ANTIBIOGRAM OF ANAEROBIC BACTERIAL ISOLATES FROM PATIENTS WITH EAR INFECTIONS

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DOCTOR OF PHARMACY (PHARM.D) DEGREE

APRIL, 2024

CERTIFICATION

This is to certify that this work was carried out by ZETHAR OFUFEKE MOMOH in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin city, Nigeria.

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Certification Of Thesis On Plagiarism

We the undersigned attest and declare that the thesis of Zethar Ofufeke Momoh

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DEDICATION

To my beloved parents, Prof. Musa and Mrs. Bola Momoh, I express my deepest appreciation for enabling and supporting me throughout this significant journey. Your encouragement and provision of resources have been invaluable. I also dedicate this research project to God Almighty, whose unwavering guidance has been my constant companion on this path.

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ABSTRACT

Background: Ear infections, particularly acute otitis media, are commonly encountered in clinical practice, often requiring antibiotic therapy. While aerobic bacteria have been extensively studied in these infections, anaerobic bacteria play a significant role that is sometimes overlooked. This study aimed to identify anaerobic bacterial isolates from patients with ear infections and determine their susceptibility profile to commonly used antibiotics.

Methods: The study evaluated forty three patients from the Ear, Nose and Throat clinic of the University of Benin Teaching Hospital, Benin city over a period of two months. Clinical and demographic data of the patients were also collected which included age, gender, alcohol history, smoking history, medical history, among others. Ear swab specimens were collected and processed for anaerobic culture. Isolates were identified using standard microbiological techniques, and antimicrobial susceptibility testing was performed using the standard agar disc diffusion and minimum inhibitory concentration method.

Results: A total of 76.47% of our study participants were female, and 23.53% were male. Participants aged 16-25 had the highest ear infection occurrence (38.24%). *Pseudomonas spp*, a facultative anaerobe, dominated the ear infections (47.06%) compared to other organisms isolated, which was more sensitive to Azithromycin(87.5%).

Conclusion:.Patients aged 16-25 were more susceptible to ear infections. This study highlights the presence of anaerobic bacteria in ear infections and their varying susceptibility profiles to commonly used antibiotics. Understanding the antimicrobial susceptibility patterns of anaerobic isolates is crucial for guiding appropriate antibiotic therapy, especially in cases of treatment failure or recurrent infections.

Keywords: Ear infections, Demographics, Anaerobic bacteria, Antimicrobial susceptibility.

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the study

The ear serves a dual function, acting as a sensory organ for hearing and as a key component of the vestibular system for balance. It is a sensitive organ of the human body and is mainly concerned with detecting, transmitting and transducing sound. Maintaining a sense of balance is another important function performed by the human ear as earlier stated. Ear infections, particularly otitis media, are common ailments affecting individuals across all age groups, with children and infants being particularly susceptible. These infections often stem from bacterial origins, playing a significant role in their development and persistence. Traditionally, medical focus has centred on aerobic bacteria in the study of ear infections. However, recent studies have illuminated the substantial impact of anaerobic bacteria in these cases. Anaerobic bacteria, thriving in the oxygen-deprived environment of the ear canal, find an optimal setting for colonisation and growth. Once established, these microorganisms can lead to persistent and recurrent ear infections (Wong et al., 2019). Furthermore, anaerobic bacteria possess specific virulence factors that enable them to adhere to and invade the mucosal lining of the ear, exacerbating inflammation and worsening the associated symptoms. Despite their significant contribution to ear infections, anaerobic bacteria often receive less attention in clinical practice. This oversight can be attributed to the limitations of diagnostic techniques that predominantly detect aerobic bacteria, as well as a lack of awareness among healthcare providers regarding the prevalence and significance of anaerobic infections in ear-related conditions (Ferguson et al., 2018). Understanding the susceptibility patterns of anaerobic bacteria in patients with ear

infections is essential for effective management and treatment of these conditions. However, research specifically focusing on the antimicrobial susceptibility profiles of anaerobic bacteria isolated from ear infection patients remains limited. Furthermore, the emergence of antibiotic resistance presents additional challenges in the treatment of anaerobic bacterial infections, underscoring the need for a thorough understanding of their susceptibility profiles (Jones and Smith, 2020). This study is aimed to bridge these gaps in the field of ear infection microbiology. By exploring the susceptibility of anaerobic bacteria, the study is aimed to provide insights into evidence-based approaches to antibiotic therapy for individuals affected by ear infections. Through this research, healthcare providers can tailor treatment strategies more effectively to manage ear infections and ultimately improve patient outcomes.

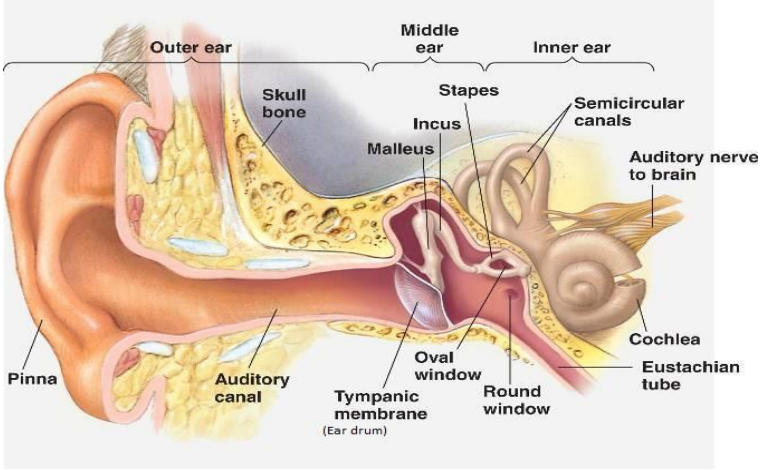


Fig 1.1 A well labelled pictorial presentation of the human ear

As illustrated above, the human ear consists of three parts: external ear, middle ear and internal ear. The outer or external ear includes the visible part of the ear, known as the pinna or auricle, and the ear canal (external auditory canal). The ear canal is lined with skin and small hairs that help trap dirt and debris. It ends at the eardrum (tympanic membrane), a thin, sensitive

membrane that separates the outer ear from the middle ear. The middle ear is an air-filled space located behind the eardrum. It contains three small bones called ossicles: the malleus (hammer), incus (anvil), and stapes (stirrup). These bones amplify sound vibrations and transmit them to the inner ear. The inner ear is a complex structure that contains the cochlea, responsible for hearing, and the vestibular system, responsible for balance. The cochlea is a spiral-shaped organ filled with fluid and tiny hair cells. When sound vibrations reach the cochlea, these hair cells convert them into electrical signals that are sent to the brain via the auditory nerve. The vestibular system consists of three semicircular canals that detect changes in head position and movement, helping us maintain balance.

Ear infections can be either bacterial or viral infections. They can occur in your middle ear which is the part of the ear just behind the eardrum, as well as the outer and inner ear. They often clear up on their own but can be painful due to inflammation or fluid buildup. They can be chronic or acute, acute ear infections are painful but short in duration. Chronic ear infections either don't clear up or recur many times. They can cause damage to the middle and inner ear, which is infrequently permanent. Therefore, ear infections can also be classified as middle, inner and outer ear infections. Otitis externa or outer ear infections affect the outer ear canal and are often referred to as swimmer's ear. They are commonly caused by water exposure, which creates a moist environment ideal for bacterial or fungal growth. Middle ear infections occur when the middle ear becomes infected and inflamed. This can happen due to a viral or bacterial infection, often following a cold or respiratory infection and may cause fluid buildup behind the eardrum. Inner ear infections are less common but can be more serious. Among the diverse array of microorganisms capable of causing these infections, certain bacteria stand out for their notable roles in ear pathology. Gram-positive cocci, a group ubiquitous on the skin and mucous membranes, is a prominent culprit in acute otitis media. Its pathogenicity lies in its ability to colonise the middle ear cavity, often leading to the accumulation of pus and severe inflammation, which exerts pressure on the eardrum resulting in intense ear pain, fever, and sometimes even hearing loss. In cases of otitis externa, commonly referred to as swimmer's ear, Gram-positive cocci find an ideal breeding ground in the moist, warm environment of the external ear canal, inciting irritation, itching, and inflammation often manifesting as redness, swelling, and discharge. The bacterium's ability to adhere to damaged skin surfaces exacerbates the

inflammatory response, contributing to the characteristic discomfort associated with this condition. Another significant group, gram-negative bacilli, notorious for their role in respiratory infections, is also a significant contributor to otitis media. In the middle ear, gram-negative bacilli can cause inflammatory responses, leading to the formation of middle ear effusions and subsequent symptoms of pain and pressure. Individuals with chronic otitis media, especially those with compromised immune systems, may find gram-negative bacilli as a persistent foe, causing recurrent infections and exacerbating the condition over time. A third group, gram-negative bacteria ubiquitous in water and soil environments, thrives in the damp confines of the external ear canal. In the context of otitis externa, particularly in individuals exposed to water activities, these gram-negative bacteria are common instigators. Their ability to produce biofilms and adhere to the epithelial lining of the ear canal results in the classic symptoms of swimmer's ear: itching, redness, swelling, and purulent discharge, with severe cases progressing to cellulitis causing significant pain and discomfort. While not primary pathogens in ear infections, gram-positive, spore-forming bacteria can opportunistically exploit disrupted ear microbiota. In instances of compromised immunity or altered ear flora, these gram-positive bacteria may seize the opportunity to cause infections, with their ability to produce enzymes and antibiotics further complicating the infection process and leading to persistent symptoms of ear pain, inflammation, and discharge. The mechanisms of pathogenicity of these organisms may be as a result of their ability to adhere to and colonise the delicate tissues of the ear. Gram-positive cocci employ adhesins and surface proteins to bind to host cells, initiating the infection cascade. Similarly, gram-negative bacilli utilise fimbriae and pili to establish a foothold in the ear canal, evading host immune defences. Upon colonisation, these bacteria trigger robust inflammatory responses, characterised by the release of cytokines and chemokines, leading to tissue damage, swelling, and the accumulation of inflammatory exudates, contributing to the hallmark symptoms of ear infections. Similarly, some strains of gram-positive bacteria and gram-negative bacteria are capable of producing toxins that directly damage host tissues, further exacerbating inflammation, compromising the integrity of the ear canal, and prolonging the course of infection. Ear infections can also be caused by a blocked Eustachian tube which can occur as a result of allergies, colds, sinus infections, excess mucus, smoking and change in pressure, or develop from infected adenoids, glands on the roof of the mouth behind the nose that help protect the body from infections, with infections spreading from these glands to the nearby ends of the tubes.

Common Symptoms generally associated with ear infections include ear pain, itching, redness, swelling of the ear canal, fever, hearing loss, feeling of fullness or pressure in the ear, severe dizziness (vertigo), nausea, vomiting, hearing loss, and ringing in the ears (tinnitus). These symptoms may occur in one or both ears and pain is usually more severe with a double ear infection, which is an infection in both ears. Along with symptoms seen in adults, such as ear pain and drainage, young children and babies may show other signs of an ear infection; rubbing or pulling their ear, not reacting to certain sounds, frequently losing balance, fussiness or restlessness and loss of appetite.

1.2 The Pathophysiology of Ear Infections:

Ear infections, encompassing conditions such as otitis externa, otitis media, and rarer inner ear infections, are intricate processes influenced by a myriad of factors. Delving into the pathophysiology of these infections unveils a cascade of events that contribute to their development and progression, each stage marked by specific mechanisms and clinical manifestations.

1.2.1 Outer Ear Infections (Otitis Externa):

The journey of otitis externa, commonly known as swimmer's ear, often begins with an initial insult to the delicate skin lining the external ear canal. This could be due to excessive moisture from swimming or bathing, trauma from scratching, or the introduction of foreign objects. In this vulnerable environment, bacteria find an ideal breeding ground. These bacteria thrive, adhering to the damaged skin epithelium. This initial microbial invasion triggers a localised inflammatory response within the ear canal, with immune cells such as neutrophils and macrophages recruited to the site of infection. This inflammatory cascade manifests as redness, swelling, itching, and pain in the external ear. As the infection progresses, the ear canal may fill with purulent discharge, indicative of a more severe inflammatory response.

1.2.2 Middle Ear Infections (Otitis Media):

Otitis media, a common affliction, often begins with dysfunction of the Eustachian tube, the narrow passage connecting the middle ear to the back of the throat. This dysfunction can occur due to various factors, including allergies, respiratory infections, or anatomical abnormalities.

When the Eustachian tube fails to equalise pressure in the middle ear, it creates an environment conducive to bacterial growth. Here, these pathogens seize the opportunity to induce an inflammatory response within the middle ear, characterised by increased vascular permeability and the accumulation of fluid (effusion). Patients often experience symptoms such as ear pain, pressure, and sometimes hearing loss. In severe cases, the pressure from fluid buildup can cause the eardrum to bulge and eventually rupture, leading to the discharge of pus and temporary relief of pain.

1.2.3 Inner Ear Infections:

While less common, infections of the inner ear, such as labyrinthitis or vestibular neuritis, can have significant consequences on balance and hearing. These infections are often viral in nature, with viruses such as herpes simplex virus (HSV) or varicella-zoster virus (VZV) implicated. The viral invasion triggers an inflammatory response within the labyrinth of the inner ear, leading to damage of the delicate hair cells responsible for balance and hearing. Patients may present with severe vertigo, nausea, vomiting, hearing loss, and ringing in the ears (tinnitus). The inflammatory damage to the inner ear structures can result in prolonged symptoms and complications. Ear infections often involve a complex interplay of multiple bacterial species, sometimes alongside viral pathogens. In these polymicrobial infections, bacteria may interact synergistically, enhancing each other's virulence and inflammatory responses. This can lead to more severe symptoms and complications. Conversely, some bacteria may compete for resources within the ear canal, altering the dynamics of the infection and affecting its progression. The balance of these interactions can influence the severity and duration of the infection, as well as the effectiveness of treatment strategies. In cases where ear infections persist or recur frequently, they can lead to chronic inflammation and structural damage to the ear. Prolonged middle ear infections, particularly in children, may result in conductive hearing loss due to the buildup of fluid and damage to the middle ear bones (ossicles). Untreated infections can progress to mastoiditis, an infection of the mastoid bone behind the ear. This severe condition can cause intense pain, swelling, and potentially life-threatening complications if left unaddressed.

The pathophysiology of ear infections reveals a multifaceted journey marked by microbial invasion, inflammatory responses, and potential complications. From the initial damage to the

delicate ear structures, to the intricate interplay of microbial invaders and the host immune response, each stage unfolds with its unique mechanisms and clinical implications. Understanding these processes is paramount for accurate diagnosis, effective treatment strategies, and the prevention of complications associated with these common yet potentially debilitating conditions that affect individuals of all ages.

1.3 Epidemiology, Risk factors and complications of ear infections

Ear infections, particularly otitis media, represent one of the most prevalent medical conditions affecting individuals of all age groups, with children and infants being especially vulnerable. According to epidemiological data, approximately 80% of children experience an acute ear infection at some point in their childhood (Smith et al., 2017). The incidence of ear infections tends to be higher among infants who are bottle-fed compared to those who are breastfed, highlighting the role of feeding practices in susceptibility to these infections (Brown et al., 2019), this difference may be attributed to the position of the bottle during feeding, which can contribute to the improper drainage of fluids from the Eustachian tubes. Various factors contribute to the epidemiology of ear infections. The anatomy of the Eustachian tubes in young children, characterised by their short and narrow structure, makes it easier for pathogens to enter and infect the middle ear. This anatomical feature, combined with an immature immune system, renders young children more prone to ear infections (Greenwood et al., 2020). Furthermore, the use of pacifiers, particularly when shared or improperly cleaned can harbour bacteria, and has been associated with a higher risk of ear infections in infants (Clark et al., 2016). Moreover, children who have recently had a cold or flu may be more vulnerable to secondary bacterial infections in the ears. Additional risk factors include being male, low birth weight, and lack of access to healthcare. Male children tend to have a slightly higher incidence of ear infections, possibly due to differences in Eustachian tube anatomy or immune responses. Low birth weight infants may have underdeveloped immune systems, making them more susceptible to infections. Furthermore, children in daycare settings, where exposure to viruses and bacteria is common, have an increased risk of ear infections.

Various environmental factors also come into play in the development of ear infections. Changes in altitude, temperature, and humidity can affect the pressure in the middle ear, potentially leading to fluid buildup and infection. Exposure to cigarette smoke, whether through direct smoking or passive exposure, is another significant risk factor in both children and adults (Johnson and Patel, 2018). The harmful chemicals in smoke can irritate the delicate lining of the Eustachian tubes, making them more susceptible to infection. Additionally, exposure to cigarette smoke, whether through active smoking or secondhand smoke, has been strongly linked to an increased incidence of ear infections in both children and adults (Johnson and Patel, 2018). The chemicals present in cigarette smoke can irritate the delicate lining of the Eustachian tubes, leading to inflammation and increased susceptibility to infections. It is also important to consider the impact of recent illnesses or previous ear infections on the epidemiology of new infections. Individuals who have recently recovered from a cold or flu are more susceptible to secondary bacterial infections, including ear infections (Taylor et al., 2019). Similarly, individuals with a history of chronic conditions such as allergies or sinusitis may experience recurrent ear infections due to underlying inflammation and compromised immune responses. Moreover, demographic factors such as gender, socioeconomic status, and access to healthcare can influence the epidemiology of ear infections. Males tend to have a slightly higher incidence of ear infections compared to females, although the reasons for this difference are not fully understood (Harris et al., 2015). Socioeconomic factors, including crowded living conditions and limited access to medical care, can contribute to a higher prevalence of ear infections in certain populations (Wilson et al., 2018).

While most ear infections resolve without complications, there are potential risks associated with untreated or recurrent infections. One of the primary concerns is hearing loss, which can occur if the infection causes damage to the delicate structures of the inner ear. In young children, especially those with chronic fluid in the middle ear, untreated ear infections can lead to speech or language delays.

In more severe cases, complications such as mastoiditis and meningitis may arise. Mastoiditis is an infection of the mastoid bone located behind the ear, which can cause intense pain, swelling, and require hospitalisation for treatment. Meningitis, a bacterial infection of the membranes

covering the brain and spinal cord, is a rare but serious complication of untreated ear infections. Symptoms of meningitis include fever, severe headache, stiff neck, and confusion, requiring immediate medical attention. In some instances, an ear infection can lead to a ruptured eardrum. This occurs when the pressure from fluid buildup becomes too great, causing the eardrum to tear. While this may sound alarming, a ruptured eardrum often heals on its own without intervention. However, it can be painful and may require medical management to prevent infection and promote healing.

1.4 Management of ear infections

One of the most commonly recommended methods for relieving ear pain is the application of a warm compress. By applying a warm cloth or heating pad to the affected ear, individuals can experience soothing relief. The gentle heat helps to reduce pain and inflammation, easing the discomfort associated with the infection. This simple yet effective remedy can be applied for about 15-20 minutes several times a day. In addition to warm compresses, over-the-counter (OTC) pain medications such as ibuprofen, or acetaminophen (Tylenol) can provide significant relief. These medications help to reduce inflammation and alleviate discomfort, making the healing process more comfortable. It is important to follow the recommended dosage instructions and consult a healthcare provider, especially when giving medication to children. For targeted relief, ear drops are another popular option for managing ear infections. These drops, available over the counter or as prescription formulations, often contain ingredients such as benzocaine or hydrocortisone. These substances help to numb the ear and reduce inflammation, providing quick and effective relief from pain and discomfort. Ear drops can be administered according to the instructions provided on the packaging or as directed by a healthcare professional. When congestion and pressure in the Eustachian tubes contribute to ear pain, decongestants can be beneficial. OTC decongestants like pseudoephedrine (Sudafed) help to reduce swelling and promote drainage, alleviating discomfort and improving symptoms. However, it is important to use decongestants cautiously, especially in individuals with certain medical conditions or when taking other medications. Simple lifestyle adjustments can also aid in the management of ear infections. Avoiding sleeping on the affected ear can help to prevent exacerbation of pain and discomfort. Instead, individuals can opt to sleep on the opposite side or use a soft pillow to

support the head. This simple measure can alleviate pressure on the ear and promote faster healing. Another important aspect of managing ear infections is keeping the affected ear dry. Moisture in the ear can prolong the infection and increase discomfort. Therefore, it is essential to take precautions such as using ear plugs or a cotton ball coated with petroleum jelly to prevent water from entering the ear canal, especially during bath time or swimming.

In addition to these relief measures, adequate hydration and rest are crucial for the body to fight off infections effectively. Drinking plenty of fluids helps to thin mucus and promote drainage, while rest allows the body to focus its energy on healing. By taking proactive steps and following these management strategies, individuals can effectively alleviate the symptoms of ear infections and support the body's natural healing process.

However, in cases where symptoms persist or worsen, it is advisable to seek medical attention. A healthcare provider can assess the severity of the infection, prescribe appropriate medications such as antibiotics if necessary, and provide further guidance on managing symptoms. This is particularly important for children, elderly individuals, or those with underlying health conditions.

1.5 Anaerobic Bacteria Ear Infections;

The broad classification of bacteria as anaerobic, aerobic, or facultative is based on the types of reactions they employ to generate energy for growth and other activities. In their metabolism of energy-containing compounds, aerobes require molecular oxygen as a terminal electron acceptor and cannot grow in its absence. Anaerobes, on the other hand, cannot grow in the presence of oxygen. Oxygen is toxic for them, and they must therefore depend on other substances as electron acceptors. Their metabolism frequently is a fermentative type in which they reduce available organic compounds to various end products such as organic acids and alcohols. Facultative organisms, known for their versatility, include a range of bacteria such as facultative gram-positive cocci, facultative gram-positive rods, and facultative gram-negative rods. They

have the unique ability to utilize oxygen as a terminal electron acceptor for metabolism, yet they can also switch to using other compounds in the absence of oxygen. These bacteria, found in various environments, can be linked to ear infections when contaminated ear drops or poor ear hygiene practices are involved. Obligate anaerobes, which strictly require an oxygen-free environment for survival, include anaerobic gram-positive cocci and anaerobic gram-negative rods. Exposure to oxygen can be lethal for these bacteria, and they are often associated with chronic or complicated cases of ear infections, such as chronic otitis media and mastoiditis. Among the bacteria commonly associated with ear infections, aerobic bacteria like aerobic gram-positive cocci are frequently implicated. These bacteria, along with aerobic gram-negative rods, can be found in chronic ear infections, often alongside other anaerobic bacteria. Ear infections caused by these groups can lead to complications such as mastoiditis and meningitis, posing significant threats to patient health. Furthermore, in ear infections, anaerobic gram-positive rods and anaerobic gram-negative rods play crucial roles. These bacteria are known for their ability to form biofilms, making infections difficult to treat and contributing to recurrent episodes of otitis media with purulent discharge. Therefore, these diverse groups of bacteria, including facultative organisms, obligate anaerobes, aerobic bacteria, and anaerobic bacteria, create a complex microbial environment in ear infections. Understanding the roles of these bacterial groups is essential for effective treatment and management of ear infections, particularly in cases of chronic or recurrent infections.

The following are the reasons why anaerobes thrive well in the environment; The middle ear environment, particularly the cavity, especially in chronic infections, can become low in oxygen due to poor ventilation and obstructed Eustachian tubes. This anaerobic environment created, favours the growth of anaerobic bacteria. Anaerobic bacteria also readily form biofilms, complex

communities of bacteria encased in a protective matrix. Biofilms shield bacteria from host defences and antibiotics, making them challenging to eradicate. Lastly, anaerobes often coexist with aerobic bacteria in ear infections. Synergistic interactions between these organisms can worsen the infection and complicate treatment.

The initial step involved in the diagnosis of anaerobic ear infections involves a thorough clinical evaluation. This includes a detailed review of the patient's medical history, focusing on previous ear infections, treatments, and any associated symptoms. Symptoms of anaerobic ear infections are the same as the classical examples stated earlier, which often include persistent ear pain, foul-smelling discharge (otorrhea), hearing loss, and fever. During the physical examination, the use of an otoscope is adopted, to inspect the ear canal, eardrum, and surrounding tissues. This examination helps identify any visible signs of inflammation, infection, or discharge. A particular attention is given to the nature of the discharge, its quantity, and any abnormalities observed in the ear structures. To confirm the presence of bacteria and identify the specific pathogens involved, laboratory tests are essential. A key component of the diagnostic process is the collection of a sample of the ear discharge (otorrhea). This sample is carefully swabbed from the ear canal and sent to the laboratory for analysis. In the laboratory, the ear discharge sample undergoes various tests to pinpoint the bacteria causing the infection. One of the primary tests is a culture and sensitivity analysis. This involves placing the sample on specialised growth media under specific conditions, including anaerobic conditions for anaerobic bacteria. The culture allows the bacteria to grow, enabling identification and assessment of their susceptibility to antibiotics. Additionally, a Gram stain of the ear discharge sample provides preliminary information about the type of bacteria present. Anaerobic bacteria typically appear as Gram-

positive rods or cocci under the microscope. This stain aids in the initial characterization of the infection and guides further testing and treatment decisions.

In cases of severe or chronic ear infections, imaging studies such as a computed tomography (CT) scan of the temporal bone may be recommended. The CT scan provides detailed images of the ear structures, helping to assess the extent of infection, identify complications such as mastoiditis or abscess formation, and guide surgical interventions if necessary. Specialized tests, such as polymerase chain reaction (PCR) testing, may also be employed to detect the DNA of specific anaerobic bacteria rapidly and accurately. This molecular technique enhances the precision of bacterial identification, aiding in targeted treatment approaches.

Once the bacteria causing the infection are identified, treatment can be tailored with antimicrobial susceptibility testing. This testing determines which antibiotics are effective against the specific pathogens. Commonly prescribed antibiotics for anaerobic ear infections include metronidazole, clindamycin, amoxicillin-clavulanate, and cefoxitin. In severe cases or when complications arise, such as the formation of abscesses, surgical intervention or ear drainage (myringotomy) may be necessary to remove infected tissue or fluid. Follow-up visits are essential for monitoring the patient's response to treatment, ensuring the resolution of symptoms, and preventing recurrence of the infection.

1.6.Antimicrobial susceptibility testing for anaerobes

Antimicrobial susceptibility testing for anaerobic bacteria is a critical component of microbiological diagnostics, aiding clinicians in determining the most effective treatment strategies for infections caused by these organisms. This testing process is often complex,

requiring specialised methods. In routine clinical practice, conducting susceptibility testing for every patient's isolate is not always feasible due to resource constraints and time considerations. However, there are specific scenarios where such testing becomes imperative. For instance, in cases of severe and life-threatening infections such as endocarditis or brain abscesses, knowing the susceptibility of the causative anaerobic bacteria is crucial for selecting the appropriate antibiotic therapy. Similarly, infections that do not respond to initial empirical treatment or those requiring prolonged antibiotic therapy, especially in cases involving bones, joints, or implanted devices, necessitate susceptibility testing to guide further management. Furthermore, the lack of available data on the susceptibilities of certain anaerobic organisms or the presence of known resistance patterns against certain antibiotics underscores the importance of susceptibility testing. Additionally, when dealing with particularly virulent anaerobes with unpredictable resistance patterns or isolates obtained from normally sterile body sites, conducting susceptibility testing becomes paramount.

In clinical microbiology, standardised testing methods established by reputable organisations such as the Clinical Laboratory Standards Institute (CLSI), the European Committee for Antimicrobial Susceptibility Testing (EUCAST), and others are followed to ensure accuracy and reliability of results. Among the various methods available, the disk diffusion method, also known as the Kirby-Bauer method, is widely used for its simplicity and cost-effectiveness. This method involves placing antibiotic-soaked paper disks on an agar plate inoculated with the bacterial isolate, allowing the antibiotics to diffuse into the agar and form zones of inhibition around the disks. The size of these zones is measured and compared to established interpretive criteria to determine susceptibility.

Another commonly used method is the broth microdilution method, conducted in 96-well microtiter plates. Here, antibiotics are serially diluted in broth medium, creating a range of concentrations for testing. The Minimum Inhibitory Concentration (MIC) is then determined as the lowest concentration of antibiotic that inhibits bacterial growth. While effective for testing certain anaerobic species, the broth microdilution method may present challenges with others due to inconsistent growth in broth.

The agar dilution method, considered the gold standard for antimicrobial susceptibility testing of anaerobic bacteria, involves incorporating antibiotics directly into nutrient agar plates. Following inoculation with standardised bacterial cells, the plates are incubated in an anaerobic environment, and the MIC is determined as the lowest inhibitory concentration.

1.7 Justification Of Statement

Anaerobic bacteria are recognized as significant pathogens in ear infections, particularly those that are chronic or persistent, contributing to prolonged and recurrent infections resistant to conventional treatments (Ahmed & Patel, 2021). Despite the limited research on anaerobic bacterial isolates in ear infections, understanding their antimicrobial susceptibility profiles is crucial for guiding treatment decisions (Baker & Evans, 2018). This knowledge empowers clinicians to administer targeted antibiotics, reducing the risks of treatment failure or recurrence (Clark & Young, 2021). Furthermore, amid the growing concern of antimicrobial resistance, especially among anaerobes, this research was aimed to provide insights for developing well-informed empirical antibiotic therapy strategies (Evans & Baker, 2018). The potential outcomes of this study could also bridge existing knowledge gaps, offering insights into new treatment avenues such as innovative antimicrobial agents or combination therapies for anaerobic ear infections (Johnson & Wilson, 2018). Ultimately, these advancements have the potential to improve patient outcomes and lessen the burden of ear infections on healthcare systems, particularly in low/middle-income countries like Nigeria (Smith & Brown, 2021).

1.8 Aim And Objective Of Study

Therefore, this study was aimed to address this gap by conducting a comprehensive analysis of anaerobic bacteria isolates and their susceptibility profiles in patients with ear infections from the Ear, Nose and Throat department of UBTH.

Specific objectives include;

- To investigate the prevalence and demographic distribution of ear infections among patients attending the Ear, Nose, and Throat Clinic of the University of Benin Teaching Hospital.
- To isolate and identify anaerobic bacterial species associated with ear infections in the study population.
- To characterise the antibiotic susceptibility profiles of the isolated anaerobic bacterial strains using agar disc diffusion and agar dilution method.

CHAPTER TWO

2.0. MATERIALS AND METHOD

2.1 Materials

2.1.1 Reagents and Chemicals

Glucose, lactose, maltose, mannitol, sucrose, galactose, Crystal violet, Lagols's Iodine and oil immersion, Methylated spirit (SPC Co. Ltd. Nigeria) Acetone, Safranin, Sodium Hydroxide pellets, Pyrogallol crystals (Loba Chem Pvt Ltd. India).

2.1.2 Culture Media

Sodium Thioglycolate, Mueller Hinton agar, Blood agar, Nutrient agar, Nutrient Broth, MacConkey Agar, Mannitol Salt Agar.

2.1.3 Equipment

Hot air oven, incubator, autoclave, compound light microscope, refrigerator (Thermocool, UK), digital weighing scale, anaerobic culture chamber (DFT Technologies, Chennai-India).

2.1.4 Glassware and other apparatus

Beakers, conical flasks, bottles (MacCartney, Universal and Bijoux), measuring cylinders. glass stirrer, glass slides, Petri dishes and Pasteur pipette (All glass wares were products of Pyrex, England), Sterile syringes, Bunsen burner, wire loop, disinfectant, cotton wool, Pooled Human plasma, sterile swab sticks, surgical gloves, surgical blades, slide (Micropoint, China), foil paper, micropipette (OEM Manufacturers) and anaerobic Chamber (Mcintosh and Filde's).

2.2 Methods

2.2.1 Study Area

This cross-sectional study was carried out in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, in association with the Department of Ear, Nose and Throat, University of Benin Teaching Hospital, a tertiary health care centre that has an ENT clinic attached to it that attends to both in-patient and out-patient.

2.2.2 Study Design, exclusion and inclusion criteria

A cross-sectional prospective study of 43 consecutive patients (extrapolated from a study by Rezai et al, 2015) and estimated to be 100 patients although study limitations led to assessment of just 43 participants), visiting the Ear, Nose and Throat clinic, at the University of Benin Teaching Hospital (UBTH), was done following ethical approval from the Institutional review boards and informed consent obtained from all participants enrolled in the study during the specified study period, spanning from January 2024 to March 2024.

The inclusion criteria for this study includes patients of all ages with ear infections, patients diagnosed with acute or chronic otitis media or otitis externa, patients presenting to the ENT clinic or healthcare facility with ear infection symptoms such as ear pain, discharge, hearing loss, or fever and their willingness to participate in the study and provide consent for sample collection and testing. The exclusion criteria on the other hand, include patients who have taken antibiotics within the past two weeks, as this may affect bacterial cultures and susceptibility testing and patients who decline to participate in the study or provide consent for sample collection and testing.

2.2.3 Ethical Considerations

Informed consent was sought and obtained from participants, while assent was obtained from patients aged 18 years and below, following informed consent from their parents, after obtaining ethical approval from the Health Research Ethics Committee of the University of Benin Teaching hospital (PROTOCOL NUMBER: ADM/E 22/A/VOL. VII/14838152180).

Participants were appropriately interviewed and informed about the purpose of the study. This included obtaining participants' demographic data and medical history, and specimen collection which was done by inserting an already labelled sterile swab stick into the ear and rotating it, to collect adequate specimens for microbiological analysis. Participants were assured that there were no risks or harms associated with participating in the study and that they had the right to withdraw from the study if they felt uncomfortable with the procedure before obtaining informed consent. The principles of voluntary participation, anonymity and confidentiality were maintained throughout the study. Participants had the right to decide whether they wanted to take part, to withdraw at any time or to refuse to provide information on unclear points. The participants' information was treated confidentially and they were not asked for their address in the questionnaire.

2.2.4 Data Collection

A semi-structured questionnaire was employed to gather various details from each study participant. This information encompassed socio-demographic aspects such as name in code for confidentiality, gender, history of smoking and alcohol usage, medication history, presence of hereditary or underlying health conditions (like diabetes, hypertension, asthma, rhino-sinusitis, GERD, etc.), Patients with ear infections were assessed for the frequency of episodes, duration of

symptoms, and any associated pain or discomfort. Details were gathered on previous treatments, including antibiotics, ear tubes, hearing aids or ear drops, and any exposure to potential risk factors such as loud noises, smoke and other environmental pollutants. Patient's details on lifestyle factors such as swimming, method of cleaning and frequency were also collected.

2.2.5 Specimen collection

Firstly, the necessary equipment in the examination room was prepared, this included sterile swab sticks, specimen containers, sterile gloves, antiseptic solution, and cotton wool. A brief period was dedicated to thoroughly explain the procedure to the patient, as it was essential to ensure their comfort and cooperation. Patients were positioned comfortably, with the affected ear facing upwards to facilitate specimen collection. With the sterile gloves already worn, a sterile swab stick was carefully inserted into the external auditory canal, and gently rotated against the walls of the canal to collect the specimen. It is important to mention that in cases of a double ear infection, separate sterile swab sticks are utilised for each ear. Also, based on the visibility of the site of infection inside the canal, the sterile swab stick was taken a bit further into the inner ear to ensure an accurate specimen collection and not mere debris. This procedure was performed by the doctors present at the study centre. After collecting the specimen, the swab stick was retrieved from the ear canal carefully, to avoid touching the surrounding skin to prevent contamination. The swab stick was immediately placed into a sterile specimen container containing five millilitre thioglycolate broth, ensuring the cap was tightly sealed to maintain sterility. The Sodium Thioglycolate broth was prepared following the manufacturer's instruction, by dissolving 4.7g of powdered Sodium Thioglycolate broth into a 200ml pre calibrated clean conical flask and a small amount of distilled water was added to dissolve the powder with the aid

of a stirring rod, before completely adding the distilled water. The conical flask was then sealed with foil paper, and put in the autoclave at a temperature of 121°C for 15 minutes to sterilise and ensure the elimination of any contaminants. Each specimen container was properly labelled with the patient's ID, including the date and time of collection for accurate tracking and analysis of the specimens. All specimens were transported to the Pharmaceutical Microbiology Laboratory within 2 hours and stored at the appropriate temperature for further microbiological studies

2.2.6 Characterization and Identification of Isolates

After incubation in sodium thioglycolate broth for 24 hours, the specimens were cultured into 10% blood agar plates, which meant 10 ml of blood in 100ml of Nutrient Agar. Using a cotton wool and disinfectant, the work bench was swabbed appropriately and the bunsen burner put on to provide a sterile environment. The 100ml nutrient agar was prepared following the manufacturer’s instructions by accurately weighing 2.8g of Nutrient Agar into a 100ml pre calibrated clean conical flask and a small amount of distilled water was added to dissolve the powder with the aid of a stirring rod, before completely adding the distilled water. The conical flask was then sealed with foil paper, and put in the autoclave at a temperature of 121°C for 15 minutes to sterilise. The 10 ml blood was carefully measured out, by cutting out the ends with a heated surgical blade with the first part poured away into a disinfectant jar. Thereafter, the 10 ml blood and 100 ml of already sterilised Nutrient agar base was mixed and 5 ml was poured into each sterile Petri dish and covered with their lids. The poured plates were allowed to sit for about 10-15 minutes and put into the autoclave at 121°C for 15 minutes to sterilise. The sterile swab stick from the container was used to collect a sample of the bacteria to be streaked. The inoculating loop was then sterilised by passing it through the flame of a Bunsen burner, it

became red-hot. This sterilisation process ensures that only the desired bacteria are transferred to the agar plate. With the loop cooled briefly by touching it to the edge of the agar away from the intended streaking area, the streaking process began, the lid of the agar plate was lifted just enough to expose a portion of the agar surface. Starting at the edge of the agar, the loop was used to streak back and forth across one section of the plate in a zigzag pattern, known as quadrant streaking. After streaking one section, the lid was closed, and the plate turned 90 degrees, the loop was then used to streak from the end of the first section into the second section, again in a zigzag pattern. This process was repeated for the remaining sections of the plate. The purpose of quadrant streaking was to dilute the bacterial sample across the plate, allowing for the growth of individual colonies with different sizes and densities. Each quadrant represented a different dilution of the original sample, aiding in the isolation of pure colonies. Once the plate had been streaked, it was closed and placed in the anaerobic jar bacteria and was left to incubate for 24-48 hours, to provide enough time for the bacterial colonies to become visible. Specimens were cultured in 10% blood agar because they help to identify bacteria through their hemolytic activity, distinguishing between alpha, beta, and gamma hemolysis patterns. Additionally, the distinct growth patterns and pigment production of different bacteria on blood agar facilitate their identification.

Furthermore, isolates were chosen for subculture into nutrient agar plates, based on colony morphologies present. Where two or more colonies on the blood agar plate appeared to be identical, only one colony was sub-cultured in the nutrient agar plate and incubated for 18-24 hours. However, where two colonies appeared to be different on the blood agar plate, both of these distinct colonies were selected for subculturing onto different nutrient agar plates. The purpose of this was to further investigate and isolate the different types of bacteria present. Once

the isolates from the incubated plates were chosen, they were suspended in a solution with a standard density of 0.5 McFarland. This standardised solution ensured a consistent inoculum size of 10^8 colony-forming units (CFU) per millilitre. To adjust the inoculum size for further testing, this suspension was then diluted 1:100, resulting in an inoculum size of approximately 10^6 CFU/ml. These blood agar plates were meticulously examined to identify bacterial colonies that may exhibit distinct characteristics such as colour, size, shape, or texture, providing clues about the types of bacteria present. Once identified, a sterile inoculating wire loop was employed to carefully pick up a single bacterial colony from the blood agar plate and ensured the isolation of a pure culture for subsequent study. The selected colony was then transferred onto a freshly prepared sterilised nutrient agar plate (prepared following the manufacturer's instructions as stated earlier), using the sterilised wire loop. This transfer was done using the streak plate method(as explained earlier) with precision to avoid contamination and to encourage the growth of individual bacterial colonies on the nutrient agar surface. After streaking, the inverted nutrient agar plates were on the workbench and incubated for 24 hours at room temperature of 37°C. This incubation period allowed the transferred bacterial colony to grow and form visible colonies on the nutrient agar and provided a clearer view of the characteristics and behaviour of the isolated bacteria.

Colonial characteristics of these bacterial colonies grown on the nutrient agar plates, were observed to be coloured, indicating pigment production, some were slimy in texture, some grew in chains, some were round and circular. Others were also observed to be smooth and shiny, some also had a dull and opaque appearance.

The biochemical identification of the different colonies observed on the nutrient agar plates, included tests like urease, oxidase, catalase, coagulase, indole and citrate tests. These tests provided valuable insights into the metabolic capabilities and physiological traits of the bacteria, aiding in their classification and differentiation. The Catalase test served as a fundamental assay to differentiate bacteria based on their ability to produce the enzyme catalase. The procedure began with a small amount of bacterial growth transferred onto a clean glass slide. A drop of 3% hydrogen peroxide (addition of 4.5 mL distilled water to 0.5 mL concentrated 30% hydrogen peroxide resulted in a 3% solution). was then added to the bacterial sample. For the bacteria species that possessed the enzyme catalase, it was seen to catalyse the breakdown of hydrogen peroxide into water and oxygen. The release of bubbles (oxygen) upon addition of hydrogen peroxide indicated a positive catalase reaction. This reaction is particularly useful in distinguishing between catalase-positive organisms such as *Staphylococcus* species and catalase-negative organisms like *Streptococcus* species. The Oxidase test aids in identifying bacteria that produce cytochrome c oxidase, an enzyme involved in the electron transport chain. To perform the test, a piece of filter paper soaked in oxidase reagent, often N,N,N',N'-tetramethyl-p-phenylenediamine, was utilised. Bacterial growth was applied to the filter paper, and colour changes observed. A rapid colour change to purple within 10-30 seconds indicated a positive oxidase reaction. This reaction was indicative of the presence of cytochrome c oxidase and is typically observed in bacteria such as *Pseudomonas* and *Neisseria* species. The Indole test is used to detect the ability of bacteria to produce indole, a byproduct of tryptophan metabolism. Bacterial growth was inoculated into a tube of tryptone broth, which provided a source of tryptophan. After incubation, Kovac's reagent, containing p-dimethylaminobenzaldehyde, was added to the tube. A positive reaction was indicated by the formation of a cherry-red colour ring

at the top of the tube. This colour change signified the presence of indole and is commonly observed in bacteria such as *Escherichia coli* and *Proteus* species. Furthermore, Citrate Utilisation test assesses whether bacteria can utilise citrate as the sole carbon source for growth. Bacterial growth was inoculated into the citrate broth, which contained sodium citrate as the only carbon source. If the bacteria possess the enzyme citrate-permease, they can transport citrate into the cell and subsequently metabolize it. A change in the colour of the medium from green to blue (after the addition of the indicator Bromothymol Blue) indicated a positive citrate utilization test. This reaction is often used to differentiate bacteria such as *Klebsiella pneumoniae*, which are capable of utilizing citrate, from others that cannot. The coagulase test is primarily employed to differentiate *Staphylococcus aureus*, a pathogenic bacterium, from other coagulase-negative staphylococci (CoNS) that lack this virulence factor. The procedure involved transferring a small amount of the bacterial culture onto a clean, sterile glass slide, a human plasma, serving as the coagulase reagent, was added to the bacterial culture. The mixture was gently mixed and observed for clot formation, positive coagulase test was indicated by the formation of a visible clot within two minutes. On the other hand, the urease test is utilised to detect the ability of bacteria to produce the enzyme urease, which catalyzes the hydrolysis of urea to ammonia and carbon dioxide. The procedure involved inoculation of the bacterial culture into the urease agar medium containing urea as the sole nitrogen source. The medium was then incubated at room temperature of 37°C, for a period of about 24-48 hours. After the incubation period, the urease activity of the bacteria was assessed by observing a colour change in the medium. A positive urease test was indicated by a pink or red colour change in the medium, resulting from the release of ammonia and an increase in pH due to urea hydrolysis. This test is particularly

valuable in differentiating bacterial species, such as *Proteus* species and certain strains of *Klebsiella pneumoniae*, which are known for their urease activity.

Gram staining method is one of the most widely used and fundamental techniques in microbiology, allowing for the differentiation of bacteria into two major groups: Gram-positive and Gram-negative. Developed by the Danish scientist Hans Christian Gram in 1884, this staining technique provides valuable information about the cell wall composition of bacteria, aiding in their classification and identification. The Gram staining procedure began with the preparation of a bacterial smear (spread of the bacterial and a drop of water) on a glass slide, which was then heat-fixed to the slide, by passing the slide quickly through the flame of a Bunsen burner, to ensure the cells adhere firmly. The slide was then flooded with crystal violet, a primary stain, and allowed to stand for a brief period, usually around one minute. During this time, all the bacterial cells on the slide absorbed the crystal violet and appeared purple or blue-violet. Thereafter, the slide was rinsed with a decolorizing agent, such as ethanol or acetone. This step is crucial because it differentiates between Gram-positive and Gram-negative bacteria based on the permeability of their cell walls. Gram-positive bacteria have thick peptidoglycan layers in their cell walls, which retained the crystal violet stain even after decolorization. In contrast, Gram-negative bacteria have thinner peptidoglycan layers and an outer membrane that was disrupted by the decolorizing agent, causing the crystal violet to be washed away.

Following decolorization, the slide was counterstained with safranin, a contrasting or secondary dye. Safranin stained the decolorized Gram-negative bacteria pink or red, while the Gram-positive bacteria retained their purple colour from the crystal violet. This allowed for the clear differentiation between the two types of bacteria under a microscope. The Gram staining method

is invaluable in microbiology laboratories for several reasons. Firstly, it provides rapid preliminary information about the morphology and cell wall characteristics of bacterial isolates. This information aids in the selection of appropriate culture media and further biochemical tests for identification. Secondly, it is an essential tool in diagnosing bacterial infections, as it can help clinicians determine the type of bacteria present and guide the choice of antibiotic therapy.

2.3 Method of Anaerobiosis

At the base of the anaerobic glass jar, a small piece of absorbent wool was placed on one side of the glass dish. 5.0 grams of pyrogallol (CHO) crystals were weighed and positioned on the wool. Using a sterile pipette, 10 mL of 4% NaOH solution (prepared by weighing 4g of sodium hydroxide pellets into a 100ml pre calibrated conical flask, where 100ml of distilled water was gently added as it is a corrosive substance, the dissolution process was done with the aid of a stirring rod to facilitate the process), was added to the pyrogallol crystals with the aid of a sterile pipette. Subsequently, all the inoculated culture plates were placed upside down on the opposite side of the glass jar. To create an anaerobic environment for the plates, the anaerobic glass jar was tightly sealed and gently rocked to mix the already prepared chemicals (pyrogallol + sodium hydroxide) inside the glass jar (Ananthanarayan and Paniker, 2006). Thereafter, the plates were allowed to incubate for a period of about 48 hours.

2.4. Antimicrobial susceptibility test(AST)

The isolates underwent antimicrobial susceptibility testing using the Minimum Inhibitory Concentration (MIC) method on Mueller-Hinton agar. The MIC method is a standardised laboratory procedure used to determine the lowest concentration of an antimicrobial agent that inhibits visible growth of a microorganism. This method aids in assessing the effectiveness of various antibiotics against specific bacterial isolates.

Firstly, the bacterial isolates obtained from the ear infection specimens underwent preparation for the AST procedure. This involved subculturing the isolates into 2 mL tubes of nutrient broth, providing a suitable growth medium for the bacteria. The purpose of this step was to ensure that the bacterial isolates are in an active and replicating state for the subsequent testing. The turbidity of the subculture was a critical aspect of the inoculum preparation. Turbidity refers to the cloudiness or density of the bacterial suspension and is usually adjusted to match the 0.5 McFarland standard. This standard serves as a reference for the density of the inoculum. To achieve the desired turbidity, the subculture was visually compared against the 0.5 McFarland standard. Throughout the process, thorough mixing of the inoculum was essential to ensure an even distribution of bacterial cells throughout the suspension. This uniformity helped in obtaining consistent and reliable results during susceptibility testing. Test antimicrobial agents were selected based on ear infection treatment guidelines from the ENT clinic, this included amoxicillin, augmentin, ciprofloxacin and septrin. Stock solutions of the antibiotics (analytical grade) to be tested were then prepared according to manufacturer's instructions, a 1 in 20 dilution was done to prepare varying concentrations of the different analytical grade ensuring standard concentrations, and using sterile distilled water. During the dilution process the number of petri dishes were considered, and was multiplied by the weight of the analytical grade in 1ml. Water was used as the solvent for dilution, and it involved dilution of the analytical grade with 6 ml of water where 6 plates are required, or 3ml when 3 plates are required. This means that, as stated earlier both the volume of water and weight of analytical grade used depended on the number of plates to be prepared. Thereafter, 19 ml of already prepared 500ml Mueller hinton agar(prepared following the manufacturer’s instructions, which involved dissolving 19g of Mueller hinton agar into 500 ml of distilled water, melting in the autoclave for 5 minutes and proceeded to sterilize in the autoclave at a temperature of 121°C for 15 minutes), was measured into a precalibrated sterile bottle, and 1 ml of the resulting solution of both the solute and solvent was pipetted using a calibrated 1 ml micropipette, into the 19ml Mueller hinton agar. This was mixed thoroughly

and poured into one petri dish, the same method was repeated for the remaining plates and the other antibiotic analytical grade. After the plates were allowed to set and solidify, they were all transferred into the hot air oven for drying, at a regulated temperature of 50°C with the lids separated and both inverted with their inner surfaces faced downwards. The plates were then divided and labelled according to specimen numbers and codes for easy identification. A sterile wire loop was used to apply the standardised inoculum to the corresponding portions of the plates for the MIC test. All plates were incubated at 37°C for 24 hours in duplicate. After incubation, the MIC values were obtained and recorded accordingly. The results were interpreted as either susceptible (GROWTH) or resistant (NO GROWTH) based on visual observation. Additionally, the interpretations were compared with published guidelines for antimicrobial susceptibility testing of commonly occurring pathogens, obtained from clinical isolates (EUCAST, 2015).

Inhibition Zone Diameter(IZD) refers to the measurement of the diameter of the clear zone that forms around an antibiotic disk on an agar plate during antimicrobial susceptibility testing. This clear zone, known as the "zone of inhibition," indicates that the bacteria are susceptible to the antibiotic and that their growth is inhibited within this area. Firstly, the bacterial isolates obtained from the ear infection specimens underwent preparation for the AST procedure. This involved subculturing the isolates into 2 mL tubes of nutrient broth, providing a suitable growth medium for the bacteria. The purpose of this step was to ensure that the bacterial isolates are in an active and replicating state for the subsequent testing. The turbidity of the subculture was a critical aspect of the inoculum preparation. Turbidity refers to the cloudiness or density of the bacterial suspension and is usually adjusted to match the 0.5 McFarland standard. This standard serves as a reference for the density of the inoculum. To achieve the desired turbidity, the subculture was visually compared against the 0.5 McFarland standard. Throughout the process, thorough mixing of the inoculum was essential to ensure an even distribution of bacterial cells throughout the suspension. This uniformity helped in obtaining consistent and reliable results during

susceptibility testing. Test multi-antibiotics discs were selected based on ear infection treatment guidelines from the ENT clinic, this included perfloxacin, ofloxacin, azithromycin, levofloxacin, cefotaxime, streptomycin, ciprofloxacin, amoxicillin, augmentin and gentamicin. Thereafter, 500ml of Mueller hinton agar was prepared in a conical flask following the manufacturer's instructions, which involved dissolving 19g of Mueller hinton agar into 500 ml of distilled water, melting in the autoclave for 5 minutes and proceeded to sterilise in the autoclave at a temperature of 121°C for 15 minutes. After sterilisation, 5ml of Mueller hinton agar was poured into sterile petri dishes, the plates were allowed to set and solidify, and transferred into the hot air oven for drying, at a regulated temperature of 50°C with the lids separated and both inverted with their inner surfaces faced downwards. After the standardised inoculum was poured using a sterile and flamed glass spreader, it was evenly distributed across the surface and corner of the agar plate. These inoculated plates were allowed to stand for about 3 minutes to ensure proper fixation of the organism, unto the agar surface. Using a forceps, the multi antibiotics disc was placed aseptically on the surface of the plates containing the agar and isolates inoculum, and the plates inverted. All plates were done in duplicates and control plates containing the inoculum only with no antibiotic disc, were prepared to ensure the viability of isolates on Mueller hinton agar. All plates were incubated at 37°C for 24 hours. After incubation, the inhibition zone diameter values in millimetre were obtained and recorded accordingly. The results were interpreted as either susceptible or resistant published guidelines for antimicrobial susceptibility testing of commonly occurring pathogens, obtained from clinical isolates (EUCAST, 2015).

2.5 Data Analysis

A total of six patient demographics variables were coded and entered from the semi-structured questionnaire into the Statistical Package for Social Sciences (SPSS) version 21.0 software (SPSS Inc Chicago IL USA). These included age, sex, occupation, smoking history, alcohol history and medication history. These variables were defined as string variables in the software.

Additionally, multiple choice questions including those describing frequency of earring wearing, previous microbial infections, symptoms of ear infections and present/past medication history were also defined as string variables. Inhibitory zone diameters were entered and defined as numeric data prior to the analysis with respect to EUCAST breakpoint. Descriptive statistics were used to report percentage frequencies of patient demographics, medication history, presence of hereditary or underlying health conditions (like diabetes, hypertension, rhinosinusitis, GERD etc.). Differences between groups were considered significant as P value < 0.05. Standard diagnostic indices including quantitative antibiogram (IZD) and MIC values, positive predictive diagnostic (Gram staining test) efficacies were all calculated according to standard procedures.

CHAPTER THREE

3.0. RESULTS

3.1. Association of sex with patient characteristics

Table 3.1 present the association of sex with patient's characteristics in this study with statistically significant relationships between the variables measured:

More female and male participants were in the age range of 16-25, followed by those >60years category with age 36-55 years being the least encountered category. Also, more males and females fell under the unemployed class with retired participants being the least encountered. Furthermore, most of the study participants presented with no history of chronic disease prior to visiting the dental clinic, and those who did were females suffering from Gastroesophageal reflux disease (GERD), and few cases of diabetes and hypertension were also recorded. Table 3.1, also highlights that a significant number of the study participants had never swam, smoked or taken alcohol and females had a higher frequency status of those currently smoking, swimming or drinking than their male counterparts. Additionally, the table shows how frequently the study participants clean their ears, both males and females clean their ears about two to three times weekly, and few of them never cleaned their ears. Most of the females and males clean their ears using cotton buds and only few females use matchstick/biro tips. Furthermore, the table also shows the symptoms most common with the sexes, pain was found to be the most common with fever being the least encountered. Majority of them had pain inside the ear, with 17 persons having discharge. The longest duration of these symptoms was recorded to be >21 days. Lastly, table 3.1 describes that most of the males and females have had previous microbial infection and use antibiotics more than three times in a month.

TABLE 3.1Relationship between sex and patient characteristics

Characteristics	Values	Male	Female	Total	P-Value
Age of patient	0-15yrs	1	5	6	0.109
	16-25yrs	1	12	13	
	26-35yrs	1	3	4	
	36-55yrs	0	1	1	
	56-60yrs	0	2	2	
	>60yrs	5	3	8	
Occupation	Unemployed	2	14	16	0.156
	Self-employed	2	4	6	
	Employed	3	3	6	
	Retired	1	0	1	
	Student	0	5	5	
Medical history	Nil	4	18	22	0.168
	Hypertension	2	0	2	
	Diabetes	1	1	2	
	Rhinosinusitis	0	0	0	
	GERD	1	7	8	
Smoking history	Stopped smoking	3	2	5	0.105
	Never smoked	5	23	28	
	Still smoking	0	1	1	
Alcohol history	Stopped drinking	4	1	5	0.003
	Never drank	2	20	22	
	Still drinking	2	5	7	
Swimming history	Stopped swimming	1	0	1	
	Never swam	6	25	31	0.115
	Still swimming	1	1	2	
Ear cleaning habit	2-3 times	4	12	16	
	Once a month	2	12	14	0.326
	Never	2	2	4	
Ear cleaning instruments	Cotton buds	8	22	30	0.238
	Biro tips	0	4	4	

Wearing of earrings	Ceremonies Everyday Once a month Never	0 1 0 7	2 17 2 5	2 18 2 12	0.006
Pain location	Inside the ear Outside ear Both	8 0 0	23 0 3	31 0 3	0.314
Presenting symptoms	Pain Discharge Fever itching	5 1 1 1	20 3 0 3	25 4 1 4	0.330
Previous ear infection	Yes No	6 2	20 6	26 8	0.911
Affected ear	Right ear Left ear Both ears	2 4 2	8 10 5	10 17 7	0.878
Pus discharge	Yes No	2 6	15 11	17 17	0.106
Symptoms duration	<7days 8-14 days >21days	3 1 4	8 9 9	11 10 13	0.477
Previous microbial infection	Yes No	7 2	19 6	26 8	0.668
Frequency of antibiotics use	Never Once a month Twice a month Three times >three times	0 0 2 2 4	7 6 6 2 5	7 6 8 4 9	0.101

3.2: **Association of Facultative Anaerobic Bacterial isolates with patient characteristics**

Table 3.2. introduces the association of anaerobic bacterial isolates with patient characteristics, however there were no statistically significant relationship/ Participants with the age range of 16-25 had more anaerobic isolates from their infected ears. Same was observed for the unemployed class with the retired class having the least. Also, those with no history of chronic diseases had more of these microbial isolates as well as those who wore earrings everyday. Conversely, subjective data from study participants showed that patients who never drank alcohol, never smoked or swam, had a higher number of microbial isolates. It was also recorded that all the microbial isolates majorly caused pain inside of the ear, only few were found on the external ear. Additionally, the isolates were more from patients who had suffered previous microbial infections and symptoms lasted more than 21 days. The number of microbial isolates obtained from patients who used antibiotics more than three times in a month were more than those who used antibiotics rarely.

TABLE 3.2 Relationship between facultative anaerobic bacterial isolates and patient characteristics

Characteristics	Values	Baci.	Kleb.	Pseu.	Staph.	P value
Age of patient	0-15yrs	2	0	2	2	
	16-25yrs	2	1	7	3	
	26-35yrs	1	0	3	0	0.641
	36-55yrs	0	0	1	0	
	56-60yrs	0	1	1	0	
	>60yrs	4	1	2	1	
Occupation	Unemployed	3	0	8	5	
	Self-employed	2	0	2	0	
	Employed	3	0	3	0	0.642
	Retired	0	0	1	0	
	Student	1	1	2	1	
Medical history	Nil	5	2	13	5	
	HTN	2	0	0	0	
	DM	1	0	0	0	0.458
	RS	1	0	0	0	
	GERD	0	1	3	1	
Smoking history	Stopped smoking	3	1	0	1	
	Never smoked	6	2	15	5	0.403
	Still smoking	0	0	1	0	
Alcohol history	Stopped drinking	2	1	1	1	
	Never drank	4	2	12	4	0.474
	Still drinking	3	0	3	1	
Swimming history	Stopped swimming	0	1	0	0	
	Never swam	8	2	15	6	0.127
	Still swimming	1	0	1	0	
Ear cleaning habit	2-3 times a week	3	2	8	3	
	Once in a month	5	1	7	1	0.436
	Never	1	0	1	2	
Ear cleaning instrument	Cotton buds	9	3	12	6	0.531
	Biro tips/match sticks	0	0	4	0	

Wearing of earrings	Ceremonies	1	1	0	0	
	Everyday	3	1	11	3	0.469
	Once a month	0	0	1	1	
	Never	5	1	4	2	
Pain location	Inside the ear	9	3	14	5	
	Outside the ear	0	0	0	0	0.890
	Both	0	0	2	1	
Presenting symptoms	Pain	5	3	14	3	
	Discharge	2	0	1	1	0.429
	Fever	0	0	1	0	
	itching	2	0	0	2	
Previous ear infection	Yes	7	3	11	5	0.909
	No	2	0	5	1	
Pus discharge	Yes	2	0	12	3	0.082
	No	7	3	4	3	
Symptoms duration	<7days	4	1	4	2	
	8-14 days	3	0	5	2	0.444
	>21days	2	2	7	2	
Previous microbial infection	Yes	8	2	11	5	0.989
	No	1	1	5	1	
Frequency of antibiotics use	Never	1	0	5	1	0.857
	Once in a month	2	0	3	1	
	Twice in a month	2	2	2	2	
	Three times	1	0	2	1	
	>three times	3	1	4	1	

KEY: Baci: *Bacillus. spp,* Kleb=*Klebsiella spp*, Pseu=*Pseudomonas spp*, Staph=*Staphylococcus aureus*. HTN = hypertension, DM= diabetes mellitus , RS= Rhinosinusitis

3.3. Association of sex with microbial isolates

Table 3.3 shows the relationship between gender and microbial isolates: Females had more species of *Bacillus, Klebsiella, Pseudomonas and Staphylococcus* compared to males although not statistically significant. *Bacillus* was more encountered in males and *Pseudomonas* in females, although the least encountered in both genders was *Klebsiella*.

TABLE 3.3 Relationship between facultative anaerobic bacterial isolates and gender

Isolates	**Male**	**Female**	**Total**	**P value (CI=95%)**
Bacillus spp	4	5	9	0.357
Klebsiella spp	1	2	3	
Pseudomonas spp	2	14	16	
Staphylococcus spp	1	5	6	
Total	8	26	34	

3.4 Frequency Distribution of Facultative Anaerobic Isolates

Among the four organisms isolated under anaerobic conditions, as depicted in table 3.2, *Pseudomonas spp.* continued to exhibit the highest occurrence rate at 47%. Following this, *Bacillus spp*. accounted for 26.47% of the isolates, while *Staphylococcus aureus* (17.7%) and *Klebsiella spp.* (8.83%) displayed lower frequencies of occurrence.

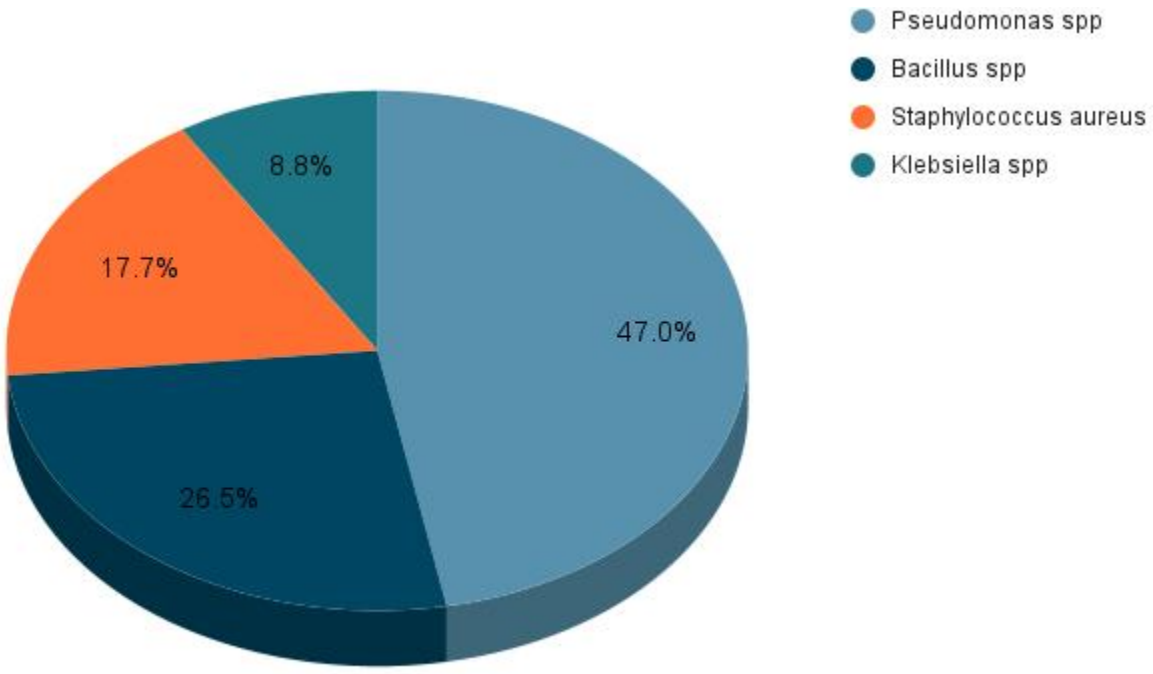
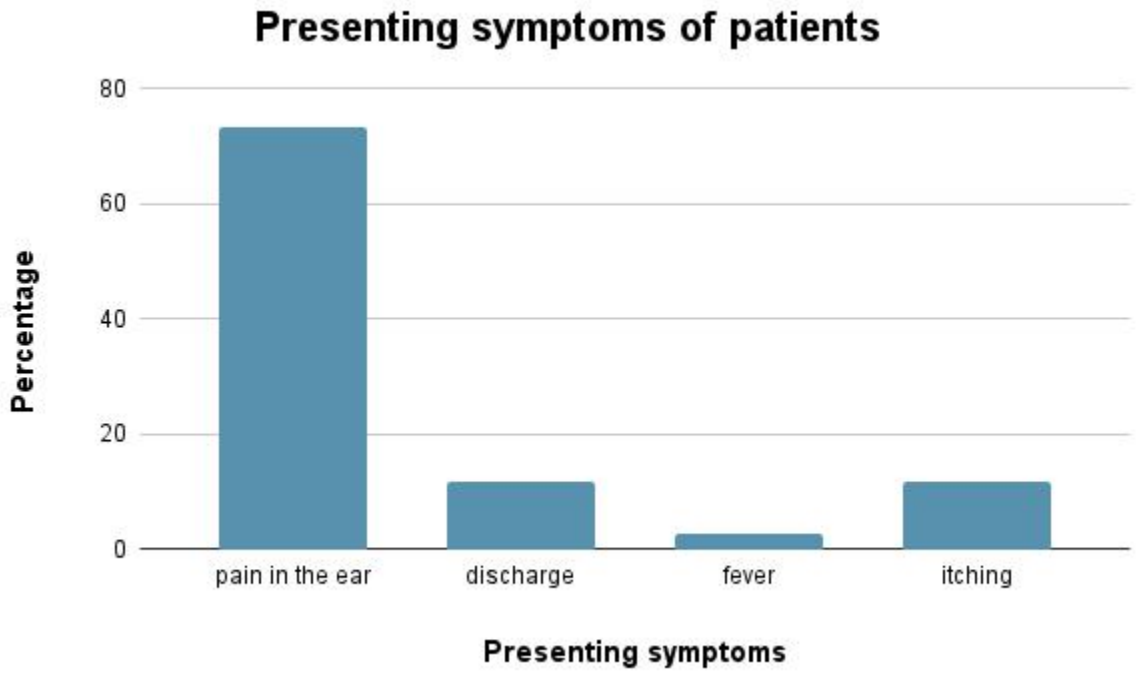


Fig 3.4: Frequency Distribution Of Facultative Anaerobes

3.5 Frequency distribution of symptoms

The bar chart below shows the symptoms with the highest percentage, as seen in the study participants. About 70% of them complained of pain in the ear and only a small fraction of the study population had a fever.



3.6. Morphological Characteristics of Facultative Anaerobic Isolates

In this investigation, biochemical tests revealed that all 34 of the clinical isolates were catalase and citrate positive, 6 were coagulase positive, 23 were oxidase positive, 9 were urease positive, and none were indole positive. Gram-negative bacilli were found to be the most common bacterial isolate, while Gram-positive cocci were found to be the least common bacterial isolate, accounting for 18 and 6 isolates, respectively. The colonial characteristics and hemolysis pattern on blood agar plates were also recorded in table 3.5.

TABLE 3.6**:** Morphological characteristics of facultative anaerobic isolates

MORPHOLOGICAL TEST	INFERENCE	BACI. SPP	KLEB. SPP	PSEU. SPP	STAPH. SPP	TOTAL ISOLATES
Colonial characteristics	Raised	9	3	0	6	18
	Flat	0	0	16	0	16
Gram stain inference	GNB	0	2	16	0	18
	GPC	0	0	0	6	6
	GPB	9	0	0	0	9
Blood agar inference	Alpha	1	1	3	0	5
	Beta	7	2	12	1	22
	Gamma	1	0	1	5	7
Catalase test	Positive	9	3	16	6	34 +VE
Coagulase test	Positive	0	0	0	6	6 +VE
Oxidase test	Positive	7	0	16	0	23 +VE
Indole test	Positive	0	0	0	0	0
Urease test	Positive	0	3	0	0	3+VE
Citrate test	Positive	9	3	16	6	34 +VE

KEY: BACI SPP: *Bacillus. spp,* KLEB SPP=*Klebsiella spp*, PSEU SPP=*Pseudomonas spp*, STAPH SPP=*Staphylococcus aureus*, +VE= Positive, -VE= Negative, GPC= Gram positive cocci, GPB= Gram positive bacilli, GNB= Gram negative bacilli

3.7 Antimicrobial susceptibility profile of facultative anaerobes using IZD

Table 3.7 showed the inhibition zone diameter of the anaerobic isolates, indicating their resistance and sensitivity percentage to the different antibiotics, which included, Fluoroquinolones(represented by perfloxacin and ciprofloxacin), Macrolides(azithromycin), Cephalosporins(cefotaxime), Penicillins(amoxicillin and amoxicillin/clavulanic acid combination), and Aminoglycosides(Gentamicin). This table indicates that all the *Bacillus spp*, were sensitive to perfloxacin, with the highest percentage resistance recorded as 33.3% as seen in cefotaxime and gentamicin. However, *Klebsiella spp,* was 100% resistant to perfloxacin, amoxicillin and augmentin. However, about a quarter of the total isolates were sensitive to azithromycin, ciprofloxacin, cefotaxime and gentamicin with a 33.3%. On the other hand, *Pseudomonas spp,* were 81.25% sensitive to the fluoroquinolones, with the highest sensitivity recorded with gentamicin, and were more resistant to the penicillins with 43.75%. The highest sensitivity percentage of *staphylococcus spp*, was recorded to be 100%, 83.3% and 100% with ciprofloxacin, perfloxacin and gentamicin respectively. However, they were more resistant to amoxicillin, and a uniform percentage distribution of sensitivity and resistance was recorded with augmentin to be each 50%.

	PERFLOXACIN (30ug)		AZITHROMYCIN (12ug)		CEFOTAXIME (10ug)		CIPROFLOXACIN (30ug)	
Isolates	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive
Bacillus spp n=9	0 (0%)	9 (100%)	2 (22.2%)	7 (77.8%)	4 (33.3%)	5 (55.6%)	1 (11.1%)	8 (88.9%)
Klebsiella spp n=3	3 (100%)	0 (0%)	2 (66.7%)	1 (33.3%)	2 (66.7%)	1 (33.3%)	2 (66.7%)	1 (33.3%)
Pseudomonas spp n=16	3 (18.75%	13 (81.25%)	2 (6.25%)	14 (87.5%)	10 (50%)	6 (37.5%)	3 (18.75%)	13 (81.25%)
Staphylococcus spp n=6	1 (16.7%)	5 (83.3%)	2 (33.3%)	4 (66.7%)	2 (16.7%)	4 (66.7%)	0	6 (100%)

TABLE 3.7a Antimicrobial susceptibility profile of facultative anaerobes using IZD

	AMOXICILLIN (30ug)		AUGMENTIN (10ug)		GENTAMICIN (30ug)	
Isolates	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive
Bacillus spp n=9	2 (22.2%)	7 (77.8%)	2 (22.2%)	7 (77.8%)	3 (33.3%)	6 (66.7%)
Klebsiella spp n=3	3 (100%)	0	3 (100%)	0	2 (66.7%)	1 (33.3%)

TABLE 3.7b Antimicrobial susceptibility profile of facultative anaerobes using IZD

Pseudomonas spp n=16	7 (43.75%)	9 (56.25%)	7 (43.75%)	9 (56.25%)	2 (12.5%	14 (87.5%)
Staphylococcus spp n=6	4 (66.7%)	2 (33.3%)	3 (50%)	3 (50%)	0	6 (100%)

3.8 Antimicrobial susceptibility profile of facultative anaerobes using MIC

Table 3.8 showed the inhibition zone diameter of the anaerobic isolates, indicating the lowest concentration of the antibiotics that inhibit their growth. *Bacillus spp* as indicated on the table, had an MIC of 25ug with amoxicillin and augmentin, 0.25 for ciprofloxacin and 10ug for septrin, this means that at these varying concentrations there was at least one no growth record for *Bacillus spp*. However, *Klebsiella spp*, had an MIC of 50ug for amoxicillin, 25ug for augmentin, 1ug for ciprofloxacin and 20ug for septrin, at concentrations lesser than these, visible growth of the isolates were recorded. *Pseudomonas spp* and *Staphylococcus spp*, had an MIC for augmentin to be 12.5ug, 0.25ug for ciprofloxacin and 20ug for augmentin. However, they had different MICs following the use of amoxicillin, which gave 12.5ug for *Pseudomonas spp* and 50ug for *staphylococcus spp*.

Table 3.8 Antimicrobial susceptibility profile of facultative anaerobes using MIC

	AMOXICILLIN(ug)						AUGMENTIN(ug)					
Bacterial isolates	100	50	25	12.5	6.25	mean MIC	100	50	25	12.5	6.25	Mean MIC
Bacilli n=9	9 NG	2 G 7 NG	7 G 2 NG	9 G	9 G	25	9 NG	9 NG	3 G 6 NG	9 G	9 G	25
Kleb. n=3	3 NG	3 NG	3 G	3 G	3 G	50	3 NG	3 NG	3 NG	3 G	3 G	25
Pseud. n=16	16 NG	1 G 15 NG	9 G 7 NG	15 G 1 NG	16 G	12.5	16 NG	16 NG	4 G 12 NG	14 G 2 NG	16 G	12.5
Staph. n=6	6 NG	2 G 4 NG	6 G	6 G	6 G	50	6 NG	6 NG	4 G 2 NG	5 G 1 NG	6 G	12.5

Table 3.8 Antimicrobial susceptibility profile of anaerobes using MIC

	CIPROFLOXACIN (ug)							SEPTRIN (ug)						
Bacterial isolate	5	2.5	1	0.5	0.25	0.125	Mean MIC	50	20	10	5	2.5	1.25	Mean MIC
Bacillus. n=9	9 NG	9 NG	6 NG 3 G	3 NG 6 G	1 NG 5 G	9 G	0.25	9 NG	5 NG 4 G	1 NG 8 G	9 G	9G	9 G	10
Kleb. n=3	3 NG	3 NG	1NG 2 G	3 G	3 G	3 G	1	3 NG	2 NG 1 G	3 G	3 G	3 G	3 G	20
Pseud. n=16	16 NG	14 NG 2 G	10 NG 6 G	8 NG 8 G	5 NG 11 G	16 G	0.25	16 NG	6 NG 10 G	16 G	16 G	16 G	16 G	20
Staph. n=6	6 NG	6 NG	6 NG	4 NG 2 G	2 NG 4 G	6 G	0.25	6 NG	1 NG 5 G	6 G	6 G	6 G	6 G	20

CHAPTER FOUR

4.0. DISCUSSION

4.1. Epidemiology of study participants

4.1.1. Demographics of study participants and bacteria isolates

The demographics of study participants offered profound insights into the prevalence and potential risk factors associated with ear infections caused by anaerobes across distinct age groups. This analysis is particularly crucial given that individuals aged 16-25 were found to exhibit the highest frequency of ear infections, followed by those over 60 years old and children aged 0-15 years. These findings illuminate varying patterns of susceptibility and contributing elements to the prevalence of anaerobic ear infections in these age groups. Children, despite being recognized in numerous studies as more susceptible to ear infections caused by anaerobes due to their immature immune systems, frequent upper respiratory infections, enlarged adenoids, horizontal and shorter eustachian tubes did not emerge as the highest frequency group in this study. This seeming discrepancy can be attributed to several factors, including the limited representation of this age group in the study sample, time limitations had also constrained the ability to recruit a larger sample size, which could have provided a more comprehensive understanding of the prevalence across different age groups. With a total sample size of 43 participants, the representation of each age group might have been uneven, potentially influencing the observed frequencies. However, the considerable prevalence of ear infections caused by anaerobes among individuals aged 16-25 underscores a critical period of vulnerability. This age range is characterised by significant life transitions, including heightened social interactions, alterations in diet and lifestyle, and exposure to potential risk factors such as

smoking and excessive alcohol consumption. From the table of results, it was discovered that a lot of them cleaned their ears with cotton buds and some even went as far as using objects like matchsticks or biro tips in their ears. The human ear canal is remarkably self-cleaning, equipped with tiny hairs and a production of earwax (cerumen) that naturally traps dirt and debris. This waxy substance gradually migrates outwards, carrying unwanted particles with it. However, the common misconception that ears require frequent cleaning with cotton swabs or other objects can lead to a detrimental cycle. Firstly, cotton swabs can easily push earwax further inwards, causing impaction. This blockage creates a warm, moist environment ideal for bacterial growth. Additionally, the delicate skin lining the ear canal is easily scratched by aggressive cleaning, introducing potential entry points for bacteria and increasing the risk of infection. There was also a higher frequency of patients with ear piercings and everyday wearing of the earrings. Ear piercings can add a touch of personal style, but some types of earrings can harbour bacteria and compromise ear health. Dangling earrings, particularly those made of materials that trap moisture or harbour dirt, can irritate the piercing site and potentially introduce bacteria into the canal. Likewise, heavy earrings can stretch or tear the earlobe, creating an open wound susceptible to infection, a practice most common amongst women and hence, explains why more women were predisposed to ear infections than men in this study. Additionally, nickel, a common metal found in many inexpensive earrings, can also trigger allergic reactions in some individuals, manifesting as redness, itching, and swelling around the piercing, creating a perfect entry point for infection. Moreover, the lack of awareness or adherence to proper ear hygiene practices in this demographic may further contribute to the heightened incidence of ear infections. Similarly, the vulnerability of older adults over 60 years old to ear infections caused by anaerobes is underscored by factors such as compromised immune systems and age-related changes in ear

structure, such as thinner and drier cerumen, fragile ear canal skin, Eustachian tube dysfunction, chronic inflammation in the middle ear, presbycusis (age-related hearing loss), and vestibular changes, collectively create an environment that is more vulnerable to anaerobic bacterial colonisation and ear infections in older adults. This demographic often experiences a higher prevalence of chronic diseases, creating an environment conducive to bacterial colonisation and subsequent infections. Despite not emerging as the highest frequency group in our study, the presence of ear infections caused by anaerobes in children aged 0-15 years remains a significant concern. This age group commonly grapples with challenges related to immature immune systems, frequent upper respiratory infections, stated earlier and exposure to daycare or school environments where infections can readily propagate. Additionally, improper ear hygiene practices in children, such as the use of improper tools, can significantly contribute to the colonisation of anaerobic bacteria and subsequent infections. Most of the study participants were found to be unemployed, while there's no clear causal link between unemployment and ear infections, the potential for stress-induced immune system decline, limited healthcare access, and poorer living conditions associated with unemployment might create a slightly higher risk for ear infections in some cases. It is worthy to note that most of the study participants have either had previous ear infections or previous microbial infections. There are a couple of reasons for this, frequent ear infections can cause scarred Eustachian tubes, and make it harder for fluid to drain out of the ear, which can then lead to another infection. Also, previous infections can cause the immune system to become overwhelmed and unable to fight off the attacking bacteria.

4.1.2. Microbial isolates and sex of study participants

The research findings revealed a noteworthy trend where female patients exhibited a higher prevalence of ear infections compared to their male counterparts. This observation stands in

contrast to previous studies, as highlighted in the introduction to the topic (Smith et al., 2018; Brown and Jones, 2017), which highlighted larger ear canal size, increased sebum production, or higher levels of certain hormones. For instance, one study proposed that the larger size of the male ear canal might create a more conducive environment for bacterial growth and infection. Additionally, higher levels of sebum production in males could lead to increased colonisation of bacteria in the ear canal, potentially contributing to a higher risk of infections. Furthermore, hormonal differences, particularly the influence of testosterone, have been hypothesised to affect the immune response and susceptibility to infections in males.

However, several potential reasons could underlie this gender disparity in ear infections. Firstly, anatomical differences between males and females could play a role. It is known that women generally have narrower and more horizontal Eustachian tubes compared to men, which may hinder proper drainage and ventilation of the middle ear (Smith et al., 2015; Johnson and White, 2016). This anatomical variation could predispose females to a higher risk of middle ear infections, especially in cases of upper respiratory tract infections where bacteria or viruses can easily travel to the middle ear through the Eustachian tubes. Additionally, hormonal fluctuations in females could contribute to increased susceptibility to ear infections. Fluctuations in oestrogen levels, particularly during menstruation, pregnancy, or menopause, can affect the immune response and mucosal lining of the ear canal, potentially altering its defence mechanisms against pathogens (Jones and Versalovic, 2009). Furthermore, behavioural factors may come into play. Studies have suggested that females are more likely to engage in activities that expose them to higher risks of ear infections, such as swimming in contaminated waters, use of dangling earrings, multiple piercings or using earbuds or headphones regularly (Lee et al., 2016). These behaviours can introduce foreign particles or pathogens into the ear canal, increasing the chances of

infection. The gender differences observed in the prevalence of these bacterial species in ear infections can be attributed to various factors related to gender roles and behaviours. For instance, women were more likely to engage in activities such as swimming, which can increase their exposure to waterborne bacteria like *Pseudomonas aeruginosa*. Additionally, the use of cosmetics and earrings, particularly in women, can introduce *Staphylococcus aureus* into the ear canal. On the other hand, men may be more prone to activities or occupations that expose them to *Bacillus species* found in soil and dust. For example, individuals working in outdoor settings may have higher chances of encountering these environmental bacteria. Additionally, men are less likely to seek medical attention for minor ear discomfort, which could lead to the persistence of bacterial infections.

4.2. Frequency Distribution of Facultative Anaerobic Isolates

The frequency distribution of facultative anaerobic isolates in ear infections highlights their adaptability to varying oxygen conditions. *Pseudomonas spp*, the most prevalent at 47%, and Bacillus spp. at 26.47%, demonstrate their ability to thrive in both aerobic and anaerobic environments. *Staphylococcus aureus* (17.7%) and *Klebsiella spp*. (8.83%) also exhibit this adaptability, persisting in diverse ear canal conditions. Their facultative anaerobic nature underscores their role as significant pathogens in ear infections, guiding targeted treatment approaches. The prevalence of *Pseudomonas spp.* as the most common facultative anaerobic isolate is noteworthy, as they are known for their adaptability to a wide range of oxygen levels, allowing them to survive and thrive in both aerobic and anaerobic environments. This adaptability is crucial in the ear, where conditions can vary due to factors such as earwax buildup and changes in humidity (Derr et al., 2012). The ability of *Pseudomonas spp*. to persist in these diverse conditions contributes to their role as significant pathogens in ear infections. Moving on to the prevalence of *Bacillus spp*. As a facultative anaerobe, it indicates their capability to

survive in the ear's fluctuating oxygen levels. While not as dominant as *Pseudomonas*, *Bacillus* species can still contribute to ear infections, especially in individuals with compromised immune systems (Kotrashetti et al., 2018). Their ability to form spores allows them to persist in the environment and potentially cause chronic ear infections if not properly treated (Kotrashetti et al., 2018). The presence of *Staphylococcus aureus,* raises concerns due to its pathogenic nature. *Staphylococcus aureus* is known to cause a variety of infections, including ear infections, and its presence in the ear can lead to complications such as otitis media (Cuesta et al., 2010). Factors such as poor ear hygiene or previous infections could contribute to the colonisation of *Staphylococcus aureus* in the ear canal, leading to recurrent infections (Cuesta et al., 2010). Finally, the lower occurrence of *Klebsiella spp*. suggests a lesser role in anaerobic ear infections (Samaranayake, 2018). While *Klebsiella* species can cause infections in various parts of the body, their lower prevalence in the ear indicates that they are not primary contributors to anaerobic ear infections. However, their presence cannot be completely disregarded, especially in cases of secondary infections or individuals with specific risk factors (Samaranayake, 2018).

4.3. Morphological Characteristics of Facultative Anaerobic Isolates

The classification of bacterial colonies into raised and flat colonies highlights the diverse forms that bacterial growth can exhibit. The variations in colony morphology can be attributed to factors like bacterial species, isolates, growth conditions, and genetic factors (Finkelstein et al., 1992). Furthermore, biochemical tests are fundamental tools for characterising and identifying bacterial species based on their metabolic capabilities (Talaiekhozani et al., 2013).

The presence of both raised and flat isolates suggests the existence of different bacterial populations within the samples. These variations in colony morphology can be indicative of the genetic diversity and adaptations of bacterial species to their environment (Kassen et al., 2013).

The presence of catalase activity (catalase positive) in 34 isolates suggests that these bacteria can break down hydrogen peroxide into water and oxygen. This characteristic is often associated with a range of bacteria, including both Gram-positive and Gram-negative species which becomes a useful means of identification for these bacteria as was employed in this study (Reiner, 2010). Isolates displaying coagulase activity are typically associated with *Staphylococcus* species, particularly *Staphylococcus aureus*. Coagulase-positive strains have the ability to clot blood plasma and are known for their pathogenic potential (virulence) (Kateete et al., 2010). Also, the presence of oxidase activity in isolates obtained, indicates that these bacteria possess the enzyme cytochrome c oxidase (Shields and Cathcart, 2010). This characteristic was used to differentiate between bacteria in this study. Isolates capable of utilising citrate as a sole carbon source suggest the presence of specific metabolic pathways, such as the citrate fermentation pathway. This was also used in bacterial identification tests for the isolates in this category in this study. Furthermore, all the isolates tested negative to indole chemical test, which indicates the lack of the enzyme tryptophanase, required to produce indole from tryptophan. The urease test used to detect the presence of the enzyme urease, which catalyses the hydrolysis of urea into ammonia and carbon dioxide, helped in differentiating bacterial species based on their ability to hydrolyze urea, and only *Klebsiella spp*. tested positive.

The results of the blood hemolysis patterns among the isolates revealed distinct characteristics, shedding light on the hemolytic properties of the bacterial species. Among the isolates, *Bacillus* and *Pseudomonas* displayed a predominant pattern of beta-hemolysis on blood agar. Beta-hemolysis, characterised by a clear zone surrounding the colonies, indicates the production of potent hemolysins capable of completely breaking down red blood cells. This suggests that both *Bacillus* and *Pseudomonas* species possess virulence factors that contribute to their pathogenicity,

enabling them to cause tissue damage and spread within the host. In contrast, *Staphylococcus aureus* exhibited a gamma-hemolysis pattern on blood agar. Gamma-hemolysis, where there is no visible hemolysis around the colonies, indicates that these *Staphylococcus aureus* isolates do not produce hemolysins capable of lysing red blood cells. This finding is consistent with some strains of *Staphylococcus aureus* that are known to lack hemolytic activity. Despite this, *Staphylococcus aureus* remains a significant pathogen in the ear, due to other virulence factors it possesses, such as toxins and adhesins. Interestingly, *Klebsiella* isolates showed a majorly beta-hemolysis pattern on blood agar. Beta-hemolysis in *Klebsiella* suggests the production of hemolysins, which may contribute to its pathogenicity and ability to cause tissue damage. This finding aligns with the known virulence factors of *Klebsiella* species, such as capsular polysaccharides and adhesins, which aid in its colonisation and infection. However, the presence of some isolates displaying alpha-hemolysis, particularly among *Klebsiella*, *Pseudomonas* and *Bacillus*, is noteworthy. Alpha-hemolysis, characterised by a greenish or brownish discoloration around the colonies, indicates partial lysis of red blood cells. This suggests the production of enzymes or factors that cause partial breakdown of blood cells, which could play a role in the pathogenicity of these isolates.

4.4 Antimicrobial susceptibility profile of facultative anaerobes using IZD

Antimicrobial susceptibility profile of facultative anaerobes using the Inhibition Zone Diameter (IZD) method provided insights into the effectiveness of various antibiotics against these bacterial isolates. The IZD method, also known as the disc diffusion method, is a standard technique used in microbiology laboratories to assess the susceptibility of bacteria to antibiotics. The interpretation of the results is based on the size of the inhibition zone surrounding the

antibiotic disc, with larger zones indicating greater susceptibility to the antibiotic. IZD can also provide insights into whether an antibiotic exhibits bactericidal or bacteriostatic effects against a particular bacterial isolate. In general terms, bactericidal antibiotics work by killing the bacteria, while bacteriostatic antibiotics inhibit bacterial growth without necessarily killing the bacteria outright. For bactericidal antibiotics, the presence of a large zone of inhibition indicates that the antibiotic has effectively killed the bacteria, leading to a clear area where no bacterial growth occurs. The larger the zone, the more effective the antibiotic is at killing the bacteria. This is typically seen with antibiotics that disrupt essential bacterial processes, such as cell wall synthesis, protein synthesis, or DNA replication. On the other hand, for bacteriostatic antibiotics, the zone of inhibition may not be as large as with bactericidal antibiotics. Instead of killing the bacteria outright, bacteriostatic antibiotics inhibit their growth and reproduction. This can result in a zone of inhibition where bacterial growth is visibly reduced but not completely halted. The effectiveness of bacteriostatic antibiotics can depend on the concentration of the antibiotic and the susceptibility of the bacterial strain.

The fluoroquinolones (perfloxacin and ciprofloxacin), a class of antibiotics, are considered broad-spectrum antibiotics. This means they are effective against a wide range of bacteria, both Gram-positive and Gram-negative. They exhibit a potent bactericidal effect by targeting specific enzymes crucial for bacterial DNA replication and repair. These antibiotics act on bacterial DNA gyrase and topoisomerase IV, essential enzymes involved in the supercoiling and relaxation of DNA during replication. Upon entry into bacterial cells, fluoroquinolones bind to the A subunit of DNA gyrase and topoisomerase IV, initiating a multi-step process of inhibition. This binding prevents the enzymes from cleaving and resealing the DNA strands properly, leading to the formation of a stable complex known as the cleavage complex. This complex, in turn, results in

the accumulation of breaks in the bacterial DNA, disrupting the normal replication and transcription processes. As bacterial DNA replication is impaired and DNA breaks accumulate, the affected bacteria are unable to replicate their DNA effectively. This interference with essential bacterial processes ultimately leads to the inhibition of bacterial growth and, ultimately, cell death. The bactericidal action of fluoroquinolones stems from their ability to cause irreversible damage to bacterial DNA, rendering the bacteria unable to survive and reproduce. The concentration of pefloxacin used in the susceptibility testing was 30 µg, which is within the standard range for testing this antibiotic. The results indicated that pefloxacin showed effectiveness against *Bacillus spp*, the majority of *Pseudomonas spp,* and most *Staphylococcus aureus* isolates. However, it exhibited limited efficacy against the *Klebsiella spp*. strains, with all three isolates showing resistance. The resistance of *Klebsiella spp.* to pefloxacin could be due to several factors, including the development of specific mechanisms by the bacteria to evade the effects of the antibiotic. *Klebsiella spp*. are known to possess various resistance mechanisms, such as efflux pumps that can actively pump out the antibiotic from inside the cell, reducing its effectiveness. Additionally, *Klebsiella* can also develop mutations in the target enzymes, DNA gyrase, and topoisomerase IV, which reduces the binding affinity of pefloxacin to these enzymes, rendering the antibiotic less effective in inhibiting bacterial growth and this can occur as a result of the prolonged use and exposure to pefloxacin. However, the bactericidal or bacteriostatic effects can also vary based on the organism's susceptibility profile, the concentration of the antibiotic, and the duration of exposure. The ciprofloxacin 30ug susceptibility testing results showed varying degrees of effectiveness against the bacterial isolates. While it generally exhibited good sensitivity, the presence of resistant strains emphasises the importance of individual susceptibility testing before prescribing antibiotics. For ear infections caused by

Bacillus, Pseudomonas, and *Staphylococcus,* ciprofloxacin would be a suitable treatment option in most cases. However, for *Klebsiella* infections, alternative antibiotics might be considered due to the presence of resistant strains. Due to the increasing prevalence of fluoroquinolone-resistant *Klebsiella* strains, current treatment guidelines often recommend alternative antibiotics for *Klebsiella* infections.

Azithromycin, another antibiotic present in the multi antibiotic disc, is a macrolide which exerts antimicrobial effects by inhibiting bacterial protein synthesis, which makes them bacteriostatic. This inhibition occurs through binding to the 50S subunit of the bacterial ribosome, a cellular structure responsible for protein synthesis. During this step, the ribosome moves along the mRNA (messenger RNA) strand, synthesizing the protein, macrolides prevent this translocation step of protein synthesis. by binding to the ribosome. By interfering with translocation, they prevent the formation of new peptide bonds between amino acids. This inhibits the elongation of the growing peptide chain, during protein synthesis. Specifically, macrolides bind to the A site of the 50S ribosomal subunit, blocking the incoming aminoacyl-tRNA from attaching to the mRNA-ribosome complex. This action prevents the addition of new amino acids to the growing polypeptide chain, ultimately leading to the inhibition of protein synthesis. Additionally, macrolides can also induce dissociation of peptidyl-tRNA from the ribosome, further halting protein synthesis. This dual mechanism of action disrupts bacterial growth and replication, ultimately leading to bacterial cell death or inhibition of bacterial growth. Macrolides are time-dependent antibiotics. Their effectiveness is related to the time they remain above the minimum inhibitory concentration in the body at a sustained level. Overall, azithromycin demonstrated varying levels of effectiveness against the organisms. It was generally more effective against *Pseudomonas* and *Bacillus* species in this study, with a higher number of sensitive isolates.

However, its efficacy against *Klebsiella* and *Staphylococcus* is limited due to the presence of resistant isolates. Therefore, azithromycin may be considered as an alternative treatment option for ear infections caused by *Pseudomonas* and some *Bacillus* strains, particularly if the isolates are sensitive. However, it would not be the first choice for *Klebsiella* or *Staphylococcus* infections due to the observed resistance in some isolates. Gram-negative bacteria like *Pseudomonas* have complex cell walls with outer membranes that act as barriers, limiting the entry of antibiotics into the cell (Nikaido, 2003). This reduced permeability can contribute to the resistance of the other strains to azithromycin. Additionally, the concentration of azithromycin used in the susceptibility testing may also play a role in the observed resistance patterns. Higher concentrations of the antibiotic might be needed to effectively inhibit the growth of certain bacteria, especially those with inherent resistance mechanisms. Considering the mechanism of action of azithromycin, which involves binding to the 50S ribosomal subunit of bacteria, inhibiting protein synthesis and ultimately leading to cell death, the observed sensitivity of some isolates indicates that they lack the specific mechanisms of resistance mentioned above. These sensitive isolates, particularly those of *Pseudomonas* and *Staphylococcus,* are likely to respond well to azithromycin treatment for ear infections caused by these bacteria. In ear infections, azithromycin is commonly used due to its effective activity against Gram-positive bacteria and a limited number of Gram-negative bacteria, which is evident in this study with the presence of resistant isolates of *Klebsiella* and *Bacillus* species, hence the importance of conducting antimicrobial susceptibility testing to guide appropriate treatment decisions. For patients with infections caused by azithromycin-resistant bacteria, alternative antibiotics with different mechanisms of action may be more effective. This emphasises the significance of understanding

the susceptibility profiles of bacterial isolates to ensure optimal treatment outcomes and to combat the emergence of antibiotic resistance.

Cefotaxime, a third-generation cephalosporin antibiotic, exerts its bactericidal effects by disrupting bacterial cell wall synthesis. It achieves this by binding irreversibly to penicillin-binding proteins (PBPs), which are enzymes involved in the final stages of peptidoglycan synthesis in bacterial cell walls. By inhibiting these PBPs, particularly the transpeptidase enzymes, cefotaxime prevents the formation of cross-links between peptidoglycan chains, essential for the structural integrity of the bacterial cell wall. This interference leads to a weakened cell wall, making the bacterial cell susceptible to osmotic lysis and ultimately resulting in cell death. *Bacillus* species displayed a mixed response, with 4 isolates demonstrating resistance to cefotaxime, while 5 isolates exhibited sensitivity to the antibiotic. *Klebsiella* isolates showed a higher level of resistance, with 2 isolates being resistant and only 1 isolate showing sensitivity to cefotaxime. A concerning trend was observed among *Pseudomonas* species, where 10 isolates were found to be resistant to cefotaxime, outnumbering the 6 isolates that displayed sensitivity. In contrast, *Staphylococcus* species exhibited a moderate level of susceptibility to cefotaxime, with 2 isolates showing resistance and 4 isolates being sensitive to the antibiotic. Cefotaxime and other third generation cephalosporin are known to be generally more effective against Gram negative than Gram positive bacteria. However, the results showed that *Pseudomonas* and *Klebsiella* species had a higher level of resistance than sensitivity, this may be due to the production of beta-lactamases or alterations in PBPs, reducing the antibiotic's effectiveness in inhibiting cell wall synthesis. *Pseudomonas* possesses an outer membrane that acts as a protective barrier. Within this membrane, porin proteins form channels that control the passage of molecules into the periplasmic space. However, some porins in *Pseudomonas*

aeruginosa are more selective, allowing only smaller molecules, such as certain nutrients, to pass through efficiently. This reduced permeability of the outer membrane becomes a challenge when it comes to antibiotic treatment, particularly with antibiotics like cefotaxime which needs to enter the bacterial cell and bind to specific targets. The decreased ability of cefotaxime to pass through the porins of *Pseudomonas aeruginosa's* outer membrane contributes to the bacterium's inherent resistance to this antibiotic. As a result, cefotaxime may struggle to enter the bacterial cell and exert its antibacterial effects effectively. This reduced permeability is one of the mechanisms by which *Pseudomonas aeruginosa* evades the antibiotic action. *Klebsiella pneumoniae* can become resistant to cefotaxime through the production of Extended-Spectrum Beta-Lactamases (ESBLs), enzymes that can hydrolyze and deactivate cefotaxime. Also, some strains of *Klebsiella* can produce carbapenemases, enzymes that confer resistance to multiple beta-lactam antibiotics, including cefotaxime. On the other hand, sensitivity to cefotaxime indicates that the antibiotic can successfully bind to PBPs, leading to cell wall damage, compromised structural integrity, and eventual bacterial cell death. Considering the potential uses for ear infections, cefotaxime, although not a first-line choice, could be a valuable option for severe or complicated cases because of its broad spectrum of activity.

Interpreting the data on amoxicillin and augmentin resistance and sensitivity at 30µg and 10µg concentration respectively, revealed distinct patterns among the bacterial species. *Bacillus species* showed notable sensitivity, with 7 out of 9 isolates being sensitive to amoxicillin, suggesting its potential effectiveness against Bacillus infections. Conversely, *Klebsiella* isolates exhibited universal resistance, with all 3 isolates showing resistance to amoxicillin, highlighting challenges in treating *Klebsiella* infections with this antibiotic. *Pseudomonas* species showed a mix of sensitivity and resistance, with 9 isolates sensitive and 7 resistant to amoxicillin,

indicating some efficacy against certain strains but with ongoing concerns about resistance. *Staphylococcus* species displayed a moderate response, with 4 isolates resistant and 2 isolates sensitive to amoxicillin, suggesting a moderate effectiveness against *Staphylococcus* infections. Amoxicillin, a beta-lactam antibiotic, inhibits bacterial cell wall synthesis by binding to penicillin-binding proteins (PBPs) in the bacterial cell wall, leading to cell lysis and death. Augmentin, a combination antibiotic containing amoxicillin and clavulanic acid, works by the same mechanism, but with the addition of clavulanic acid which inhibits beta-lactamase enzymes, enhancing amoxicillin's effectiveness against beta-lactamase-producing bacteria. This mechanism of action disrupts the integrity of the bacterial cell wall, rendering the bacteria unable to maintain their structure and ultimately leading to cell death. However, the absence of a clear increase in efficacy of Augmentin over Amoxicillin in the obtained results may be attributed to several factors, including the inherent susceptibility of the bacterial isolates to Amoxicillin alone, variations in bacterial species' response to the combination, and the specificity of Clavulanic Acid primarily targeting beta-lactamase enzymes. These factors, alongside the sample size, testing concentrations, and duration of treatment in the study, could have contributed to the observed outcomes. However, both augmentin and amoxicillin are first line treatment of ear infections, because of their broad spectrum of activity, bactericidal effects and availability of different favourable dosage formulations.

Gentamicin is an aminoglycoside antibiotic that works by irreversibly binding to the bacterial ribosome, specifically the 30S subunit, causing misreading of the genetic code and inhibiting the formation of functional proteins. This disruption of protein synthesis leads to bacterial cell death, this makes them unique among other protein synthesis inhibitors in that they are bactericidal. Aminoglycosides are concentration-dependent antibiotics. Their effectiveness is related to

achieving a certain concentration in the body, known as the peak concentration, and higher peak concentrations are associated with increased bactericidal activity. However, the resistance to gentamicin often occurs due to the modification of the bacterial ribosome, reducing the binding affinity of the antibiotic. Additionally, bacterial production of enzymes, such as aminoglycoside-modifying enzymes, can inactivate gentamicin, leading to resistance. Considering the potential uses for ear infections, gentamicin is commonly used to treat various types of bacterial ear infections, including those caused by Gram-negative organisms like *Pseudomonas aeruginosa,* certain Gram-positive organisms such as *Staphylococcus aureus* as obtained from the results. Gentamicin's potential for treating ear infections despite its ototoxicity lies in its ability to be administered in a localised manner, such as in the form of ear drops. When used in this manner, the concentration of gentamicin reaching the inner ear is significantly lower compared to systemic administration, reducing the risk of ototoxicity. The ototoxic effects of gentamicin, which can lead to damage to the inner ear structures responsible for hearing and balance, are primarily associated with systemic use or high systemic doses. However, when administered directly into the ear canal, gentamicin's concentration is largely confined to the site of infection, limiting its systemic absorption. Additionally, the structure of the ear canal provides a barrier that prevents significant systemic absorption of gentamicin when applied topically. This means that the risk of ototoxicity is greatly minimized compared to systemic administration. Moreover, the benefits of gentamicin in treating severe or persistent ear infections often outweigh the potential risks of ototoxicity when used in a localized form. The antibiotic's potency against a wide range of bacterial pathogens, particularly those commonly implicated in ear infections, makes it a valuable option in situations where other treatments have failed or are not effective.

4.5 Antimicrobial susceptibility profile of facultative anaerobes using MIC

MIC is the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism after overnight incubation. By determining the MIC values for various antibiotics, we can ascertain the susceptibility or resistance of facultative anaerobes to these drugs.

From the results obtained, MIC using amoxicillin was done in varying concentrations of 100ug, 50ug, 25ug, 12.5ug and 6.25ug. *Bacillus* showed moderate sensitivity with a MIC of 25 µg, suggesting effectiveness with caution, while *Klebsiella* exhibited higher resistance at 50 µg, indicating the need for alternative antibiotics. *Pseudomonas* displayed sensitivity at 12.5 µg, making amoxicillin a potential treatment option in select cases. *Staphylococcus*, with a MIC of 50 µg, demonstrated higher resistance, particularly notable for methicillin-resistant strains. These results underscore the importance of tailored treatment plans, judicious antibiotic use, and ongoing surveillance to address antibiotic resistance effectively, ensuring optimal patient care and minimising the spread of resistant strains.

The results of Minimum Inhibitory Concentration (MIC) testing using varying concentrations of Augmentin (amoxicillin-clavulanate) against *Bacillus, Klebsiella, Pseudomonas*, and *Staphylococcus* provided crucial insights into the susceptibility profiles of these bacterial species to the antibiotic combination. For Bacillus, the mean MIC of 25µg for Augmentin indicated a moderate level of sensitivity. This suggested that Bacillus strains require a relatively moderate concentration of the amoxicillin-clavulanate combination to inhibit their growth. While not highly resistant, this moderate MIC value implies that Augmentin could be a viable treatment option for Bacillus infections. However, clinicians may need to consider the dosage and duration of treatment to ensure effectiveness. Moving on to *Klebsiella*, the mean MIC of 25µg for Augmentin suggested a similar moderate level of susceptibility, which indicated that *Klebsiella*

strains also require a moderate concentration of the amoxicillin-clavulanate combination for inhibition. While not highly sensitive, this finding implied that Augmentin may still have utility in treating ear infections caused by *Klebsiella*, particularly in cases where other antibiotics are not suitable due to resistance or other factors. In the case of *Pseudomonas,* the mean MIC of 12.5µg for Augmentin indicated a good level of susceptibility, which suggested that *Pseudomonas* strains showed a relatively better response to the amoxicillin-clavulanate combination compared to some other antibiotics. The lower MIC value implies that Augmentin could be an effective treatment choice for *Pseudomonas* ear infections, particularly those that are not highly resistant. Finally, *Staphylococcus* exhibited a mean MIC of 12.5µg for Augmentin, indicating a good level of susceptibility. This suggests that *Staphylococcus* strains required a moderate concentration of the amoxicillin-clavulanate combination for inhibition. This finding implies that Augmentin could be a suitable treatment option for *Staphylococcus* ear infections, including methicillin-resistant strains (MRSA) which often show resistance to other antibiotics. The implications of these results are significant for clinical practice. Augmentin, with its combination of amoxicillin and clavulanate, offers a broad spectrum of activity against a range of bacterial species. The moderate MIC values observed for *Bacillus, Klebsiella, Pseudomonas*, and *Staphylococcus* suggest that Augmentin could be a valuable treatment option for these infections. Clinicians may consider Augmentin as an empiric therapy choice for suspected bacterial infections caused by these organisms, especially when the specific pathogen is unknown or in cases where there is a possibility of mixed infections.

The results of Minimum Inhibitory Concentration (MIC) testing using ciprofloxacin against facultative anaerobic isolates, including *Bacillus, Klebsiella, Pseudomonas*, and *Staphylococcus*, held significant implications for the treatment of ear infections caused by these bacterial species.

Starting with *Bacillus*, the low mean MIC of 0.25µg suggested that ciprofloxacin is a highly effective treatment option for *Bacillus*-related ear infections. Given the antibiotic's ability to penetrate tissues effectively, it offers promise in targeting these infections, providing clinicians with a potent tool against this facultative anaerobic isolate commonly associated with ear-related ailments. However, *Klebsiella* had a mean MIC of 1µg indicating a somewhat lower susceptibility to ciprofloxacin compared to *Bacillus*. However, this still signifies a considerable level of sensitivity, suggesting that ciprofloxacin remains a viable treatment choice for *Klebsiella*-related ear infections. Its broad spectrum of activity and ability to reach high tissue concentrations make it a valuable option, particularly when considering cases of otitis media or other ear infections where *Klebsiella* is implicated. For *Pseudomonas*, the finding of a low mean MIC of 0.25 µg underscores the effectiveness of ciprofloxacin against this challenging pathogen. *Pseudomonas* is a common culprit in chronic and difficult-to-treat ear infections, often due to its resistance mechanisms. The high susceptibility to ciprofloxacin observed here presents a significant advantage in the treatment arsenal for *Pseudomonas*-related ear infections. Clinicians may rely on ciprofloxacin to target these infections effectively, providing relief to patients suffering from persistent or recurrent ear issues. Finally, *Staphylococcus,* including methicillin-resistant strains (MRSA), displayed a low mean MIC of 0.25 µg for ciprofloxacin. This finding is particularly noteworthy considering the prevalence of *Staphylococcus* in ear infections, especially those associated with otitis externa or middle ear infections. The high sensitivity to ciprofloxacin suggests its utility as a potent treatment option, offering clinicians an effective means to combat *Staphylococcus*-related ear infections, including those caused by resistant strains.Therefore, ciprofloxacin is a versatile and reliable antibiotic choice for facultative anaerobic isolates such as *Bacillus, Klebsiella, Pseudomonas*, and *Staphylococcus*. Its ability to

penetrate ear tissues effectively, coupled with its broad spectrum of activity and low MIC values against these pathogens, positions it as a valuable tool in the management of various ear-related ailments. Clinicians can consider ciprofloxacin as a first-line or empiric therapy option for suspected bacterial ear infections, aiming to provide prompt and effective treatment while minimising the risk of complications or recurrence. However, there are limitations associated with the use of ciprofloxacin, this includes its ability to cause severe reactions such as tendonitis or central nervous system effects, particularly in vulnerable populations like children or the elderly.

The results of Minimum Inhibitory Concentration (MIC) testing using Septrin (sulfamethoxazole-trimethoprim) against *Bacillus, Klebsiella, Pseudomonas,* and *Staphylococcus* provided important insights into the susceptibility profiles of these bacterial species and their implications for ear infections treatment. Beginning with *Bacillus*, the mean MIC of 10µg for Septrin indicated a moderate level of susceptibility. This suggests that *Bacillus* strains may respond to Septrin treatment, but at a slightly higher concentration. While Septrin could potentially be effective against *Bacillus*-related ear infections, clinicians must consider its moderate susceptibility and possibly higher dosage requirements. For *Klebsiella, Staphylococcus* and *Pseudomonas*, the mean MIC of 20µg for Septrin suggests a moderate to low level of susceptibility. This indicated that *Klebsiella, Staphylococcus* and *Pseudomonas* strains may exhibit reduced sensitivity to Septrin, requiring a higher concentration of the antibiotic for inhibition. While Septrin could still be considered as a treatment option for *Klebsiella, Staphylococcus* and *Pseudomonas* related ear infections, its effectiveness might be limited, particularly in cases where higher MIC values are observed. For ear infections treatment, the moderate to low susceptibility of *Bacillus, Klebsiella, Pseudomonas,* and *Staphylococcus* to

Septrin raises considerations for its use. Septrin may be a potential treatment option for these bacterial species, especially in cases where other antibiotics are contraindicated or unavailable. However, the observed MIC values suggest that Septrin might be more effective against some strains than others, highlighting the importance of bacterial identification and susceptibility testing before prescribing. Limitations associated with Septrin use in ear infections treatment must also be acknowledged. Firstly, the potential for antibiotic resistance development poses a concern, particularly with prolonged or inappropriate use of Septrin. Bacterial strains may develop resistance mechanisms, rendering Septrin less effective over time. Secondly, Septrin is known to have a higher risk of adverse effects, such as allergic reactions or gastrointestinal disturbances, which can impact patient tolerance and adherence to treatment. Additionally, Septrin may not be suitable for certain patient populations, including pregnant women, infants, and individuals with folate deficiencies. Special caution should be exercised in these cases to avoid potential complications. Lastly, the variability in susceptibility among different bacterial strains emphasises the need for individualised treatment plans and careful monitoring of patient response.

CHAPTER FIVE

CONCLUSION

In conclusion, frequent wearing of earrings, history of previous ear infections, and lifestyle factors such as swimming, alcohol intake and smoking were associated with high levels of ear infections in the study population. Notably, more females and individuals aged 16-25 were more encountered, and exhibited a significant frequency of ear infections than their male counterparts and the other age groups in this study. *Pseudomonas spp* was more prevalent in this study, total of 16 isolates (47%) and was found to be more sensitive to Azithromycin(87.5%), fluoroquinolones (81.25) as well as Amoxicillin and Amoxicillin clavulanic combination with a mean MIC of 12.5ug.

However, the implications of this research hold promise for improved clinical outcomes and enhanced antibiotic stewardship practices. By integrating these susceptibility profiles into the decision-making process, clinicians can navigate the complexities of ear infections with greater precision and efficacy. This project not only deepens our understanding of the microbial landscape within the ear but also explains the importance of safe and meticulous research practices in the ENT clinic setting. As we continue to refine our approach, guided by these findings, we make way for a future where patients with anaerobic ear infections receive optimal and personalised care, marking a significant stride towards improved quality of life and well-being.

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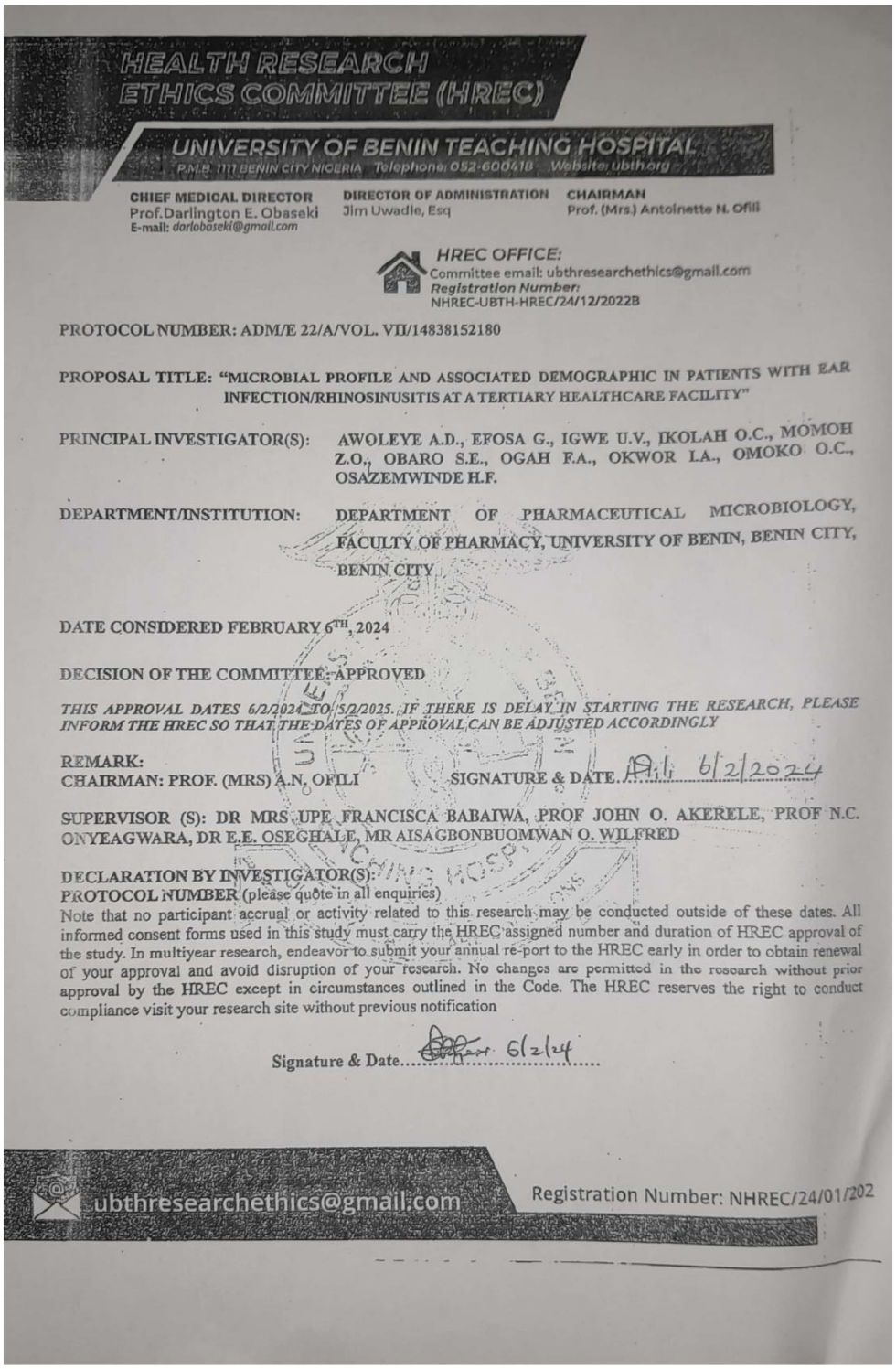
APPENDIX

Ethical Approval



HEALTH RESEARCH ETHICS COMMITTEE (HREC)

UNIVERSITY OF BENIN TEACHING HOSPITAL

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Registration Number:
NHREC-UBTH-HREC/24/12/2022B

PROTOCOL NUMBER: ADM/E 22/A/VOL. VII/14838152180

PROPOSAL TITLE: "MICROBIAL PROFILE AND ASSOCIATED DEMOGRAPHIC IN PATIENTS WITH EAR INFECTION/RHINOSINUSITIS AT A TERTIARY HEALTHCARE FACILITY"

PRINCIPAL INVESTIGATOR(S): AWOLEYE A.D., EFOSA G., IGWE U.V., IKOLAH O.C., MOMOH Z.O., OBARO S.E., OGAH F.A., OKWOR I.A., OMOKO O.C., OSAZEMWINDE H.F.

DEPARTMENT/INSTITUTION: DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY, BENIN CITY

DATE CONSIDERED FEBRUARY 6TH, 2024

DECISION OF THE COMMITTEE: APPROVED

THIS APPROVAL DATES 6/2/2024 TO 5/2/2025. IF THERE IS DELAY IN STARTING THE RESEARCH, PLEASE INFORM THE HREC SO THAT THE DATES OF APPROVAL CAN BE ADJUSTED ACCORDINGLY

REMARK:
CHAIRMAN: PROF. (MRS) A.N. OFILI SIGNATURE & DATE. A.N.O. 6/2/2024

SUPERVISOR (S): DR MRS UPE FRANCISCA BABAIWA, PROF JOHN O. AKERELE, PROF N.C. ONYEAGWARA, DR E.E. OSEGHALE, MR AISAGBONBUOMWAN O. WILFRED

DECLARATION BY INVESTIGATOR(S):
PROTOCOL NUMBER (please quote in all enquiries)
Note that no participant accrual or activity related to this research may be conducted outside of these dates. All informed consent forms used in this study must carry the HREC assigned number and duration of HREC approval of the study. In multiyear research, endeavor to submit your annual re-port to the HREC early in order to obtain renewal of your approval and avoid disruption of your research. No changes are permitted in the research without prior approval by the HREC except in circumstances outlined in the Code. The HREC reserves the right to conduct compliance visit your research site without previous notification

Signature & Date..... 6/2/24

ubthresearchethics@gmail.com Registration Number: NHREC/24/01/202

SEMI STRUCTURED QUESTIONNAIRE; PATIENTS WITH EAR INFECTION

Section A: Personal Information

1 .PATIENT I.D: _____ Date: _____

2.Age: 0-15yrs 16-25yrs 26-35yrs 36-45yrs 46-55yrs 56-60yrs >60yrs

3. Sex:M F Phone: _____ Height: __ (cm) Weight:__(kg) Occupation: _____

Section B: Health and Lifestyle History

1. Smoking history:

Never smoked Stopped smoking Still smoking

2. Alcohol history:

Never drank Stopped drinking Still drinking

3. Medical History: (Please provide details)

_____

Section C: Medication History

4.Past Medication History: (Please provide details)

_____

5.Present Medication History: (Please provide details)

_____

Section D; Social Activities

1.Swimming history

Never swam stopped swimming still swimming

2.Ear cleaning habit;

Do you clean your ears?

Yes Not often Never

3.What do you use in cleaning your ears

Cotton buds Biro tips feather other objects,please specify_____

4.Recently pierced your ears

Yes no

5.How often do you wear earrings

Only for events everyday weekly once a month

Section E; Ear infection symptoms

1.What are your present symptoms?

Pain in the ear discharge poor hearing fever Other symptoms,please specify

_____

2.Have you had previous ear infection?

Yes no

If yes, which ear did it affect?

Right left both ears

3.Have you experienced any change in air movement ?

Yes no

4.Do you have any nasal congestion or discharge?

Yes no

Section F: Microbial Infection History

1. Have you had previous microbial infections that warranted the use of antibiotics?

Yes No If yes,

what type of infection; respiratory infection

Peptic ulcer urinary tract infection wound infection others_____

2. how often do you use antibiotics for any infection in a year

Never once twice three times > three times

3. do you complete your antibiotics therapy

yes no I stop the drug once I feel better

4. Have you used gentamicin,amikacin,streptomycin or tobramycin antibiotics injection before?

Yes no

Section G; comorbidities

1. Do you have any existing medical conditions or comorbidity?

Asthma COPD rhinitis/sinusities HIV GERD structural abnormality others_____

Section H: Antimicrobial Susceptibility Profile

1. Have you previously undergone antimicrobial susceptibility testing prior to sinusitis treatment?

Yes No

2. How effective was the treatment based on the results of the susceptibility test? Please describe.

_____

Section I: Additional Comments

1. Do you have any additional comments, concerns, or information you'd like to share regarding your experience with sinusitis or its treatment?

_____

2. Is there anything else you believe is important for the research team to know?

_____

Section J: Consent

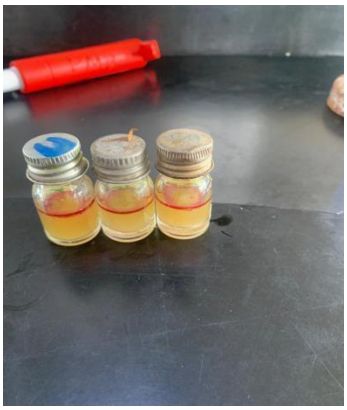
4.do you agree to participate in this research study?

Yes No

Signature: _____ Date: _____

PICTURES FROM THE LABORATORY:

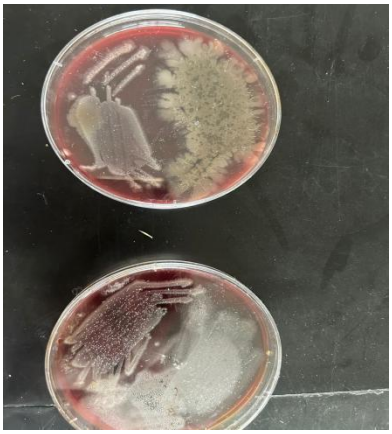
Positive test for citrate (green to blue)



Positive test for indole(red ring formed at the top)

Taking readings of inhibition zone diameter

Antimicrobial susceptibility using MIC(broth dilution)



Hemolytic pattern of isolates on blood agar

age of patient

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0-6yrs	2	5.9	5.9	5.9
	7-15yrs	4	11.8	11.8	17.6
	16-20yrs	4	11.8	11.8	29.4
	21-25yrs	9	26.5	26.5	55.9
	26-30yrs	2	5.9	5.9	61.8
	31-35yrs	2	5.9	5.9	67.6
	46-55yrs	1	2.9	2.9	70.6
	56-60yrs	2	5.9	5.9	76.5
	>60yrs	8	23.5	23.5	100.0
	Total	34	100.0	100.0	

location of pain

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	inside ear	31	91.2	91.2	91.2
	both	3	8.8	8.8	100.0
	Total	34	100.0	100.0	

patients ear cleaning habit

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	16	47.1	47.1	47.1
	not often	14	41.2	41.2	88.2
	never	4	11.8	11.8	100.0
	Total	34	100.0	100.0	

patients occupation

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	business	1	2.9	2.9	2.9
	civil se	6	17.6	17.6	20.6
	counsell	1	2.9	2.9	23.5
	matron	1	2.9	2.9	26.5
	nill	16	47.1	47.1	73.5
	retired	1	2.9	2.9	76.5
	sale rep	1	2.9	2.9	79.4
	student	4	11.8	11.8	91.2
	Student	1	2.9	2.9	94.1
	trader	2	5.9	5.9	100.0
	Total	34	100.0	100.0	

organism detected

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Bacillus cerus	1	2.9	2.9	2.9
	Bacillus subtilis	8	23.5	23.5	26.5
	Klebsiella pnemoniae	1	2.9	2.9	29.4
	Klebsiella pneumoniae	2	5.9	5.9	35.3
	Pseudomonas aeruginosa	16	47.1	47.1	82.4
	Staphylococcs aureus	1	2.9	2.9	85.3
	Staphylococcus aureus	5	14.7	14.7	100.0
	Total	34	100.0	100.0	

swimming history

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	never swam	31	91.2	91.2	91.2
	stopped swimming	1	2.9	2.9	94.1
	still swimming	2	5.9	5.9	100.0
	Total	34	100.0	100.0	

alcohol history

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	never drank	22	64.7	64.7	64.7
	stopped drinking	5	14.7	14.7	79.4
	still drinking	7	20.6	20.6	100.0
	Total	34	100.0	100.0	