetelet sought to identify a mathematical relationship between height and weight that could describe the "average man" in population studies. His index, derived from dividing a person's weight in kilograms by the square of their height in meters (kg/m^2) (Eknoyan, 2008), was originally intended as a demographic and statistical measure rather than a diagnostic or clinical tool for individuals.

In the 1970s, Ancel Keys and colleagues critically evaluated various indices to determine which best reflected relative body fat across diverse populations. Their comparative analysis confirmed that Quetelet's formula correlated most strongly with actual body fat percentages, leading to the coining of the modern term *Body Mass Index (BMI)* (Keys *et al.,* 1972). Ever since then onward, BMI has become a standard, simple, and cost-effective anthropometric measure widely used in clinical practice and epidemiological research for categorizing individuals based on body weight relative to height.

The World Health Organization (WHO) formally adopted BMI in the year 1995, as a universal index for classifying underweight, normal weight, overweight, and obesity, defining the respective categories as follows:

- **Underweight:** < 18.5 kg/m²
- **Normal weight:** 18.5 – 24.9 kg/m²
- **Overweight:** 25.0 – 29.9 kg/m²
- **Obese:** ≥ 30 kg/m²

(WHO, 2025).

These categories have become the global reference for assessing population health risks related to malnutrition and obesity, facilitating surveillance of non-communicable diseases such as diabetes, cardiovascular disorders, and metabolic syndrome (McBurney and Cho, 2024).

Although BMI remains a valuable and practical screening tool, it has notable limitations. It does not differentiate between lean muscle and fat mass, nor does it indicate fat distribution or body composition (Nuttall, 2015). Consequently, individuals with identical BMI values may present markedly different metabolic and cardiovascular risks. This limitation has led researchers to explore complementary indicators, such as waist circumference and waist-to-hip ratio, which provide additional insights into visceral adiposity and metabolic health (Hussein and Tawfeeq, 2024).

Recent advances in gut microbiome research have expanded the conceptual understanding of BMI beyond simple anthropometric assessment. Studies have demonstrated that BMI is closely linked to alterations in the gut microbial ecosystem, which influences energy metabolism, nutrient absorption, and systemic inflammation (Aranaz *et al.*, 2021; Cheng *et al.,* 2022). Individuals with elevated BMI often exhibit changes in microbial composition particularly an increased Firmicutes/Bacteroidetes ratio that promote greater energy harvest

from food and contribute to adiposity (Duan *et al.,* 2021; Karačić *et al.,* 2024). Similarly, *Escherichia coli* and other commensal bacteria have been implicated in modulating host metabolism, with some strains exacerbating obesity and insulin resistance under high-fat diet conditions (Ju *et al.*, 2023; Takeuchi *et al.,* 2023).

Moreover, the gut microbiota plays a dual role in obesity and antibiotic resistance, forming part of the broader "gut resistome," which represents all antibiotic resistance genes within the microbiome (Fri *et al.,* 2024). These findings suggest that BMI may serve not only as an indicator of nutritional status but also as a potential marker of microbial and metabolic imbalance, thereby linking nutritional health with microbial ecology and antibiotic resistance (Islam *et al.*, 2023; Enache *et al.*, 2024).

2.2 *Escherichia coli* **as a Commensal and Pathogen.**

Escherichia coli (*E. coli*) is a Gram-negative, facultative anaerobic bacterium classified under the family Enterobacteriaceae and belongs to the coliform group. It is one of the earliest colonizers of the human gastrointestinal tract (GIT), establishing itself shortly after birth and becoming one of the most abundant commensal bacteria in the gut microbiota (Nji *et al.,* 2021; Tawfick *et al.,* 2022; Mazumder *et al.,* 2023). As a commensal organism, *Escherichia coli* contributes to several critical physiological functions, including aiding in nutrient metabolism, synthesizing essential vitamins such as vitamin K and B complex, and protecting the gastrointestinal tract against colonization by pathogenic microorganisms (Basavaraju and Gunashree, 2022; Cheng *et al.,* 2022; Hu *et al.,* 2022). Furthermore, commensal *Escherichia coli* plays role in maintaining microbial homeostasis and modulating immune responses

within the host, making it an integral component of the human gut ecosystem (Sankararaman *et al.,* 2023; Fri *et al.,* 2024).

Despite its beneficial roles, *Escherichia coli* is highly versatile and can transition into a pathogenic state under certain conditions. Strains that acquire virulence factors through horizontal gene transfer are capable of causing a wide spectrum of diseases. These include enteric infections such as those caused by enterotoxigenic *Escherichia coli* (ETEC), enteropathogenic *Escherichia coli* (EPEC), and enterohemorrhagic *Escherichia coli* (EHEC), as well as extraintestinal infections such as urinary tract infections, sepsis, and neonatal meningitis caused by uropathogenic and extraintestinal pathogenic *Escherichia coli* (ExPEC) (Mazumder *et al.,* 2023; Rana *et al.,* 2024). The ability of *Escherichia coli* to act both as a commensal and as a pathogen underscores its remarkable genetic adaptability and metabolic versatility (Tawfick *et al.,* 2022; Wollein Waldetoft *et al.,* 2023).

Even non-pathogenic strains are of public health importance because they can harbor and disseminate antibiotic resistance genes (ARGs) within the gut microbiome. Such genes may be transferred to pathogenic counterparts under selective pressure, contributing to the spread of antibiotic resistance in human populations (Nji *et al.,* 2021; Lee *et al.,* 2023; Fri *et al.,* 2024). The gut microbiota of individuals with altered nutritional status, such as those with high or low Body Mass Index (BMI), have been related with differential carriage of resistant bacteria (Aranaz *et al.,* 2021; Companys *et al.,* 2021). Studies have shown that obesity or malnutrition can influence gut microbial composition, including commensal *Escherichia coli*, potentially affecting both metabolism and the resistome the collective pool of antibiotic resistance genes (Aranaz *et al.,* 2021; Ju *et al.,* 2023; Takeuchi *et al.,* 2023).

From a public health perspective, *Escherichia coli* presents a dual challenge. While it is essential for maintaining intestinal health and metabolic balance, pathogenic strains remain major contributors to morbidity and mortality globally, particularly among children under five years of age, immunocompromised individuals, and vulnerable populations in low- and middle-income countries (Murray *et al.,* 2022; Onah and Umar, 2024). Furthermore, commensal *Escherichia coli* in apparently healthy individuals may silently carry and disseminate ARGs, making the organism a valuable sentinel for monitoring community-level antibiotic resistance (Sali *et al.,* 2021; Tawfick *et al.,* 2022).

In light of its dual role, continuous surveillance of *Escherichia coli* in healthy populations is critical. Understanding the relationship between commensal *Escherichia coli*, BMI, and antibiotic resistance provides insights into the interplay between host nutritional status, microbial ecology, and antibiotic resistance, thereby informing strategies to reduce the community spread of resistant bacteria (Nji *et al.,* 2021; Islam *et al.,* 2023; Enache *et al.,* 2024).

2.3 Antibiotic Resistance: A Global Challenge

Antibiotic resistance (AR) has emerged as one of the most pressing global health challenges of the 21st century, threatening the effectiveness of antibiotics and complicating the treatment of infectious diseases (Murray *et al.,* 2022; Alabi *et al.,* 2025). Bacteria, including commensal and pathogenic strains, employ a range of mechanisms to evade the effects of antibiotics. These mechanisms include enzymatic degradation of antibiotic agents, modification of drug targets, activation of efflux pumps, and reduced permeability of the bacterial cell wall (Fri *et al.,* 2024; Rana *et al.,* 2024). The rapid evolution of such resistance

traits has been exacerbated by the widespread misuse and overuse of antibiotics in human medicine, veterinary practice, agriculture, and self-medication, particularly in low- and middle-income countries such as Nigeria (Iheanacho and Eze, 2022; Onah and Umar, 2024; Popoola *et al.,* 2024; Onukansi *et al.,* 2025).

The World Health Organization (WHO, 2025) identifies antibiotic resistance as one of the top ten global health threats, highlighting its impact on morbidity, mortality, and healthcare costs. Resistant infections prolong hospital stays, limit treatment options, and increase the financial and societal burden of disease. Nji *et al.* (2021) reported high prevalence of antibiotic-resistant commensal *Escherichia coli* in healthy individuals, demonstrating that resistance is not confined to clinical settings but is also circulating within communities. Studies also indicate that young adults, including university students, frequently engage in irrational antibiotic use due to factors such as academic stress, over-the-counter availability, and limited awareness, further facilitating the dissemination of resistant organisms (Popoola *et al.,* 2024; Onukansi *et al.,* 2025).

Among resistant organisms, *Escherichia coli* has gained particular attention due to its dual role as a commensal and opportunistic pathogen. Certain strains, such as extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*, exhibit resistance to third-generation cephalosporins and are often co-resistant to multiple other antibiotic classes, complicating both hospital- and community-based management (Tawfick *et al.,* 2022; Mazumder *et al.,* 2023). Even non-pathogenic commensal strains can act as reservoirs of resistance genes, transferring them to pathogenic strains under selective pressure and thereby sustaining the spread of resistance within human populations (Sali *et al.,* 2021; Lee *et al.,* 2023).

Recent research has highlighted that host factors, including nutritional status and Body Mass Index (BMI), may influence the carriage of antibiotic-resistant bacteria. Alterations in gut microbiota associated with obesity, overweight, or undernutrition can affect the abundance and diversity of commensal *Escherichia coli*, which in turn may modulate the gut resistome the collective pool of antibiotic resistance genes (Aranaz *et al.*, 2021; Ju *et al.*, 2023; Takeuchi *et al.*, 2023; Fri *et al.*, 2024). Individuals with high BMI often show changes in microbial composition that favor the persistence of resistant bacteria, suggesting a complex interplay between host metabolic status, microbiota, and antibiotic resistance (Sankararaman *et al.*, 2023; Enache *et al.*, 2024).

Given these dynamics, antibiotic resistance is not merely a clinical issue but a multifaceted public health problem that requires integrated approaches, including rational antibiotic stewardship, continuous surveillance, public education, and investigation into host-related factors such as BMI and lifestyle behaviors (Onah and Umar, 2024; Alabi *et al.*, 2025; WHO, 2025). Addressing these challenges is crucial for containing the spread of resistant organisms in both healthy and vulnerable populations.

2.4 Relationship between BMI and Gut Microbiota

The human gastrointestinal tract hosts a highly complex and dynamic community of microorganisms collectively referred to as the gut microbiota (Thursby and Juge 2017). These microbes perform essential functions, including nutrient metabolism, immune regulation, and maintaining the integrity of the gut barrier (Cheng *et al.*, 2022; Islam *et al.*, 2023; Sankararaman *et al.*, 2023). Emerging evidence indicates that variations in Body Mass Index (BMI) are associated with distinct gut microbial profiles, highlighting a strong

interplay between host nutritional status and microbiome composition (Aranaz *et al.,* 2021; Companys *et al.,* 2021; Hu *et al.,* 2022).

Individuals with higher BMI, particularly those who are overweight or obese, often exhibit reduced microbial diversity and significant shifts in microbial composition, including an increased Firmicutes to Bacteroidetes ratio and higher abundance of certain metabolically active taxa (Duan *et al.,* 2021; Karačić *et al.,* 2024; Liu *et al.,* 2021). Conversely, lean individuals tend to harbor microbiota that favor energy-efficient metabolism and maintenance of healthy adiposity (Palmas *et al.,* 2021; McBurney and Cho, 2024; Augustynowicz *et al.,* 2025). These variations influence host energy harvest and storage, as certain microbial populations enhance caloric extraction from the diet, predisposing individuals to weight gain and obesity, while others support metabolic regulation that favors leanness (Ju *et al.,* 2023; Takeuchi *et al.,* 2023).

Beyond metabolism, BMI-associated alterations in gut microbiota have been linked to differences in the gut resistome (Liang *et al.,* 2025) in collection of antibiotic resistance genes within the microbial community. Studies suggest that individuals with obesity may harbor gut microbiota enriched in antibiotic resistance genes, whereas undernutrition or malnutrition can impair immune defenses, making the host more susceptible to colonization by resistant bacteria (Vliex *et al.,* 2022; Enache *et al.,* 2024; Fri *et al.,* 2024). Commensal organisms such as *Escherichia coli* can act as reservoirs for these resistance genes, which may be mobilized to pathogenic strains under selective pressure, emphasizing the dual impact of BMI on metabolic and microbial dynamics (Tawfick *et al.,* 2022; Mazumder *et al.,* 2023).

These findings collectively suggest that BMI is not only a measure of nutritional status but also a determinant of gut microbial ecology and the carriage of antibiotic-resistant bacteria. The reciprocal relationship between BMI and the gut microbiome provides a biological rationale for investigating how variations in BMI influence the prevalence and resistance profiles of commensal *Escherichia coli*, particularly among populations with diverse nutritional statuses such as university students (Aranaz *et al.,* 2021; Ju *et al.,* 2023; Enache *et al.,* 2024). Understanding this relationship could inform strategies aimed at mitigating the spread of antibiotic resistance while addressing obesity and undernutrition as interconnected public health challenges.

2.5 Antibiotic Resistance in Commensal *Escherichia coli*

Commensal *Escherichia coli* are natural inhabitants of the human gastrointestinal tract (GIT), contributing to gut homeostasis, nutrient metabolism, and protection against pathogenic colonization (Tawfick *et al.,* 2022; Ju *et al.,* 2023). Although they are generally non-pathogenic in healthy individuals, commensal *Escherichia coli* can serve as important reservoirs of antibiotic resistance genes, which can be mobilized to pathogenic strains through horizontal gene transfer (Mazumder *et al.,* 2023; Fri *et al.,* 2024; Rana *et al.,* 2024). This makes them a valuable indicator for monitoring the circulation of antibiotic resistance (AR) within communities.

Exposure to antibiotics whether through medical prescriptions, self-medication, or agricultural use creates selective pressure on commensal bacteria, driving the acquisition and persistence of resistance determinants (Popoola *et al.,* 2024; Alabi *et al.,* 2025; Onukansi *et al.,* 2025). Studies conducted in different populations have consistently reported resistance

among commensal *Escherichia coli*, including resistance to commonly used antibiotics such as ampicillin, tetracycline, and cotrimoxazole (Nji *et al.,* 2021; Tawfick *et al.,* 2022).

In Nigeria, irrational antibiotic use including self-medication, incomplete therapy, and over-the-counter access has been identified as a major driver of antibiotic resistance in both pathogenic and commensal bacteria (Iheanacho and Eze, 2022; Onah and Umar, 2024; Onukansi *et al.,* 2025). University students, due to academic pressures, mobility, and easy access to antibiotics, are particularly prone to such practices, potentially facilitating the emergence and spread of resistant commensal strains within campus communities (Popoola *et al.,* 2024; Onukansi *et al.,* 2025).

Evidence from local and regional studies demonstrates that multidrug-resistant commensal *Escherichia coli* are widespread in healthy populations. Nji *et al.* (2021) reported a high prevalence of antibiotic-resistant *Escherichia coli* in apparently healthy individuals across community settings, highlighting the role of commensals as silent reservoirs of resistance genes. The presence of these strains in the gut microbiota reveals the potential for resistant bacteria to disseminate within populations, bridging community and hospital environments and complicating infection control measures (Tawfick *et al.,* 2022; Mazumder *et al.,* 2023; Lee *et al.,* 2023).

Monitoring antibiotic resistance in commensal *Escherichia coli* provides critical insights into population-level exposure to antibiotics and serves as an early warning system for emerging resistance patterns. Studying these bacteria among university students, such as at UNIBEN, offers a window into the hidden burden of antibiotic resistance in young, mobile populations and underscores the need for targeted interventions aimed at promoting rational antibiotic use

and limiting the spread of resistance genes in the community (Alabi *et al.*, 2025; Onukansi *et al.*, 2025; WHO, 2025).

2.6 BMI and Antibiotic Resistance of Commensal Bacteria

While relationship between Body Mass Index (BMI) and gut microbiota composition is well established, comparatively fewer studies have examined how nutritional status influences the carriage of antibiotic-resistant bacteria. Recent research indicates that BMI-related alterations in the gut microbiota may create ecological conditions that favor the persistence and dissemination of resistant strains (Fri *et al.*, 2024; Vliex *et al.*, 2022; Enache *et al.*, 2024).

Obesity, in particular, has been associated with changes in gut microbial diversity and composition, including an enrichment of microbial taxa carrying antibiotic resistance genes (Aranaz *et al.*, 2021; Ju *et al.*, 2023; Takeuchi *et al.*, 2023). Such shifts may increase the size and diversity of the gut resistome, enabling commensal bacteria, such as *Escherichia coli*, to act as reservoirs for resistance determinants. In addition, the metabolic and inflammatory environment associated with obesity may facilitate the horizontal transfer of resistance genes among gut microbes, further amplifying the risk of resistance propagation (Companys *et al.*, 2021; Islam *et al.*, 2023; Sankararaman *et al.*, 2023).

At the other extreme, undernutrition and malnutrition are linked to impaired gut barrier function, altered microbial communities, and weakened host immunity (Enache *et al.*, 2024; McBurney and Cho, 2024; Augustynowicz *et al.*, 2025). Such conditions may similarly promote colonization and persistence of antibiotic-resistant commensals, highlighting that both high and low BMI can influence the gut resistome in ways that increase the likelihood of resistant bacteria establishing within the host.

The dual burden of malnutrition and obesity, increasingly observed in young adult populations, may therefore amplify the risk of carriage and spread of antibiotic-resistant commensals (Aranaz *et al.,* 2021; Companys *et al.,* 2021; Hu *et al.,* 2022). Despite these insights, there is limited data specifically linking BMI to antibiotic resistance in commensal *Escherichia coli*, particularly within African populations and among apparently healthy individuals such as university students (Nji *et al.,* 2021; Onukansi *et al.,* 2025).

Understanding the relationship between BMI and the carriage of resistant commensal bacteria is crucial for identifying host-related risk factors that contribute to antibiotic resistance. Such knowledge can guide public health strategies aimed at mitigating the spread of resistant organisms, particularly in populations vulnerable to both nutritional extremes and irrational antibiotic use (Popoola *et al.,* 2024; Alabi *et al.,* 2025; WHO, 2025).

2.7 Antibiotic Resistance in Nigeria: The Student Population

Nigeria is currently facing a significant and growing challenge of antibiotic resistance, driven largely by irrational and widespread use of antibiotics across human, animal, and environmental sectors (Iheanacho and Eze, 2022; Onah and Umar, 2024; Alabi *et al.,* 2025). The easy availability of antibiotics without prescription, self-medication practices, and incomplete therapeutic courses are prevalent, creating selective pressure that promotes the emergence and persistence of resistant bacteria (Popoola *et al.,* 2024; Onukansi *et al.,* 2025).

University students constitute a demographic of particular concern in the Nigerian context. This population often exhibits high levels of antibiotic misuse, influenced by academic stress, peer behavior, mobility, and limited knowledge of antibiotic resistance (Popoola *et al.,* 2024; Onukansi *et al.,* 2025). Surveys conducted in tertiary institutions indicate that students

frequently engage in self-medication and inappropriate use of antibiotics, practices that may facilitate the colonization and persistence of resistant commensal bacteria in their gastrointestinal tract (Alabi *et al.*, 2025; Onukansi *et al.*, 2025).

Evidence from Nigerian studies has highlighted the high prevalence of antibiotic resistance among commensal *Escherichia coli* isolated from apparently healthy individuals. Nji *et al.* (2021) reported widespread resistance to commonly used antibiotics, including ampicillin, tetracycline, and cotrimoxazole, mirroring patterns observed in clinical isolates. Similarly, Oyetayo and Ojo (2017) observed multidrug-resistant *Escherichia coli* in community samples, underscoring the continuous exchange of resistance determinants between hospital and community environments. These findings suggest that commensal *Escherichia coli* in healthy individuals can act as silent reservoirs, capable of spreading resistance within the broader population (Tawfick *et al.*, 2022; Mazumder *et al.*, 2023).

The student population is particularly significant due to their high mobility, social interactions, and frequent contact with diverse environmental and community settings. Carriage of resistant commensal *Escherichia coli* in this group poses a potential risk for wider dissemination of antibiotic resistance, not only within university campuses but also into surrounding communities (Alabi *et al.*, 2025; Onukansi *et al.*, 2025). Investigating the relationship between BMI, antibiotic use, and carriage of resistant *Escherichia coli* among students provides critical insights for public health interventions aimed at reducing antibiotic resistance and promoting rational antibiotic use in young adults.

2.8 Knowledge Gap

The interplay between body mass index (BMI), gut microbiota, and antibiotic resistance has been increasingly recognized in global research, yet evidence from Nigeria remains limited. International studies indicate that BMI influences gut microbial composition and the gut resistome, with obesity associated with enriched microbial communities carrying antibiotic resistance genes, while undernutrition alters gut ecology in ways that may favor colonization by resistant bacteria (Aranaz *et al.,* 2021; Ju *et al.,* 2023; Sankararaman *et al.,* 2023; Takeuchi *et al.,* 2023; Fri *et al.,* 2024; Augustynowicz *et al.,* 2025). Additionally, variations in the Firmicutes/Bacteroidetes ratio and other microbial signatures have been linked to different BMI categories, highlighting the role of microbial ecology in metabolic and immune modulation (Companys *et al.,* 2021; Liu *et al.,* 2021; Karačić *et al.,* 2024; McBurney and Cho, 2024).

In Nigeria, research has predominantly focused on clinical isolates of *Escherichia coli*, with limited attention to commensal strains from healthy individuals (Iheanacho and Eze, 2022; Onah and Umar, 2024). This gap restricts understanding of how healthy carriers contribute to the maintenance and dissemination of antibiotic resistance within communities. Studies have documented multidrug-resistant commensal *Escherichia coli* in healthy populations, demonstrating that resistance determinants may circulate silently between community and hospital environments (Nji *et al.,* 2021; Tawfick *et al.,* 2022; Mazumder *et al.,* 2023; Rana *et al.,* 2024). Despite these findings, little is known about the relationship between BMI and the carriage of resistant commensal bacteria in the Nigerian context.

University students represent a unique and vulnerable population. This group frequently engages in self-medication and irrational antibiotic use, which may increase the carriage of resistant commensal bacteria (Popoola *et al.,* 2024; Alabi *et al.,* 2025; Onukansi *et al.,* 2025). Yet, there is no systematic study examining whether BMI influences antibiotic resistance in commensal *Escherichia coli* among Nigerian students. Understanding this relationship is critical, as it could reveal host-related factors contributing to antibiotic resistance and inform targeted interventions for populations facing the dual burden of undernutrition and obesity (Enache *et al.,* 2024; Yarahmadi *et al.,* 2024).

Furthermore, although commensal *Escherichia coli* are recognized globally as reservoirs of transferable resistance genes (Tawfick *et al.,* 2022; Wollein Waldetoft *et al.,* 2023; Fri *et al.,* 2024), data from sub-Saharan Africa remain sparse. This lack of information represents a significant knowledge gap, particularly regarding how BMI may influence the carriage and dissemination of resistance genes in apparently healthy individuals. Addressing this gap is essential for understanding the hidden reservoirs of antibiotic resistance in the community and for guiding public health strategies aimed at mitigating antibiotic resistance in Nigeria (Onah and Umar, 2024; Alabi *et al.,* 2025; WHO, 2025).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Study Area

The study was carried out at Medical Microbiological Laboratory in University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria, with samples collected from undergraduate students at University of Benin (UNIBEN) within and outside the campus.

3.2 Study Design

This research adopted a cross-sectional laboratory-based experimental design. The study involved the collection of stool samples from apparently healthy students, isolation of commensal *Escherichia coli*, and subsequent determination of the relationship between the Body Mass Index (BMI) of participants and the antibiotic resistance profile of the isolates.

3.3 Ethical Approval

Ethical approval was obtained from the University of Benin Ethical Committee. Informed consent was obtained from each participant, and confidentiality of personal data was strictly maintained. Participants were assigned identification codes instead of names

3.4 Duration of Study

Sample collection commenced on the 6th of August, 2025 and continued until 24th of September, 2025.

3.5 Sample Collection

About 70 Fresh stool specimens were collected. A total of 9 samples were collected in wide-mouthed sterile universal containers, while 61 samples were collected using sterile swab

sticks. Each sample was labeled with a unique code identifier and transported promptly to the UBTH Medical Microbiology Laboratory for processing.

3.6 Body Mass Index (BMI) Evaluation

The weight of each participant was measured using a calibrated weighing scale, and height was measured using a measuring tape. BMI was calculated using the formula:

$$BMI = \frac{Weight(kg)}{Height(m^2)}$$

Participants were categorized according to the World Health Organization (WHO) classification:

- Underweight: <18.5 kg/m²
- Normal weight: 18.5–24.9 kg/m²
- Overweight: 25.0–29.9 kg/m²
- Obese: ≥30 kg/m²

(WHO, 2025)

3.7 Laboratory Procedures.

3.7.1 Microbiological Analysis.

3.7.1.1 Isolation of *Escherichia coli*

Each stool specimen was inoculated onto MacConkey agar and incubated aerobically at 37 °C for 18–24 hours. *Escherichia coli* was confirmed by its Colonial Morphology, Motility test, Gram staining and standard biochemical tests (using indole, citrate and urease tests).

3.7.1.2 Antibiotic Susceptibility Testing

Antibiotic susceptibility was performed using the modified Kirby-Bauer disk diffusion method on Mueller-Hinton agar following Clinical and Laboratory Standards Institute (CLSI, 2021) guidelines. Standard antibiotic discs included commonly prescribed agents such as Amoxicillin-Clavulonate (30µg), Cefepime (30µg), Cefotaxime (30µg), Ceftazidime (30µg), Imipenem (10µg), Meropenem (10µg), Ciprofloxacin (5µg), Ofloxacin (5µg) and Gentamicin (10µg). Zone diameters were measured and interpreted according to CLSI breakpoints as Sensitive (S), Intermediate (I), or Resistant (R).

3.7.2 Identification of *Escherichia coli.*

3.7.2.1 Colonial Morphology.

On culture media, *Escherichia coli* exhibits distinct colonial characteristics that aid in its presumptive identification:

- On Nutrient agar, colonies were large (2–3 mm in diameter after 18–24 hours), moist, smooth, low-convex, and opaque with entire edges. They were non-pigmented.
- On MacConkey agar, colonies were medium in sized, round, moist, smooth, and pink to red in color due to lactose fermentation, often surrounded by a zone of bile salt precipitation.

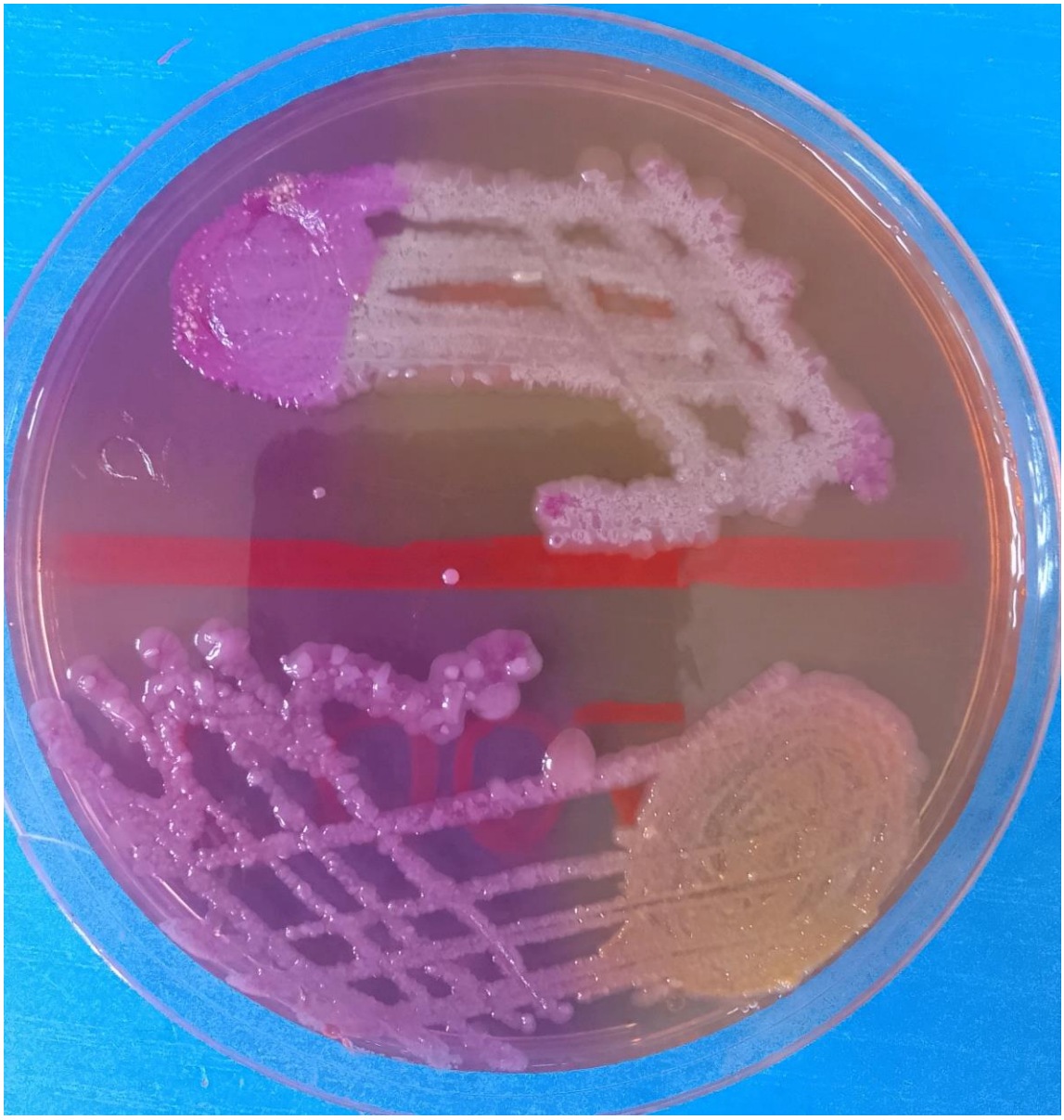

Figure 1: Colonies of growing *Escherichia coli* organisms, showing pinkish (lactose fermenter) coloration on MacConkey agar**.**

3.7.2.2 Motility test.

Motility test is a microbiological procedure used to determine whether an organism is capable of self-directed movement, usually mediated by flagella. The test was important in identifying *Escherichia coli,* as *Escherichia coli* is a motile bacterium with a peritrichous flagella.

Hanging Drop techniques was used, by placing a ring of plasticine on a clean coverslip, firmly, a drop of normal saline was placed at the center of the coverslip bounded round with plasticine ring, and a discrete colony with the presumptive colonial morphology was picked from the agar using a sterile straight wire and inoculated in the normal saline. A clean grease-free glass slide was placed gently and firmly on the surface of the plasticine ring, inverted and viewed under microscope using ×40 objective lens.

3.7.2.3 Gram Staining.

Gram staining was carried out to determine the cell morphology and Gram reaction of the isolates, as it was known to possess a thin peptidoglycan in it cellular wall, and could not retain the primary stain after being decolorized, taking up the secondary stains, and thus, appearing pinkish as rod-shaped organism.

Procedure: Smears were prepared from fresh cultures, air-dried, and heat-fixed on grease-free glass slide. They were sequentially stained with crystal violet (primary stain) for 60 seconds and rinsed in water, mordant with Lugol's iodine solution for another 60 seconds and rinsed in running water, decolorized with acetone–alcohol for few seconds and washed off in running water and then counterstained with neutral red (secondary stains) for 60 seconds, rinsed in water and air-dried. The stained smears were examined using ×100 oil immersion

objective lens under microscope. The isolates appeared as Gram-negative short rods, either singly or in pairs.

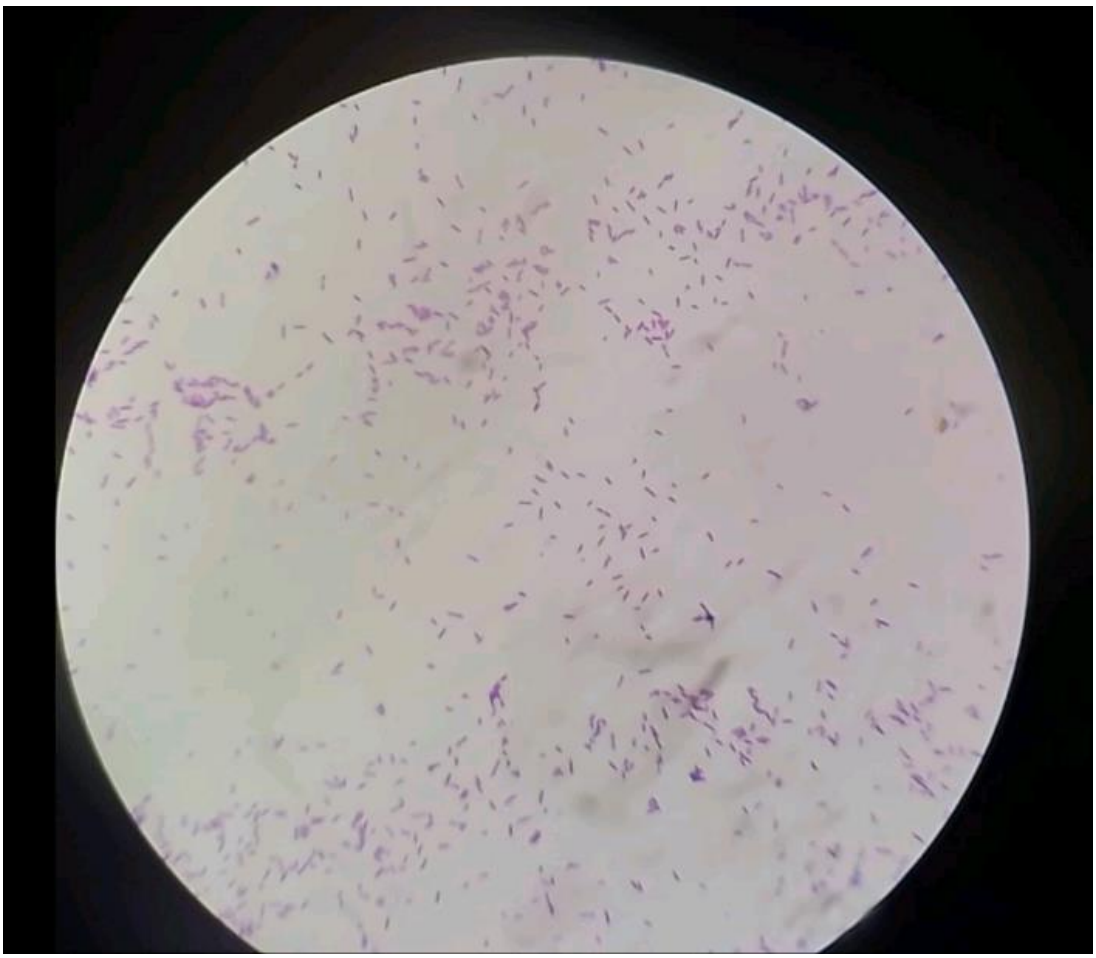

Figure 2: Gram staining reaction of *Escherichia coli* organisms under microscope using ×100 oil immersion objective lens, seen as pinkish rods.

3.7.2.4 Indole Test.

A unique biochemical test specific to differentiating *Escherichia coli* from all other coliforms. It was based on the ability of the *Escherichia coli* isolates to produce indole from tryptophan. The isolated organism was inoculated into sterile tryptophan broth and incubated at 37 °C for 18–24 hours. Following incubation, Kovac's reagent was added to each tube. The formation of red or pink ring that developed at the surface due to the action of p-dimethylaminobenzaldehye (an active agent in Kovac's reagent) reacting with the indole produced in the medium indicated a positive reaction. The isolates of *Escherichia coli* is usually positive to indole test.

3.7.2.5 Citrate Utilization Test.

The citrate utilization test was usually performed alongside with indole test to determine whether the isolates could utilize citrate as a sole source of carbon and bromothymol blue is used as an indicator in this medium, which normally turns blue when the medium is alkaline. The isolated organism was inoculated onto Simmon's citrate agar slants in a bijou bottle and incubated at 37 °C for 24 - 48 hours. A positive test was indicated by growth and a blue coloration of the medium, while a negative result showed no growth and retention of the green color. The *Escherichia coli* isolates were usually negative for citrate utilization.

3.7.2.6 Urease Test.

The urease test was used to determine if the isolated *Escherichia coli* could hydrolyze urea to ammonia. The isolated organism was inoculated onto Christensen's urea agar slants and incubated at 37 °C for 24 - 48 hours. The development of a bright pink coloration indicates a positive reaction. *Escherichia coli* isolates gave negative reactions, as no color change was observed. The indicator used here is Phenol red.

3.7.3 Identification of ESBL-producing *Escherichia coli.*

Extended Spectrum β-Lactamase (ESBL) production among *Escherichia coli* isolates was detected phenotypically using selected cephalosporins and a β-lactam/β-lactamase inhibitor combination, in accordance with CLSI (2021) guidelines. The antibiotics used were Amoxicillin-Clavulonate (30 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Cefepime (30 µg), Imipenem (10 µg), Meropenem (10 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg) and Gentamicin (10 µg).

Pure cultures of the isolates were standardized to 0.5 McFarland turbidity and lawn-inoculated on Mueller-Hinton agar plates. Antibiotic discs were placed aseptically on the agar surface, and the plates were incubated at 37 °C for 18–24 hours. After incubation, the diameters of the inhibition zones were measured.

ESBL production was identified when there was a marked reduction in zone diameters for third-generation cephalosporins (Ceftazidime, Cefotaxime) and fourth-generation cephalosporin (Cefepime), with restoration of activity in the presence of Amoxicillin-Clavulonate (synergy effect). Isolates exhibiting this pattern were recorded as ESBL producers.

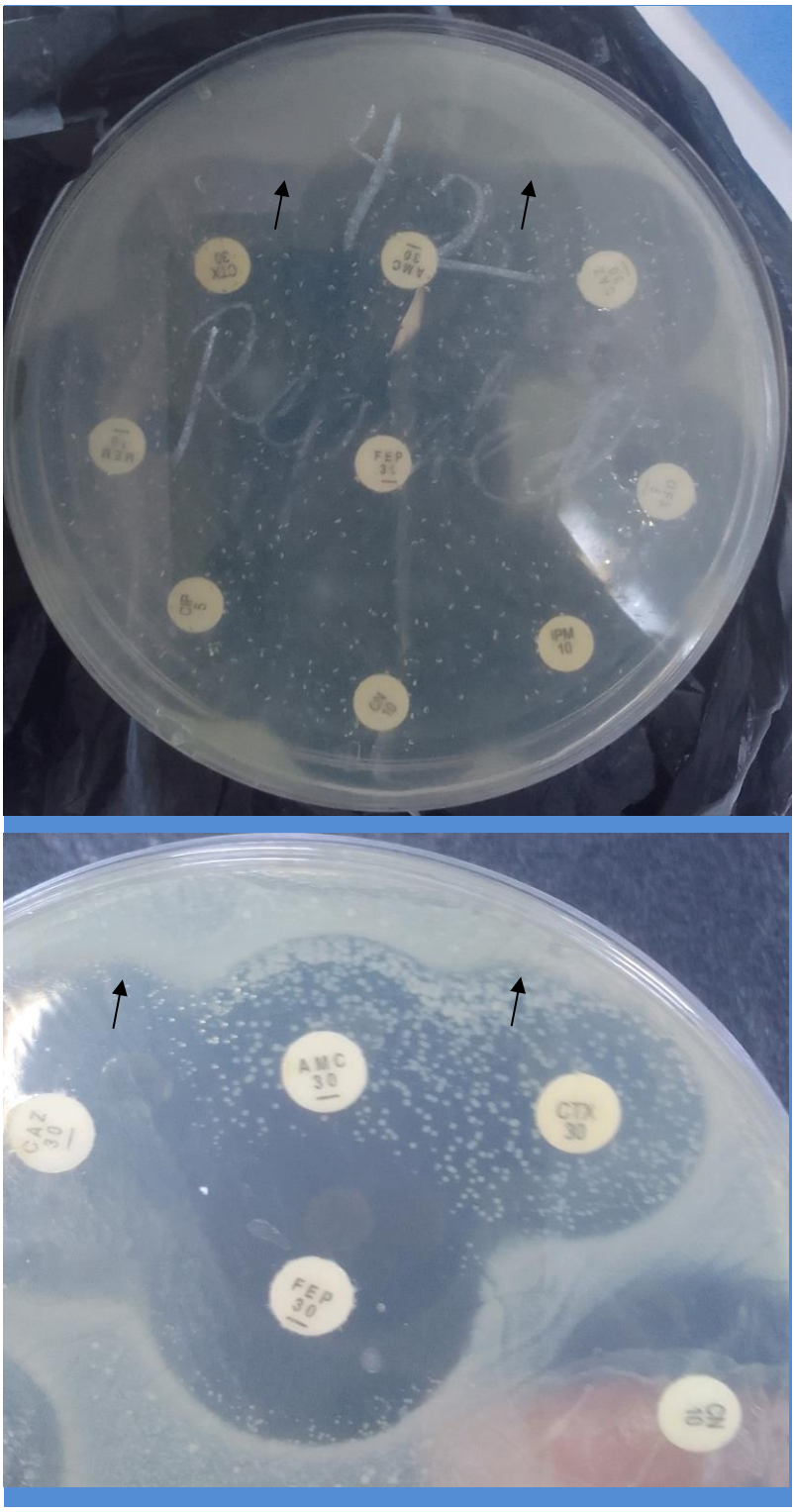

Figure 3: Arrow pointing to the Key-hole identification (Phenotypical detection) of ESBL-producing organisms.

3.8 Statistical Analysis

Data obtained were entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS, version 22). Descriptive statistics (frequency and percentage distributions) were generated. The relationship between BMI categories and antibiotic resistance patterns of *Escherichia coli* was assessed using Chi-square test at 95% confidence interval. A p-value of <0.05 was considered statistically significant.

CHAPTER FOUR

RESULT

The results obtained in this study were shown in Table 4.1 - 4.4 and Fig 4.1. Table 4.1 shows the demographic of the study participants. Obese participants were observed in Faculty of Basic Medical Science and Education.

Table 4.2, a total of 60 (85.7%) out of the 70 participants had *Escherichia coli* in their stool samples. The recovery of *Escherichia coli* from the participants was not significantly ($p = 0.696$) affected by their BMI categories (Table 4.2).

Extended Spectrum Beta-lactamases (ESBL) producing *Escherichia coli* was not detected among *Escherichia coli* strains recover from overweight and obese participants. Although, the prevalence of ESBL-producing *Escherichia coli* was higher in participants that were underweight compared to their counterpart that have normal weight (26.1% vs 11.8% respectively), the prevalence of ESBL-production did not differ significantly ($p = 0.2984$) among BMI categories of participants (Table 4.3)

Ofloxacin was the most active antibacterial agent while Cefotaxime was the most resistance antibacterial agent (Fig 4.1)

Due to the fact that only one strains of *Escherichia coli* from participant that was overweight, it was not included in the statistical analysis. Comparing the rate of resistance between participant that were underweight and those that have normal weight as well as those that were obese with those that have Normal weight, there was no significant difference ($p > 0.05$) in the rate of resistance to the various antibacterial agent and BMI categories (Table 4.4)

Table 4.1: Demography of Study Participants

	Participants tested (n = 70)	**Underweight (n = 25)**	**Normal weight (n = 41)**	**Overweight (n = 1)**	**Obese (n = 3)**
Faculty (n = 15)					
Agriculture	2	0	2	0	0
Art	4	1	3	0	0
Basic medical sciences	23	11	9	1	2
Dentistry	1	0	1	0	0
Education	5	1	3	0	1
Engineering	4	2	2	0	0
Life sciences	4	2	2	0	0
Management sciences	3	1	2	0	0
Medicine	16	5	11	0	0
Optometry	1	0	1	0	0
Pharmacy	1	0	1	0	0

Physical sciences	2	0	2	0	0
Science laboratory technology	2	2	0	0	0
Social science	1	0	1	0	0
Veterinary medicine	1	0	1	0	0
Gender					
Male	58	21	34	1	2
Female	12	4	7	0	1
Residence					
On campus	29	10	17	0	2
Off campus	41	15	24	1	1

Table 4.2: Distribution of *Escherichia Coli* among Body Mass Index (BMI) Categories of Study Participants

Body Mass Index (BMI) categories	**Number of participants tested**	**Number of *Escherichia coli* isolated**
Underweight	27	23 (85.19%)
Normal weight	39	34 (87.2%)
Overweight	01	01 (100%)
Obese	03	02 (66.67%)
Total	70	60 (85.71%)

p = 0.6896

Table 4.3: Prevalence Of ESBL-producing *Escherichia coli* among Body Mass Index (BMI) Categories of Study Participants

Body Mass Index (BMI) categories	**Number of *Escherichia coli* isolated**	**Number of positive ESBL-producing *Escherichia coli.***
Underweight	23	6 (26.09%)
Normal weight	34	4 (11.76%)
Overweight	01	0 (Ø*)
Obese	02	0 (Ø*)
Total	60	10 (16.67%)

p = 0.2984 **Ø* = Not included in the analysis.**

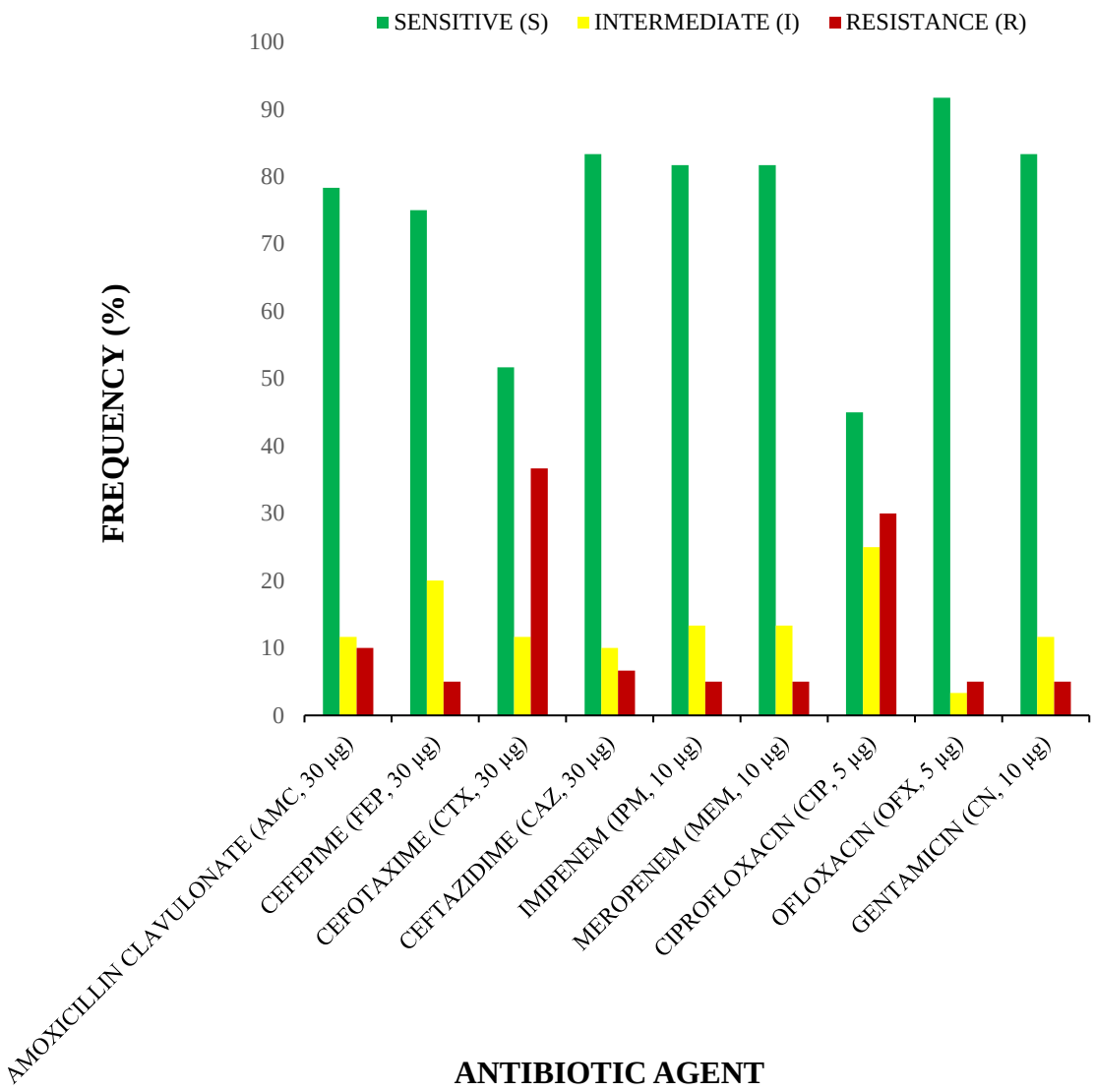

Figure 4: Susceptibility Profile of *Escherichia coli*.

Table 4.4: Relationship between Antibiotic Resistance and Body Mass Index (BMI) Categories.

Antibiotic disks	Underweight	Normal weight	p – value
Amoxicillin Clavulonate (AMC, 30 µg)			
Resistance (R)	2	3	**0.9866**
Sensitive (S) + Intermediate (I)	21	31	
Cefepime (FEP, 30 µg)			
Resistance (R)	2	0	**0.3092**
Sensitive (S) + Intermediate (I)	21	34	
Cefotaxime (CTX, 30 µg)			
Resistance (R)	12	8	**0.0523**
Sensitive (S) + Intermediate (I)	11	26	
Ceftazidime (CAZ, 30 µg)			
Resistance (R)	3	1	**0.3491**
Sensitive (S) + Intermediate (I)	20	33	
Imipenem (IPM, 10 µg)			
Resistance (R)	2	1	**0.7263**

Sensitive (S) + Intermediate (I)	21	33	
Meropenem (MEM, 10 µg)			
Resistance (R)	3	0	**0.1190**
Sensitive (S) + Intermediate (I)	20	34	
Ciprofloxacin (CIP, 5 µg)			
Resistance (R)	9	6	**0.1335**
Sensitive (S) + Intermediate (I)	14	28	
Ofloxacin (OFX, 5 µg)			
Resistance (R)	3	0	**0.1190**
Sensitive (S) + Intermediate (I)	20	34	
Gentamicin (CN, 10 µg)			
Resistance (R)	3	0	**0.1190**
Sensitive (S) + Intermediate (I)	20	34	
	Obese	**Normal weight**	**p – value**
Amoxicillin Clavulonate (AMC, 30 µg)			
Resistance (R)	1	3	**0.2127**
Sensitive (S) + Intermediate (I)	1	31	

Cefotaxime (CTX, 30 µg)			
Resistance (R)	0	8	**1.0000**
Sensitive (S) + Intermediate (I)	2	26	
Ceftazidime (CAZ, 30 µg)			
Resistance (R)	0	1	**1.0000**
Sensitive (S) + Intermediate (I)	2	33	
Ciprofloxacin (CIP, 30 µg)			
Resistance (R)	1	6	**0.3556**
Sensitive (S) + Intermediate (I)	1	28	

CHAPTER FIVE

Discussion and Conclusion

5.1 Discussion

This study investigated the relationship between Body Mass Index (BMI) and antibiotic resistance in commensal *Escherichia coli* isolated from stool samples of apparently healthy students at the University of Benin (UNIBEN). The results provide insight into the prevalence of commensal *Escherichia coli*, the occurrence of Extended Spectrum β-Lactamase (ESBL)-producing strains, and the association between BMI and antibiotic resistance patterns within a young, community-based population.

Out of 70 participants, *Escherichia coli* was isolated from 60 stool samples (85.7%), indicating high colonization rates across all BMI categories. Colonization was observed in 100% of overweight participants, 87.2% of normal-weight participants, 85.2% of underweight participants, and 66.7% of obese participants. These differences were not statistically significant ($p = 0.6896$). This aligns with previous findings showing that *Escherichia coli* is a dominant member of the human gut microbiota irrespective of host nutritional status (Tawfick *et al.,* 2022; Ju *et al.,* 2023; Fri *et al.,* 2024). The lack of a significant association suggests that BMI alone may not determine colonization, as commensal *Escherichia coli* is maintained in diverse gut environments. Similar patterns of widespread colonization in apparently healthy Nigerian populations have been reported (Mazumder *et al.,* 2023; Onukansi *et al.,* 2025).

Among the 60 isolates, 10 (16.7%) were ESBL-producers. The prevalence was highest among underweight participants (26.1%) compared to normal-weight participants (11.8%), while no ESBL-producing isolates were detected in overweight or obese participants. Although the trend suggested increased carriage among underweight individuals, the differences were not statistically significant ($p = 0.2984$). This observation is consistent with reports of community carriage of ESBL-producing *Escherichia coli* in Africa and globally (Nji *et al.,* 2021; Rana *et al.,* 2024). In Nigeria, prior studies have documented ESBL carriage among healthy individuals, highlighting the silent circulation of resistance determinants within the community (Aibinu *et al.,* 2003; Alabi *et al.,* 2025). The relatively higher proportion among underweight participants may be associated with altered gut microbial composition, compromised immunity, or increased susceptibility to colonization by resistant strains (Aranaz *et al.,* 2021; Takeuchi *et al.,* 2023; Enache *et al.,* 2024). However, the absence of statistical significance emphasizes the need for studies with larger sample sizes to clarify these associations.

Antibiotic susceptibility testing revealed that Ofloxacin was the most effective agent, whereas Cefotaxime recorded the highest resistance rate. These findings mirror global trends where fluoroquinolones retain activity against community *Escherichia coli* isolates, whereas resistance to third-generation cephalosporins is widespread (Tawfick *et al.,* 2022; Mazumder *et al.,* 2023; Rana *et al.,* 2024). In Nigeria, high cephalosporin resistance has been linked to over-the-counter availability and misuse of antibiotics (Popoola *et al.,* 2024; Alabi *et al.,* 2025; Onukansi *et al.,* 2025). The retained efficacy of carbapenems (Imipenem and Meropenem) suggests their continued value as last-resort drugs; however, the presence of

resistant strains even in healthy carriers underscores the importance of strict antibiotic stewardship.

No statistically significant associations were observed between BMI and resistance to specific antibiotics (all $p > 0.05$), although a borderline trend was noted for Cefotaxime ($p = 0.0523$). This contrasts with large-scale metagenomic studies that have reported differences in gut resistome composition according to BMI, with obesity often associated with enriched resistance genes (Sankararaman *et al.,* 2023; Takeuchi *et al.,* 2023; Fri *et al.,* 2024). The discrepancy in the present study may be due to the limited sample size in overweight and obese categories, as well as population-specific factors such as dietary patterns and antibiotic exposure (Companys *et al.,* 2021; Enache *et al.,* 2024).

The detection of resistant commensal *Escherichia coli* in apparently healthy students represents a significant public health concern. These bacteria can act as reservoirs of transferable resistance genes, posing risks of dissemination to pathogenic strains and the broader community (Tawfick *et al.,* 2022; Mazumder *et al.,* 2023; Wollein Waldetoft *et al.,* 2023). The findings suggest that environmental exposure and patterns of antibiotic use, rather than BMI alone, may be stronger determinants of resistance carriage in this population (Popoola *et al.,* 2024; Alabi *et al.,* 2025; Onukansi *et al.,* 2025).

5.2 Limitations of the Study

The small sample size, particularly, in the overweight and obese categories, reduced the statistical power to detect significant associations between BMI and antibiotic resistance. Additionally, the study was done in one institution (UNIBEN), which may limit the generalizability of the findings to other populations. This research work was limited to the

use of only cultural identification, motility and Gram biochemical reactions to presumptive identification of the *Escherichia coli organisms* and phenotypic method was used to identify ESBL production via antibiotic resistance, while molecular techniques that could have provided more detailed insights into the specific resistance genes were not employed. Furthermore, BMI, although widely used, is a relatively crude measure of nutritional status and does not fully capture differences in body composition or fat distribution that may influence gut microbiota.

5.3 Conclusion

This study demonstrated that *Escherichia coli* was highly prevalent among apparently healthy students at the University of Benin, with a detection rate of 85.7%. The distribution of *Escherichia coli* colonization across different BMI categories showed no significant variation, indicating that BMI did not strongly influence carriage in this population. Extended Spectrum β-Lactamase (ESBL)-producing *Escherichia coli* were identified in 16.7% of isolates, with the highest prevalence observed among underweight participants, although this difference was not statistically significant. Antibiotic susceptibility testing revealed that Ofloxacin was the most effective drug, while Cefotaxime exhibited the highest resistance rates among isolates. Overall, no significant relationship was found between BMI and resistance to those specific antibiotics tested, although a borderline relationship was noted with Cefotaxime. These findings suggest that BMI is not a major determinant of antibiotic resistance in commensal *Escherichia coli*, but the observed resistance patterns highlight the growing public health concern of antibiotic resistance in community settings.

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APPENDIX I

Media
The media used includes commercially dehydrated products and laboratory prepared media.

MacConkey Agar (CM7, Oxiod, England)

Constituents

Peptone	20.0grams
Lactose	10.0grams
Neutral red	0.075 grams
Bile salt	5.0grams
Sodium chloride	5.0grams
Agar	5.0grams
Distilled water	1000ml

pH 7.4±0.2 at 25°C

Preparation

- 55grams of MacConkey agar powder was weighed.
- It was suspended aseptically in 1 liter of sterile distilled water and was allowed to dissolve for 10 minutes.
- It was sterilized by autoclaving at 121°C for 15 minutes.
- The agar was cooled at 50°C, mixed and then poured aseptically into the petri dish.
- It was allowed to set and stored at 4°C.

Mueller Hinton Agar (LabMal, Academy)

Constituents

Casein hydrolysate	17.5grams

Beef infusion	2.0grams
Starch	1.5grams
Agar	17.0grams
Distilled water	1000ml

pH 7.3±0.1 at 25°C

Preparation

- 76grams of Mueller Hinton agar powder was weighed and suspended aseptically in 2 litres of sterile distilled water and was allowed to dissolve for 10 minutes.
- It was sterilized by autoclaving at 121°C for 15 minutes.
- The agar was cooled at 50°C, mixed and then poured aseptically into the petri dish.
- It was allowed to set and stored at 4°C

Citrate Koser Medium (Himedia (M069) Laboratories, India)

Constituents

Sodium ammonium phosphate	1.5grams
Potassium dihydrogen phosphate	1.0grams
Magnesium sulphate	0.2grams
Sodium citrate	3.0grams
Bromothymol blue	0.016
Distilled water	1000ml

pH 6.7±0.2 at 25°C

Preparation

- 1.71 grams of sodium citrate powder was weighed and suspended aseptically to 300ml of sterile distilled water.

- This was allowed to dissolve for 10 minutes and then mixed.
- Equal volume of the broth was dispensed into sterile bijou bottles.
- It was then sterilized by autoclaving at 121°C for 15 minutes.
- It was allowed to cool and then stored at room temperature.

Urea Agar Base (CM53, Oxford, England)

Constituents

Peptone	1.0grams
Glucose	1.0grams
Sodium chloride	5.0grams
Disodium phosphate	1.2grams
Potassium dihydrogen phosphate	0.8grams
Phenol red	0.02grams
Agar	15.0grams
Distilled water	95ml

pH 6.8±0.2 at 25°C

Preparation

- 7.58grams of urea agar base powder was suspended in 300ml of sterile distilled water.
- It was heated to boil to dissolve completely.
- It was sterilized by autoclaving at 121°C for 15 minutes.
- It was cooled to 50°C, 16ml of sterile 40%, urea solution was added aseptically and mixed.
- It was then dispensed into sterile bijou bottles and was allowed to set in a slanted position.

- It was then stored at 4°C for 2 weeks

Peptone Water (CM9, Oxiod, England)

Constituents

Peptone	10grams
Sodium chloride	5.0grams
Distilled water	100ml

pH 7.2±0.2 at 25°C

Preparation

- 4.5grams of peptone powder was weighed and added aseptically to 300ml of sterile distilled water.
- It was mixed and dispensed into sterile bijou bottles.
- It was sterilized by autoclaving at 121°C for 15 minutes.
- It was allowed to cool and stored at room temperature.

Chemical Reagent

All chemicals used in this study were of analytical grade and they include;

Normal saline

Constituents

Sodium chloride	0.85grams
Distilled water	100ml

Kovac's reagent

Constituents

p-dimethylaminobenzaldehyde	5grams
Alcohol	75ml
Hydrochloric acid (concentrated)	2.5ml

APPENDIX II

Materials

Bijou bottles

Cotton wool

Coverslips

Forceps

Glass slides

Grease pencil

Latex gloves

Petri-dishes

Straight wire

Wire loop

Equipment

Autoclave

Bunsen-burner

Hot air oven

Incubator

Refrigerator