PLASMID CURING OF MULTI-DRUG RESISTANT BACTERIAL ISOLATES FROM PATIENTS WITH SINUSITIS

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

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CERTIFICATION

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

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CERTIFICATION OF THESIS ON PLAGIARISM

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DEDICATION

To my beloved parents Mr. and Mrs Igwe, I deeply appreciate you, for giving me the enablement and providing the resources to undertake this important journey and for being as a source of encouragement through it all. I Also dedicate this Research project to God Almighty who has indeed kept and guided me all through this journey.

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ABSTRACT

Background: The nasal cavity hosts a wide array of organisms. Pathogenic organisms may find access into the sinuses leading to infection. Plasmids, which are small extracellular DNA molecules, play a role by transferring resistance encoded genes among bacteria, facilitating the spread of antibiotic resistance. By investigating the role of plasmid genes in potential treatment failures, the research seeks to broaden our understanding on how plasmids mediate antibiotic resistance. This knowledge is key for developing effective strategies to combat sinusitis diseases using antimicrobial agents.

Methods: The study evaluated forty-three patients from the Ear Nose and Throat Clinic of the University of Benin Teaching Hospital, Benin city. Patient's data collected were age, gender, alcohol history, smoking history, medical history. Isolates obtained were subjected to antimicrobial susceptibility testing and plasmid curing with acridine orange as the curing agent using standard agar disc diffusion method.

Results: A total of 58.1% of our study participants were female, and 41.9% were male. Participants aged 16-25 had the highest sinusitis occurrence (27.9%). Nasal discharge was the most encountered symptom across all participants. *Staphylococcus aureus* dominated in the nasal cavity of study participants (35.1% aerobic) compared to other organisms isolated. *Pseudomonas aeruginosa* accounted for the highest abundance (31.3%) under anaerobic condition. Multi-drug resistance was observed in 23.4% and 25% of total aerobic and anaerobic isolates respectively. Resistance to fluoroquinolones was lost post curing in *Staphylococcus spp, Klebsiella spp.*

Conclusion: Females were more susceptible to sinusitis with recurrent episodes experienced compared to males. The presence of plasmid-mediated multidrug resistance genes underscores the need for antibiograms, and rational antibiotics use in sinusitis treatment.

Keywords: Nasal cavity, Demographics, bacteria, resistance, plasmid.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Background of study;

Sinusitis is one of the most commonly diagnosed diseases in primary health care facilities. It causes substantial morbidity and it is one of the major diseases that accounts for the prescription of antibiotics (Kim,2007). Sinusitis is an inflammation of the mucous membranes lining the sinuses (Miller 2009). The sinuses are membranes air-filled tubes and pockets behind the nose and inflammation due to pathogens results in obstruction and poor drainage of the sinuses. Bacterial and viral invasion as well as allergens can irritate these sinuses causing them to get blocked and filled with fluid. A disturbance in mucocillary clearance and inflammatory factors are also potential causes of this inflammation. (Hamilos,2000). Sinusitis may be acute, sub-acute, chronic or recurrent depending on the severity (Richard,2016). Diagnosis of acute bacterial sinusitis is based on associated symptoms such as the presence of purulent nasal discharge accompanied by nasal obstruction, facial pain, pressure or fullness that persist or worsens within 10days of initial improvement (Richard,2015). According to Richard (2015), sinusitis is classified into chronic sinusitis where the duration of disease spans beyond 12weeks with or without exacerbation and acute sinusitis where duration of disease is less than 4weeks. A situation where a patient has 4 or more annual episodes of sinusitis is regarded as Recurrent acute sinusitis. In a research work done by Richard (2015) about 0.5-2.0% of sinusitis caused by virus are complicated by the presence of bacteria and this causes further obstruction of the sinuses by the deposition of nasal bacteria into the sinuses.

1.2 Epidemiology of sinusitis

Western literature has reported sinusitis to be more prevalent than arthritis or hypertension, affecting between 5% and 15% of studied populations. According to a recent analysis of US National Health Interview Survey data, sinusitis affects approximately 1 in 7 adults. (Olushola *et al.,* 2017). Women appear to be affected more than men, and the most commonly affected age group among adults is mid-40s to mid-60s (Zara *et al.,*, 2018).A study by Shi *et al.,*,2015 showed that a total of 10,636 respondents from seven cities in China participated in the study. In these seven centers, the prevalence of CRS varied from 4.8% to 9.7%, with a mean prevalence of 8.0%. In mainland China, chronic sinusitis afflicted about 107 million people. People with certain medical disorders, such as gout, asthma, allergic rhinitis, and chronic obstructive pulmonary disease, were more likely to have chronic sinusitis. The prevalence varied by age group, ethnicity, marital status, and education ($P < 0.05$), but not by household per capita income or living space ($P > 0.05$). Males had a slightly greater prevalence (8.79%) than females (7.28%) ($P = 0.004$). There were two distinct risk variables for CRS: secondhand tobacco smoke and current smoking ($P = 0.001$).

However, there are no available statistics for sinusitis in Nigeria, data is only available for few regions of Nigeria. For example, according to a research by Adegbiji *et al.,* 2022 at the University Hospital in Ekiti, southwest Nigeria, 8.3% had sinusitis with 77.6% being chronic and 22.4% being acute cases. Also, a study by Afolabi *et al.,* 2017 described sinusitis as one of the most prevalent otorhinolaryngology illnesses that accounts for 7.92% of all cases seen throughout the study period. This number is greater than that of a different study done in northwest Nigeria by Iseh and Makusidi, who discovered a prevalence of 7.3%. this result, however, is within the range described by Hopkins *et al.,* in 2007, who stated that RS affects 5% to 15% of the populations they studied and is more common than hypertension or arthritis. Several variables, including patient demographics, antibiotic usage patterns, and

geographic location, can affect the prevalence of multi-drug resistance among bacterial isolates recovered from sinusitis patients. Studies have shown varying prevalence rates worldwide, with some regions experiencing higher levels of multi-drug resistance than other. In general, sinusitis is a growing health issue that is linked to health status, allergies, smoking and medication.

1.3 Anatomy of the paranasal sinuses

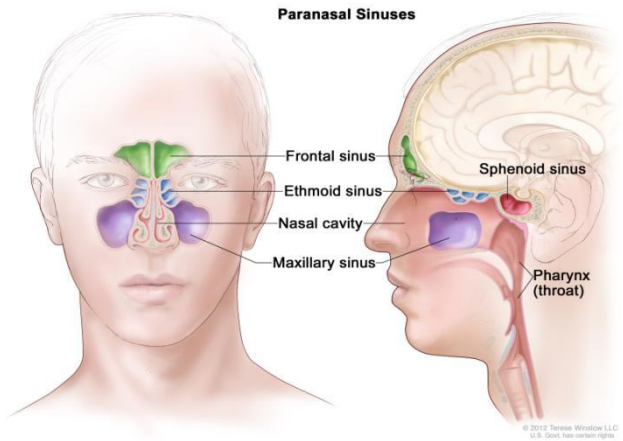

Fig.1 diagram of the paranasal sinuses (Bethesda,2023)

In a research work by Singh (2017), the sinuses were described as air-filled spaces located within the bones of the skull and exist as four (4) sets of paired sinuses. These include the maxillary sinuses which are the largest sinuses and lies beneath the eyes in the maxillary bone. It is the first sinus to develop and it is filled at birth. The maxillary sinuses open into the center of the semi-lunar hiatus found in the lateral wall of the middle nasal meatus. The anterior wall of the sinus corresponds to the facial surface of the maxillary bone and houses the infraorbital nerve which runs through the infraorbital canal along the roof of the sinus and send branches to the soft tissues of the cheek. The roof of the maxillary sinus is known as the superior wall and it is the floor of the orbit which is innervated by the infraorbital nerve and supplied by the infraorbital artery. The sinus floor is also known as the inferior wall and it is

in proximity with the posterior teeth apices from which it is separated by a layer of compact bone. Studies carried out by Cappello (2023) showed that the first molars perforate the sinus floor in 2.2% cases and the second molar in 2.0% cases. The maxillary sinus is lined with mucus producing ciliated pseudostratified columnar epithelium and these cilia perform the role of draining mucous into the nasal ostia.

The frontal sinuses are located superior to the orbit within the frontal bone. It is pyramidal in shape and this can vary depending on ethnicity and climate. The frontal sinuses are highly air filled and drain via the frontonasal duct which opens up at the hiatus semilunaris within the middle meatus of the nasal cavity. The frontal sinuses are innervated by the supraorbital nerve and supplied by the anterior ethmoidal artery.

The ethmoid sinuses are formed by a conglomerate of cells with an intricate structure through which all paranasal sinuses drain. They are located between the eyes on either side of the septum. At birth, there are about 3-4 pairs of air pockets which develops to about 5-15pairs by adulthood with a total volume of 2-3mL. The ethmoid sinuses are supplied by the anterior and posterior ethmoidal arteries from the ophthalmic artery.

The Sphenoid Sinus originates in the sphenoid bone at the center of the head .it is located centrally and posteriorly within the body of the sphenoid bone. The sinuses are divided by a bony wall and they discharge their mucus through an opening in the front wall of the sinus into the nose.

1.4 Pathophysiology of sinusitis

The function of the sinuses is to humidify and heat up inhaled air, increase resonance of speech, lighten the weight of the head, filter out foreign materials such as microorganisms, dust, pollutants and other antigens. A study done by Zele *et al.,*2011 described the nose as the first contact of the respiratory system with the external environment. This contact

predisposes the nasal mucosa to a great range of air borne pathogens and allergens. The sinuses function by draining into the intranasal meatus by small channels called Ostia. The middle meatus and the superior meatus work in conjunction with hair-like structures in the nasal cavity called the cilia to circulate mucus and filtered debris to the nasopharynx and oropharynx where they are swallowed. Ineffective clearance of these foreign materials due to viral infection are attributed to obstruction of the sinus ostia, dysfunction of the cilia or thickening of sinus secretion which result in acute sinusitis of viral origin. The nasal mucosa responds to the virus by producing mucus and recruiting mediators of inflammation, such as white blood cells, to the lining of the nose, which cause congestion and swelling of the nasal passages. The resultant sinus cavity hypoxia and mucus retention cause the cilia which move mucus and debris from the nose to function less efficiently, creating an environment for bacterial growth and proliferation in the usually sterile paranasal sinuses (Derek and Edward, 2023). If the acute viral sinusitis does not resolve, chronic sinusitis can develop due to mucus retention, hypoxia, and blockade of the ostia. This promotes mucosal hyperplasia, continued recruitment of inflammatory mediators, and the potential development of nasal polyps. However, other factors can predispose to sinusitis. The viral infection usually resolves without treatment in less than 14 days. A secondary Bacterial Infection is diagnosed If symptoms worsen after 3 to 5 days or persist for longer than 10 days and are more severe than normally experienced with a viral infection. Zele *et al.,* (2011) attributed Gram positive cocci bacteria as a frequent colonizer of the nasal cavity which has the ability to invade and survive in respiratory epithelial cells where they secrete enterotoxins which initiate an inflammatory response. The inflammation can predispose to the development of acute sinusitis by causing sinus ostial blockage. Although inflammation in any of the sinuses can lead to blockade of the sinus ostia, the most commonly involved sinuses in both acute and chronic sinusitis are the maxillary and the anterior ethmoid sinuses.

1.5 Bacterial isolates in sinusitis

According to a research by Brook (2010) the upper respiratory tract houses several pathogens which may predispose a patient to an infection such as Sinusitis. Bacterial infection often follows a viral infection and it is regarded as a complication of acute viral sinusitis. Inflammation of the sinuses may be attributed to a host of microbes which exist as a community and serve as a disease potentiator. Studies by Brook, 2010 showed that there is a variation existing between the sinuses of a healthy patient and that of patients with sinusitis. Invariably, pathogens with high propensity to cause infection have been isolated from healthy nasal cavity. The normal flora of the nasal cavity includes *Staphylococcus aureus, Staphylococcus epidermidis,* α and γ-*Streptococc*i, *Propionibacterium acne* and aerobic diphtheroid. The sterility of the sinuses is questionable as there is continuous communication and interaction between the nasal cavity which is a known bacterial reservoir and the sinuses through the ostia. This interaction enables bioburden in the nasal cavity or nasopharynx to gain access into the sinuses leading to infection. A study by Pourmousa *et al.,* 2015 showed that the most common bacterial isolated from patients with sinusitis as well as post nasal drip includes aerobic Gram-negative and Gram-positive bacteria as well as Anaerobic bacteria*(*Bhattacharyya & Kepnes, 1999).

1.6 Antimicrobial susceptibility profile

The best way to ascertain the clinical effectiveness of a particular antimicrobial agent is by subjecting isolated organisms to various antimicrobial agents with a goal of detecting organisms that may have developed or acquired resistance to a particular antimicrobial agent as well as ascertain that the drug of choice is able to halt or eliminate the particular causative organism .(Reller *et al.,* 2009). Antimicrobial susceptibility testing provides quantitative and qualitative results. Quantitative result as it determines what concentration is effective enough to prevent the proliferation of bacteria. Qualitative result as it classifies microbes into

susceptible, intermediate and resistant. To cater for qualitative result analysis and interpretation, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) expert rules have been designed as a measure to assist in the inference of susceptibility profile of clinically related pathogens. EUCAST uses clinical breakpoints obtained from clinical and microbiological evidence through susceptibility testing involving determination of Minimum Inhibitory Concentration (MIC) and Inhibitory Zone Diameter (IZD) (Leclercq *et al.,* 2013). Clinical laboratories use several methods to conduct antimicrobial testing depending on availability of equipment and the nature of experiment to be conducted. Classical antimicrobial testing involving phenotypic testing uses methods such as disc diffusion method and gradient diffusion method (Gajic *et al.,* 2022).

1.6.1 Disc diffusion method;

This is also known as Kirby-Bauer test for antibiotics sensitivity. It is performed by inoculating the surface of agar with isolated bacteria and an antibiotics-containing paper disc is placed on top and the plate can incubate at suitable temperature. The theory behind this method is that when a filter paper disc that is impregnated with a known concentration of a particular antibiotics agent is placed on the surface of an already inoculated mueller-hinton agar, water is absorbed into the disc and causes the antimicrobial agent to diffuse across the surface of the agar and attacks the bacterial isolate and forms a clear zone (Hudzicki, 2009). A clear zone infers that the antibiotics was able to kill the bacteria and this zone is known as Zone of Inhibition. The size of these zones can be related to EUCAST break-point which factors in the rate of diffusion of the antimicrobial agent as well as size of the molecules and the isolate can then be classified as resistant, intermediate or susceptible.

1.6.2 Agar dilution method;

This is one of the most suitable method for determining the minimum inhibitory concentration of a particular antimicrobial agent to clinically obtained microbes. Agar dilution involves the addition of a known concentration of antimicrobial substance into molten nutrient or mueller-hinton agar (Wiegand *et al.,* 2008). The antibiotics to be tested is first diluted in a solvent that will not produce a false positive result. Hence water is mainly used as a diluent. The antibiotics is diluted serially to produce several concentrations until the final concentration is achieved which is then incorporated into molten agar and poured. Bacteria prepared to a standard concentration is then spotted on the surface of the agar and incubated at suitable temperature. The plates are then observed for growth. The least antibiotics concentration that halted the growth of the bacteria is regarded as the minimum inhibitory concentration of that antimicrobial agent against that microbe.

1.7 Mechanism of multidrug resistance and role of plasmids

The incidence of bacterial resistance to antimicrobial agents increases at an exponential rate as against the discovery and production of newer antimicrobial agents. This poses a crucial public health issue as there are little to no effective antimicrobial drugs to combat these resistant strains of microorganisms (Kaushiki, 2022). Multi Drug Resistant (MDR) Bacteria strains in the past were linked to nosocomial infections but in recent times, these strains have become more common and this has resulted in therapeutic failure in the use of antimicrobial agents, morbidity, increase cost of treatment and irrational use of antibiotics. A study done by Rezai *et al.,* (2016) in Iran showed that empirical antibiotics therapy is often used in the treatment of sinusitis and the emergence of antibiotics resistance has countered the success rate of this treatment approach. Multi drug resistance occurs when three or more classes of antimicrobial agents cannot eradicate or inhibit the growth of a particular strain of

bacteria (Terreni *et al.,* 2021). This resistance may be innate or acquired. Innate resistance is attributed to chromosome-linked inherited genes or traits present in a bacterial cell which encode for resistance. This may express as development of efflux pumps which are membrane bound transporters that actively export multiple antibiotics from the internal cellular fluid of the bacterial cell to the outer environment (Nishino *et al.,* 2021). Poor permeability of lipopolysaccharide layer to antimicrobial agents as seen in gram negative organisms is also considered an innate mechanism of resistance. Some bacteria may secrete degrading enzymes which alter the integral structure of antimicrobial agents and render them inactive. Alteration of drug target site causes a disorientation in drug binding to these target site leading to no antimicrobial activity (Bharadwaj *et al.,* 2022). The advent of a destructive agent in the bacterial cell signals an SOS response which causes DNA repair and mutagenesis to combat oxidative stress as well as confer resistance to antimicrobial agents (Memar *et al.,* 2020).

Acquired resistance occurs through spontaneous mutation and acquisition of genetic materials such as plasmids from another bacteria (Vinayamohan *et al.*, 2022). One approach to ascertain the general mechanisms of bacterial drug resistance is to expose the bacterial cells to procedures that result in plasmid removal, since antibiotic resistance in bacteria can be either chromosomal or plasmid mediated (Churchill and Akpe,2019). Plasmid has impacted on several areas of bacterial evolution including acquisition of genes involved in virulence, ecological interaction and antimicrobial resistance (Schroeder *et al.,* 2017). These Plasmids are minute circular extrachromosomal molecules present in a host bacterial cell that are independent of the host in order to replicate (Carattoli,2011). plasmids offer no benefit towards the growth and replication of the host cell as they carry no gene for this role. Rather, they promote the survival of the organism through antibiotics resistance by mobilizing accessory genes via horizontal gene transfer (Rodríguez-Beltrán *et al.,* 2021). plasmids which

confer antibiotics resistance may encode resistance to more than one class of antibiotics and when picked up by a previously susceptible bacteria, may cause resistance to all classes of antibiotics encoded in the plasmid (Bennett,2008). Plasmids exist as Conjugative Plasmids which allow for cell-to-cell transfer of genetic material and Mobilizable Plasmids which are fewer in number and require a conjugative plasmid co-resident in the cell to encode conjugation function to permit cell-to-cell transfer of genetic material. Bacteria which can undergo horizontal gene transfer possess a sex pilus in the donor bacterium which copulates with the recipient bacterium and forms a mating bridge which allows for the for the transfer of genetic material -Plasmid (Goldlust *et al.,* 2020). Hence due to the clinical and economic disadvantages posed by these resistant plasmid genes, it is necessary and of great importance to remove or eliminate these resistant factors by the use of plasmid curing agents in an attempt to curb the wide spread of multi-drug resistance in patients with sinusitis.

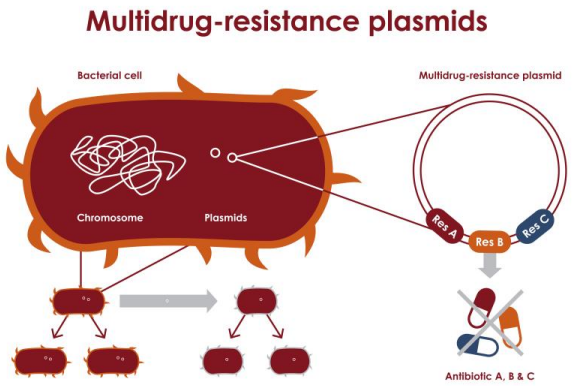

Fig. 2 Plasmid mediated resistance (Hughes and Datta,1983)

1.8 Plasmid curing agents;

Plasmids generally are unstable and may detach from their host when conditions become unfavorable. However, many are stable and warrant the use specific agents in order to eliminate them. Plasmid curing hence is a process of removing plasmid encoded antibiotics resistance function through the use of curing agents that are either physical (such as exposing host strain to increased temperature) or chemical (which involves the use of chemical agents such as intercalating dye) and offer the attribute of halting the replication of plasmids as well as knock off plasmids from host cells (Patwardhan *et al.,* 2018). Plasmid curing is used as an approach towards displaying the role of plasmid in antimicrobial resistance (Zaman *et al.,* 2010).

Curing plasmids from a bacterial strain enables a direct comparison to be conducted between the plasmid carrying and plasmid cured cells, substantiating the association between a geneti c property and carriage of that specific trait in the plasmid (Rezaee *et al.,* 2007). These curing strategies are designed to facilitate the removal of plasmids from host cells in order to obtain plasmid-free cells that can be used to evaluate plasmid-based metabolism (Andersen *et al.,* 1981). Intercalating Dyes such as acriflavine, Acridine orange, ethidium bromide and quinacrine act as curing agents (Litake, 2022). Acridine orange, which is a mutagen and a typical intercalating dye is used as a plasmid curing agent due to its ability to interact with plasmid DNA by means of intercalation due to the planar structure of acridine orange (Crémieux *et al.,* 1995). Acridine molecule fit s into the DNA base pair of plasmid DNA and results in a frame-shift mutation (Kumar and Tripathi, 1985). Acridine have been shown to attach to DNA in vitro and release free radicals when energy is transferred from dye molecules to the DNA backbone, most likely in the duplex's A +T rich areas (Graslund *et al.,* 1969). This causes the loss of replication function of plasmids DNA and coherently, loss of antimicrobial resistance profile. Acridine orange also facilitates

inactivation of repair processes stimulated by the presence of a stressor (Barker and Hardman, 1978). This repair is normal in growing culture but the presence of acridine orange causes a defect in the acridine sensitive-gene *polA* and causes loss of repair function (Southwick *et al.,* 1972). Acridine orange has been used for plasmid curing at doses ranging from 0.05 to 0.2mg/ml (Letchumanan *et al.,* 2015). Acridine orange is used also in laboratory procedures as a DNA stain and visualizing agent in fluorescence microscopy as it emits an orange-red fluorescence when intercalated into DNA. Owing to its mutagenicity, cytotoxicity and potential to cause DNA damage, acridine orange is not used for in-vivo studies.

Sodium dodecyl sulphate also known as sodium lauryl sulphate is an anionic surfactant which denatures proteins such as DNA polymerases, helicases, and primases necessary for Plasmid DNA replication. They act by binding to the hydrophobic region of these protein and cause a conformational change to the molecular structure of the protein leading to loss of function (Moriyama and Takeda, 1999). However, little study has been done towards the use of sodium lauryl sulphate as a plasmid curing agent as the chemical agent is primarily used as a denaturing agent in laboratory procedures (Di Carlo *et al.,* 2005). Although it was used at a concentration range of 0.002-10% by Sonstein and Baldwin (1971) for the curing of penicillinase plasmid in *Staphylococcus aureus.*

For plasmid DNA to replicate, the DNA gyrase enzyme is required to catalyze a winding effect called Supercoiling of DNA strand. If this supercoiling is lost due to the inhibition of gyrase enzyme, there would be a halt in plasmid DNA replication and consequently elimination of plasmids. DNA gyrase inhibitors such as quinolones, novbiocin, clorobiocin target the A and B-subunit of gyrase enzyme (Hooper *et al.,* 1983). concentration range of 1-7 µg/m coumermycin has been used in the curing of ColE1 plasmids from *E. coil K12C600* (Trevors, 1985). As a limitation, DNA gyrase inhibitors may produce a false negative result

as they may act directly on the host cell and cause a damaging effect on bacterial DNA gyrase enzyme as oppose to plasmid DNA gyrase (Hooper *et al.*, 1983).

Mitomycin C is an antimicrobial agent with the capability of causing damage to DNA via DNA cross linkage (Wei *et al.,* 2001). Mitomycin C binds to DNA molecule and forms a link between the two strands of DNA. Mitomycin C is then reduced to hydroquinone which react with purine bases such as guanine and adenosine to form a reactive intermediate (Hooper *et al.,* 1983). This process activates bacterial stress response in order to correct the pending DNA damage (Dronkert and Kanaar, 2001). This repair to DNA causes the loss of plasmid thus resulting in the loss of resistance function. Mitomycin C is often used at relatively low concentrations, typically in the range of 0.1 to 1 microgram per milliliter (µg/mL) of bacterial culture (Dörr *et al.,* 2009). the use of mitomycin C clinically is limited due to its ability to cause bone marrow toxicity (Sinawe,2023).

Rifampicin attaches directly to RNA polymerase enzyme responsible for plasmid DNA replication and segregation (Trevors, 1985) and this results in variation in plasmid expression. A research work by Bazzicalupo and Tocchini-Valentini (1972), suggested that rifampicin inhibited the first reaction in RNA transcription which is catalyzed by RNA polymerase (Boy *et al.,* 2022). When rifampicin binds to this enzyme, It alters with the synthesis of RNA from DNA templates. This, inhibition disrupts not only the transcription of chromosomal genes but also the transcription of plasmid genes, including those involved in plasmid replication and maintenance (Sutormin *et al.,* 2022). At concentration of 0.01µg/mL, Rifampicin was employed as a curing agent which led to the loss of resistance to cadmium and erythromycin (Trevors,1985). the draw back in the use of rifampicin is the incidence of cytotoxicity and the propensity to result in antimicrobial resistance (Roy *et al.,*2021).

1.9 Justification of study

The sinuses may harbor a complex community of bacteria, some of which are infectious and disease causing. These infections often prove difficult to treat due to the emergence of multi-drug resistance which may be Plasmid mediated. The lack of quick proper identification of pathogens especially in patients with sinusitis led to broad-spectrum antibiotics overuse. As a result of this dilemma, organisms became resistant to available antimicrobial agents. Also, Data about the endemic antimicrobial resistance are generally difficult to find, particularly in countries such as Nigeria where antibiotics are easily obtainable over the counter. Hence, an evidence-based knowledge regarding the local antimicrobial resistance pattern is fundamental for a guided selection strategy.

This study was aimed at evaluating microbial pattern with patient demographics as well as understanding the role of plasmids in multi-drug resistant bacteria isolated from patients with Sinusitis who visited the study center and exploring curing technique.

1.10 Aim and objective of study

This study was aimed at evaluating demographics of patients with sinusitis who visit the Ear Nose and Throat Clinic of the University of Benin Teaching Hospital and to study the plasmid profile of multi-drug resistant bacterial isolates obtained from these patients.

Specific objectives include;

- To evaluate the trends, demographic distribution and co-morbidities associated with sinusitis at the study center.
- To isolate, identify and characterize aerobic and anaerobic bacteria associated with sinusitis at the study center.
- To determine the antibiotic susceptibility profiles of the bacterial isolates

- To identify and confirm the presence of multi-drug resistant isolates through antibiotics susceptibility testing.
- To eliminate plasmids from multi-drug resistant isolates using acridine orange.
- To carry out susceptibility testing using disc diffusion method for isolates treated with acridine orange.
- To compare the antimicrobial resistance patterns of cured and uncured isolates to assess the role of plasmids in conveying resistance.

CHAPTER TWO

MATERIALS AND METHODOLOGY

2.1 Materials

2.1.1 Reagents and chemicals

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

2.1.2 Culture media

Nutrient Agar (Titan Biotech Ltd. India), Nutrient Broth (Titan Biotech Ltd. India), Blood Agar, MacConkey Agar (Hi flowwn Global Ltd, Nigeria), Mannitol Salt Agar (Titan Biotech Ltd. India), Mueller-Hinton agar (Chaitanya Agro Biotech Ltd), Sodium Thioglycollate broth(Titan Biotech Ltd. India).

2.1.3 Equipment

Hot air oven, incubator, autoclave, refrigerator (Thermocool, UK), shaker bath (Gallenkamp, England), compound light microscope, anaerobic culture chamber, digital weighing scale.

2.1.4 Glassware and others

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

2.2 Methods

2.2.1 Study area

This study was carried out at the Ear Nose and Throat (ENT) Clinic of University of Benin Teaching Hospital. A Tertiary health care center that has an ENT clinic attached to it that attends to both in-patient and out-patient.

2.2.2 Study design

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 lead to assessment of just 43 participants), visiting the study center was done following ethical approval from the Institutional review boards and informed consent obtained from all participants enrolled in the study.

2.2.3 Ethical considerations

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) , Informed consent was sought and obtained from adult participants, while assent was obtained from patients below 18 years following informed consent from their parents. Participants were duly interviewed and briefed on the scope of the study which included obtaining demographic data, medical history of participants and collection of specimens. Participants were assured of the absence of risks and harm associated with participating in the study and that they have the right to opt-out if they do not feel comfortable with the process before obtaining their informed consent. The principle of voluntary participation, maintenance of anonymity, and confidentiality was maintained throughout the study. Participants were given the right to decide whether to participate,

withdraw at any point, or decline to provide information on unclear points. Information provided by participants was treated confidentially, with no request 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, cardiovascular disease, etc.), duration of symptoms, nature of symptoms experienced, possible triggers, co-morbidity with other respiratory tract infection, Inquiries about patient social habits covered the frequency of alcohol consumption and smoking, . Participants were also evaluated regarding antibiotics use to assess rational use of antibiotics therapy.

2.2.5 Population of study, exclusion and inclusion criteria.

The study was conducted among all eligible study participants across all ages who were diagnosed with sinusitis within the designated study period (from January 2024 to March 2024). Only patients who had been evaluated and diagnosed by the doctor was included in the study while patients who had been on antibiotics 2-4 weeks prior to specimen collection were excluded from the study.

2.2.6 Specimen collection

The study evaluated a total of 43 specimens obtained from the nasal cavity of enrolled study participants who presented with sinusitis at the study center. Nasal specimen was collected from the posterior nasal vestibules of study participants using sterile swab sticks prelabelled

with patient's unique identification code. With the study participant's head tilted backwards, the sterile swab stick was gently inserted into the nasal cavity about 2cm and allowed to touch the nasal walls. The swab stick was rotated around the nasal wall and slowly removed via circular motion to collect as much specimen. This procedure was done using one swab stick per nostril depending on the amount of secretion obtained and patient compliance. This procedure was done by the physicians of the ENT Clinic. Proper aseptic techniques were observed to minimize contamination and ensure the integrity of the collected specimen. 5mL of already prepared sodium thioglycollate broth was introduced into the swab stick container aseptically. All specimens were transported within 2 hours to the Department of Pharmaceutical Microbiology laboratory for further microbiological investigations.

2.2.7 Isolation, characterization and identification of isolates

After incubation in sodium thioglycollate broth for 24 hours, the specimens were sub-cultured into 10% blood agar plate. Blood agar plates were prepared by suspending 14g of nutrient agar in 500mL of sterile water in a conical flask. The flask was swirled on the work bench to allow proper dispersion of the agar into the liquid medium. The preparation was then warmed in an autoclave until the system homogenized completely followed by sterilization at a temperature of 121°C for 15minutes. The molten agar was allowed to cool before introduction of human blood to prevent hemolysis of the red blood cells present. Under aseptic technique, 50mL of human blood was added into 450mL molten nutrient agar to make a 10% preparation and the flask was swirled gently to allow even mixing. About 20mL of the prepared blood agar still in the molten form was poured into plastic disposable petri dishes (15 x 100mm size) and the plates were allowed to set and then dried in an hot air oven at a regulated temperature of 50ºC for 10minutes with the lid separated from the plate, with lid and base inverted with their inner surfaces facing downwards. Dried Plates were

labeled properly using patient's individual identification code. Specimen from the overnight sodium thioglycollate culture were inoculated into blood agar and streaked using ziz-zag continuous streaking technique using a flamed inoculating loop. Plates intended for aerobic culture were incubated for 24 hours at 37°C in an incubator. Isolates were chosen for subculture into already prepared nutrient agar, MacConkey agar and mannitol salt agar based on colony morphological appearance on blood agar. Hemolytic properties of colonies were observed also and documented. 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 other differential agar and incubated for 18-24hours. when two colonies appeared to be different on the blood agar plate, both were sub-cultured on different agar plates.

MacConkey agar was prepared by suspending 25g of nutrient agar in 500mL of sterile water in a conical flask which was sealed using an aluminum foil sheet. The flask was swirled on the work bench to allow proper dispersion of the agar into the liquid medium. The preparation was then warmed in an autoclave until the system homogenized completely and then sterilized at a temperature of 121°C for 15minutes. The agar was allowed to cool and while in its molten form, 20mL was poured aseptically into plastic disposable plastic petri dishes and allowed to set properly and then dried in an hot air oven at a regulated temperature of 50ºC for 10minutes with the lid separated from the plate and both inverted with their inner surfaces facing downwards. Dried Plates were labeled properly using patient's individual identification code. Colonies which appeared on blood agar plates were sub-cultured into MacConkey agar and plates were incubated for 24 hours at 37°C in an incubator. Growth on this media was recorded. Same procedure was repeated using 55.5g of Mannitol salt agar in 500mL of sterile water and growth as well as colour change was recorded.

Upon isolation, Gram staining procedure as well as microscopy was carried out. A smear of the isolate obtained from nutrient agar plate was made using sterile water and heat-fixed by passing the back of the glass slide quickly through an open flame. The slide was placed on a gram staining rack and crystal violet which is the primary stain was added using a pipette with a contact time of 60 seconds and then washed with sterile water. This was followed by iodine treatment which acted as a mordant with a contact time of 60 seconds, decolorization using ethanol was followed and the slide was rinsed immediately the ethanol came in contact with the slide. A counter-stain(safranin-Red) was applied with a contact time of 60seconds and the slides were allowed to air dry. The slides were viewed under a light microscope at 40x and 100x magnification. The colour of cells, shape and arrangement were documented.

Biochemical tests were used to identify the isolates based on individual biochemical properties. Catalase test was done by making a smear of the test isolate obtained from nutrient agar with sterile water on a sterile glass slide. With the aid of a pipette, a drop of hydrogen peroxide was placed on the smear and the presence of effervescence was recorded

Coagulase test was done by preparing a thick smear of the test isolate with sterile water on a glass slide. A drop of pooled human plasma was placed on the surface of the smear and the slide was gently rocked. Development of clots or clumps was recorded

Indole test was done by dissolving 3.75g of peptone water broth into 250ml of sterile water in a conical flask which was sealed using an aluminum foil sheet. The broth was sterilized in an autoclave at 121°C for 15minutes. The broth was allowed to cool and 5mL was dispensed aseptically into bijoux bottles. A loopful of test isolate was inoculated into broth and cultured at 37°C for 24hours. Post incubation period, 0.5mL of Kovac's reagent was added using a calibrated micropipette and the formation of a pink ring was documented

Urease test was done by suspending 12g of Christensen's urea agar in 500mL of sterile water in a conical flask which was sealed using an aluminum foil sheet. The conical flask was then

heated until a homogenized system was achieved. the agar was then sterilized in an autoclave at 121°C for 15minutes and allowed to cool. 5mL of the agar was aseptically poured into a sterile MacCartney bottle and the bottle was placed in a slant position until solidification. The surface of the agar was then streaked with the test isolate and incubated for 24 - 48hours at 37°C and colour change was recorded.

Citrate test was done by suspending 12g of Simmon's citrate agar in 500mL of sterile water in a conical flask which was sealed using an aluminum foil sheet. The conical flask was then heated until a homogenized system was achieved. The agar was then sterilized in an autoclave at 121°C for 15minutes and allowed to cool. 5mL of the agar was aseptically poured into a sterile MacCartney bottle and the bottle was placed in a slant position until solidification. The surface of the agar was then streaked with the test isolate and incubated for 24 - 48hours at 37°C and colour change was recorded.

Oxidase test was carried out using Whatman's number 1 paper impregnated with oxidase reagent. The strip was moistened with sterile water and a smear of test isolate was made on the moistened spot. A rapid colour change was noted. All results obtained across all biochemical test results were documented accurately.

2.2.8 Method of anaerobiosis

The method postulated by Bauchener was adopted with minor modifications and adjustment. An anaerobic jar was employed. After sterilization using disinfectant, 5.0g of pyrogallol crystals was weighed accurately and put on one side of a glass dish which was placed at the base of the anaerobic jar. A metal gauze was used to cover the dish. All labelled inoculated blood agar plates were placed upside down over the metal gauze of the dish. 4% Sodium Hydroxide solution was prepared by dissolving 4g of NaOH pellets in 100mL of distilled water. 10mL of this 4% NaOH solution was added to the pyrogallol crystals using a

sterile pipette and the anaerobic jar was sealed properly immediately. The jar was rock gently in order to allow mixing of the chemicals (pyrogallol + NaOH) and creation of an anaerobic environment (Ananthanarayan and Paniker, 2006). The plates were was allowed to incubate for 72hours. Macroscopic, microscopic and Biochemical investigation were done to identify isolates.

2.2.9 Antimicrobial susceptibility test

Isolates obtained were subjected to antimicrobial susceptibility testing and the disc diffusion method on Mueller-Hinton agar was employed. Antimicrobial agents used were selected based on treatment guidelines adopted by the study center. Mueller-Hinton agar was prepared by suspending 19g of Mueller-Hinton agar in 500mL of sterile water in a conical flask which was sealed using aluminum foil paper. The flask was swirled on the work bench to allow proper dispersion of the agar into the liquid medium. The flask was then warmed in an autoclave until the system homogenized completely and then sterilized at a temperature of 121°C for 15minutes. The agar was allowed to cool and 20mL was poured aseptically into disposable plastic petri dishes and allowed to set properly. The plates were then dried in an hot air oven at a regulated temperature of 50ºC for 10minutes with the lid separated from the plate. Both lid and base were inverted with their inner surfaces facing downwards. Dried Plates were labeled properly with identification codes. 1mL of an overnight nutrient broth culture (which was prepared by inoculating a loopful of isolate into 2mL of prepared nutrient broth) was transferred aseptically on the surface of the already prepared Mueller-Hinton agar plate using a micropipette. With the aid of a flamed glass spreader, the culture broth was distributed evenly across the surface of the agar. The plates were allowed to stand for a few minutes to allow proper fixation unto the surface of the agar. With the aid of a flamed forceps, multi-antibiotics disc containing Pefloxacin (30µg), ofloxacin (10µg), azithromycin (12µg),

levofloxacin(20µg), cefotaxime (10µg), streptomycin (10µg), ciprofloxacin (30µg), amoxicillin (30µg), amoxicillin-clavulanic acid (10µg) and Gentamycin (30µg) was placed aseptically on the surface of the prepared agar and the plates inverted. All plates were done in duplicates and Control plates containing the isolate culture only and no antibiotics disc was prepared to ensure the viability of isolates on Mueller-Hinton Agar. All plates were incubated at 37 °C for 24 hours in an incubator. Inhibitor zone diameters in millimeter(mm) were recorded using a measuring ruler and the result interpreted as susceptible or resistant based on published guidelines for antimicrobial susceptibility testing for commonly occurring clinical pathogens obtained (EUCAST, 2015)

2.2.10 Plasmid curing

After antimicrobial testing, isolates that were resistant to three (3) or more classes of antimicrobial agents were termed multi-drug resistant and were selected for plasmid curing. According to a method outlined by Brown (2000), Nutrient broth was prepared by dissolving 6.5g of nutrient broth in 500mL of sterile water in a conical flask. The preparation was sterilized by autoclaving at 121°C for 15minutes. 2mL of this sterile nutrient broth was dispensed aseptically into sterile sample bottles and labeled appropriately with identification codes. A loopful of multi-drug resistant isolate was inoculated into the nutrient broth and incubated at 37 °C for 24 hours. Double strength nutrient broth was prepared by dissolving 13g of nutrient broth in 500mL of sterile water and 5ml was dispensed aseptically into sterile sample bottles using a micropipette. A volume of 20µL from the overnight bacterial culture was sub-cultured into the double strength nutrient broth. The broth was then supplemented with 0.1mg/mL acridine orange which was prepared by dissolving 1mg of acridine orange in 10mL of sterile water. The sample bottles were incubated at 37 °C for 72 hours in a shaker bath set to a revolution rate of 150 rpm. The procedure was repeated using 0.4mg/mL

acridine orange which was prepared by dissolving 4mg of acridine orange in 10mL of sterile water.

After the 72hours incubation period, a sterile swab stick was used to streak the surface of an already prepared mueller-hinton plate with the acridine orange - culture preparation and the plate was allowed to stand to allow proper fixation. With the aid of flamed forceps, multi-antibiotics disc was placed aseptically on the surface of the agar. All plates were incubated at 37°C for 24 hours and the inhibitory zone diameters in millimeter were recorded and the results before and after curing were compared.

2.3 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 27.0 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 patient's symptoms, frequency of episodes, family history, possible allergens or trigger were also defined as string variables. Inhibitory zone diameters were entered and defined as numeric data prior to the analysis with respect to EUCAST breakpoints. Descriptive statistics were used to report percentage frequencies of patient demographics, medication history, presence of co-morbidities (like asthma, Chronic Obstructive Pulmonary Disorder, ear infection, Gastric Esophageal Reflux Disorder (GERD), etc.). Statistical difference between variables were calculated using chi-square. Difference between groups were considered significant at P value < 0.05. Standard diagnostic indices including quantitative antibiogram (IZD) values, positive predictive diagnostic (Gram staining test) efficacy were all calculated according to standard procedure

CHAPTER THREE

RESULTS

3.1 Association of gender with patient demographics and medical history

Table 3.1a showed the frequency distribution of patient demographics. A total of 43 patients were involved in the study with a total number of 25 females (58.1%) of which the highest frequency of females (8 females) fell within the age range of 16-25years and 18males (41.9%) with most male participants falling within the age range 7-15years. The least encountered age range was 16-20years (2.3%) followed by patients above 60years (4.7%). University students accounted for 30.2% of participants (13 participants) with most being female. 25.6% were self-employed and 4.7% of total participants which was the least encountered were retired. No retired female participated in the study. Most males encountered in the study were mainly unemployed or self-employed and the least number of males observed were retired. All female encountered reported to have never smoked and 60% of females stated to have never taken alcohol. For male, 10 participants never smoked and 2 persons still smoke. More males encountered during the study do not take alcohol and with only a few still consuming alcohol.

Table 3.1b illustrated the association of gender with patient medical history with the inclusion of statistical significance. The highest number of patients (19 patients accounting for 44.2% of total population) reported symptoms which persisted for more than 14days with females having more frequency compared to males and the least duration of symptoms experienced by both genders was 11-14days. Study showed that 65.1% (28patients) had previous episodes of sinusitis which warranted treatment and out of these 28 patients, 12 patients had episodes of symptoms which occurred more than four times in a year. Investigation revealed that more females had recurrent sinusitis manifesting greater than 4 times in a year compared to their male counterpart. Interestingly, more males reported to having family history of sinusitis compared to females. Nasal discharge was the most

common symptom among all sexes (24 patients had yellow discharge,17 patients had clear discharge and 2 patients had red discharge). Among which, more females had yellow nasal discharge compared to men. Same trend was observed across clear and red nasal discharge. Conversely, other symptoms such as stuffiness, cough, itchy throat, fever, headache, jaw and facial swelling, facial pain, and mouth breathing were frequent among female compared to males. The least symptom experienced was facial and jaw swelling which was reported by only 16.3% of participants.

Trigger patterns reported by study participants included cold weather which was reported by 58.1% of study population. 51.2% (22patients) attributed dust as one of the aggravating factors. 4.7% of total participants reported pollen grains as one of the trigger agents to episodic symptoms. More female attributed dust, cold weather, smoke and pollen grain as a trigger factor to symptom episodes compared to male.

Majority of study participants reported no co-existing disease but a hand full reported certain pressing health issue. Asthma was the most encountered co-morbidity accounting for 9.3% of total participants. with even distribution across both sexes, more female were presented with GERD and ear infection compared to male. Also, more males were presented with diabetes and hypertension compared to female from result obtained.

Table 3.1a Association of gender with patient demographics

		Male	Female	Total	P –value (ci=95%)
age of patients	7- 15years	5	5	10	0.260
	16-25years	4	8	12	
	26-35years	5	6	11	
	36-45years	1	2	3	
	46-55years	0	2	2	
	56-60years	2	1	3	
	>60years	2	0	2	
smoking history	never smoked	10	25	35	0.001
	stopped smoking	6	0	6	
	still smoking	2	0	2	
alcohol history	never drank	8	15	23	0.537
	still drinking	3	4	7	
	stopped drinking	7	6	13	
Occupation	employed	0	5	5	0.088
	self employed	6	6	12	
	student	4	9	13	
	not employed	6	5	11	
	Retired	2	0	2	

Table 3.1b Association of gender with patient medical history

Medical history		**male**	**female**	**Total**	p-value cl=95%
Duration of symptoms	<5days	2	7	9	0.520
	6-10days	6	5	11	
	11-14 days	2	2	4	
	>14days	8	11	19	
		18	25	43	
Previous episodes of	Yes	8	20	28	0.016
symptoms	No	10	5	15	
		18	25	43	
Frequency of episodes	Nil	10	5	15	0.143
	once a year	1	1	2	
	twice a year	3	5	8	
	3 times a year	1	5	6	
	> 4 times a year	3	9	12	
		18	25	43	
Family history of sinusitis	Yes	7	5	12	0.173
	No	11	20	31	
FREQUENCY OF SYMPTOMS					
Nasal discharge		18	25	43	0.000
Stuffiness		13	19	32	
Cough		10	14	24	
Facial pain		7	15	22	
Itchy throat		7	15	22	
Headache		7	15	22	
Mouth breathing		9	12	21	
Fever		6	10	16	
facial and jaw swelling		3	4	7	
Others		0	3	3	

3.2 Result analysis of aerobic isolates

3.2.1 Frequency distribution of aerobic isolates

The study isolated a total of 77 bacterial isolates from the nasal cavity of patients with sinusitis via aerobic condition. As shown in Table 3.2.1, The prevalence of *Staphylococcus aureus* was the highest with a frequency of 35.1% followed by *Pseudomonas aeroginosa* with a prevalence of 20.8%. *Enterobacter aerogene*s had the least abundance of colonization with a frequency of 2.6% .

3.2.2 Morphological and biochemical characteristics of aerobic isolates

Table 3.2.2 descriptively shows the morphological and biochemical characteristics of isolates obtained from patients with sinusitis. In the morphological investigation of isolates, 55% of isolates appeared elevated on the surface of blood agar used. 44.1% of isolates had a lawn appearance on blood agar surface. Notably, 5 isolates which accounted for 6.4% of total isolates presented as slimy when picked with a sterile wire loop. Observation of hemolytic property showed that 15 isolates were α-hemolytic, 18 isolates were β-hemolytic and 35 isolates were ɣ-hemolytic. The investigation which warranted culture on differential media carefully noted 27 isolates(35%) which turned the cherry-red colour of mannitol salt agar to golden yellow and 11 isolates(14.2%) turned the agar pink. 27 isolates appeared yellow on nutrient agar, 34 appeared white and 16 appeared green on nutrient agar.

Biochemical characterization revealed that all 77 isolates obtained were catalase positive, only *Staphylococcu*s *aureu*s isolates were coagulase positive (27 isolates in total accounting for 35%). 30% of isolates were oxidase positive, no isolate was indole positive, 80.5% of isolates were citrate positive and 55.8% of isolates were urease positive. The investigation showed that 49.4% of isolates obtained were Gram Positive Cocci (a total of 38 isolates). The least encountered isolates with a frequency of 20.8% were Gram Positive Bacilli.

Table 3.2.1 frequency distribution of Aerobic isolates

	Frequency	**Percent%**
Staphylococcus aureus	27	35.1
Staphylococcus epidermidis	11	14.3
Cornybacterium spp	4	5.2
Bacillus spp	12	15.6
Klebsiella spp	5	6.5
Enterobacter aerogenes	2	2.6
Pseudomonas aeroginosa	16	20.8
Total	77	100.0

Table 3.2.2 Biochemical and morphological characteristics of aerobic isolates

MORPHOLOGICAL	INFERENCE	STAPH AURE	STAPH EPI	CORN BACT	PSEU	KLEB SPP	ENTER BACT	BACIL SPP	TOTAL ISOLATE
Colonial characteristics	RAISED	27	11	0	0	5	0	0	43
	FLAT	0	0	4	16	0	2	12	34
	NON-MUCOID	0	0	0	0	0	2	0	2
	MUCOID	27	0	0	16	5	0	12	60
Hemolytic characteristics	α	8	0	0	7	0	0	0	15
	B	13	0	0	9	0	0	5	18
	Ɣ	6	11	4	0	5	2	7	35
Appearance on MSA	YELLOW	27	0	0	0	0	0	0	27
	PINK	0	11	0	0	0	0	0	11
Appearance on Nutrient agar	YELLOW	27	0	0	0	0	0	0	27
	WHITE	0	11	4	0	5	2	12	34
	GREEN	0	0	0	16	0	0	0	16
Gram stain inference	GPC	27	11	0	0	0	0	0	38 GPC
	GNB	0	0	0	16	5	2	0	23 GNB
	GPB	0	0	4	0	0	0	12	16 GPB
Catalase test	POSITIVE	27	11	4	16	5	2	12	77 +VE
Coagulase test	POSITIVE	27	0	0	0	0	0	0	27 +VE
Oxidase test	POSITIVE	0	0	0	16	0	0	0	23 +VE
Citrate test	POSITIVE	27	0	0	16	5	2	12	62 +VE
Indole test	POSITIVE	0	0	0	0	0	0	0	2 +VE
Urease test	POSITIVE	27	11	0	0	5	0	0	43 +VE

Keyword; GPC= Gram Positive Cocci, GNB = Gram Negative Bacilli ,GPB = Gram Positive Bacilli, MSA= Mannitol Salt Agar,STAPH AUREUS= *Staphylococcus aureus*,STAPH EPI= *Staphylococcus epidermidis*, KLEB SPP=*klebsiella spp,* BACIL SPP= *Bacillus supps*,PSEUD =*Psueudomonas aeruginosa,* CORN BACT= *Corynebacterium spp*,ENTERO BACTER= *Enterobacter aerogenes*

3.2.3 Association of aerobic isolates with patient demographics

Table 3.2.3 showed the association of aerobic isolates and patient characteristics. More females had *Staphylococcus aureus* in their nasal cavity compared to males. Same trend was observed among *Bacillus spp,* and *Pseudomonas aeruginosa*. Interestingly, more males had a prevalence of *Staphylococcus epidermidis* and *Klebsiella spp* compared to females. Both sexes had equal prevalence of *Corynebacterium spp* and *Enterobacter aerogenes.* Notably, the highest frequency of aerobic isolates was obtained from patients within the age of 21-25years with *Staphylococcus aureus* being the most encountered organism within this age range. Investigation revealed that the highest microbial load obtained were from students who participated in the study with *Staphylococcus aureus* being the most prevalent isolate. Notably, *Pseudomonas aeruginos*a had the highest frequency among participants who were self-employed. Retired participants had the least number of isolates with an even distribution of *Corynebacterium spp* and *Pseudomonas aeruginosa.* Participants who never smoked or drank had the highest distribution of isolates with *Staphylococcus aureus* being the most prevalent followed by *Staphylococcus epidermidis*.

Table 3.2.3 Association of aerobic isolates with patient demographics

		STAPH AUR	STAPH EPID	CORN BACT	BACIL SPP	KLEB SPP	ENTERO BACTER	PSEUD AERO	Total	P-Value Cl=95%
sex of patients	male	9	7	2	4	2	1	5	30	0.544
	female	18	4	2	8	3	1	11	47	
Age of patients (years)	0-15	7	2	1	2	2	0	3	17	0.948
	16-25	7	5	1	5	1	0	4	23	
	26-35	6	3	1	4	2	1	5	21	
	36-45	2	1	0	1	1	0	1	6	
	46-55	2	0	0	0	0	0	1	3	
	56-60	3	0	0	0	0	1	1	5	
	>60	0	0	1	0	0	0	1	2	
Occupation	employed	3	1	0	1	1	0	2	8	0.312
	self employed	10	1	0	3	1	2	6	23	
	student	8	5	1	6	1	0	5	26	
	not employed	6	4	2	2	2	0	2	18	
	retired	0	0	1	0	0	0	1	2	
smoking history	never smoked	22	10	3	12	4	1	13	65	0.834
	stopped smoking	3	1	1	0	1	1	3	10	
	still smoking	2	0	0	0	0	0	0	2	
alcohol history	never drank	15	7	2	8	4	1	5	42	0.476
	still drinking	3	1	2	1	0	0	3	10	
	stopped drinking	9	3	0	3	1	1	8	25	

3.2.4 Association of aerobic isolates with patient medical history

Table 3.2.4 shows the association of aerobic isolates with disease characteristics in this study with statistically significant relationships between measured variables. Investigation revealed that participants who had duration of symptoms longer than 14 days had the most bioburden in their nasal cavity with *Staphylococcus aureus* being the most prevalent isolate followed by *Pseudomonas aeruginosa*. The least frequency of microbes was encountered among participants who suffered symptoms for between 11-14days. Conversely, the most isolates were obtained from patients who had previous episodes of sinusitis symptoms with the highest distribution among participants who had symptoms more than four times in a year. Interestingly, the least number of isolates were obtained from participants who reported to have a family history of sinusitis.

In regards the symptoms encountered during the course of investigation, nasal discharge which was common among all participants recorded the highest number of isolates with *Staphylococcus aureus* being the most predominant. Other symptoms recorded high numbers of isolates with facial and jaw swelling having the least number of isolates. Patients who reported cold weather as a trigger factor had the greatest number of isolates with Staphylococcus aureus being the most frequent. The least trigger encountered was associated to pollen grains which recorded the least number of bioburden in the nasal cavity of patients with sinusitis. Notably, majority of participants who had no known co-morbidities had a higher burden of microbes compared to those who had other existing disease. Participants with asthma recorded the most distribution of isolates with *Staphylococcus aureu*s being the most prevalent. The least encountered associating diseases were ear infection and GERD.

Table 3.2.4 Association of aerobic isolates with patient medical history

		STAPH AUR	STAPH EPID	CORN BACT	BACIL SPP	KLEB SPP	ENTERO BACTER	PSEUD AERO	TOTAL	P-Value cl=95%
Duration of symptoms	<5days	6	3	1	3	3	0	2	18	0.793
	6-10days	8	2	1	3	1	1	6	22	
	11-14 days	2	0	1	0	0	0	1	4	
	>14days	11	6	1	6	1	1	7	33	
Previous episodes		18	7	1	10	2	2	12	52	0.429
Frequency of episodes	nil	9	4	3	2	3	0	4	25	0.925
	once a year	1	1	0	1	0	0	1	4	
	2times a year	6	1	0	2	0	1	4	14	
	3times a year	3	2	0	2	1	0	2	10	
	> four times a year	8	3	1	5	1	1	5	24	
Family history		8	4	1	4	0	1	5	23	0.892
SYMPTOMS										
Nasal discharge		27	11	4	12	5	2	16	77	0.000
Stuffiness		20	8	3	7	4	1	12	55	
Headache		14	6	2	7	1	1	10	41	
Facial pain		15	4	3	3	4	1	8	38	
Itchy throat		11	7	2	7	2	0	9	38	
Mouth breathing		15	4	0	6	3	0	8	36	
Fever		9	5	2	5	1	0	5	27	
Facial and jaw swelling		6	1	0	2	1	0	2	12	

Keyword; STAPH AUREUS= *Staphylococcus aureus*, STAPH EPI= *Staphylococcus epidermidis*, KLEB PNEU=*klebsiella spp* BACIL SPP= *Bacillus spp* PSEUDO AERO =*Psueudomonas aeruginosa,* CORN BACT= *Corynebacterium spp*, ENTERO BACTER= *Enterobacter aerogenes*

3.2.5 Antimicrobial susceptibility testing of aerobes

Table 3.2.5 shows the antimicrobial susceptibility pattern associated with these isolates against various antimicrobial agents used in the treatment of sinusitis. 85.7% of all total isolates were susceptible to the bactericidal action of levofloxacin which is a classic example of antimicrobial agents which belong to the fluoroquinolone class. In general, majority of isolates obtained and tested were susceptible to fluoroquinolones. On the other hand, 46.8% of total obtained isolates treated with amoxicillin which is an example of penicillin antibiotics showed the least susceptibility to the lethal action of the antibiotics.

Table 3.2.5 Antimicrobial susceptibility of aerobes

Isolate	**FQ**	**AMG**	**CEF**	**MAC**	**AM/CLA**	**AMX**
S. aureus	24	21	20	14	16	12
(n=27)	88.9%	77.8%	74.0%	51.8%	59.3%	44.4%
S. epidermidis	11	11	10	10	5	6
(n=11)	100%	100%	90.9%	90.9%	45.5%	54.5%
Corynebacterium spp	4	3	3	4	2	2
(n = 4)	100%	75%	75%	100%	50%	50%
Bacillus spp	9	11	8	8	9	7
(n = 12)	75.7%	91.7%	66.7%	66.7%	75%	58.3%
Klebsiella spp	3	3	2	3	2	0
(n = 5)	60%	60%	40%	60%	40%	0%
E. aerogenes	2	1	1	1	1	1
(n = 2)	100%	50%	50%	50%	50%	50%
P aeruginosa	13	12	10	3	8	8
(n = 16)	81.3%	75%	62.5%	18.8%	50%	50%
Total	66	62	54	43	43	36
(n=77)	85.7%	80.5%	70.1	55.8%	55.8%	46.7%

Keyword; FQ= Fluoroquinolones, AMG= aminoglycoside, CEF= Cephalosporin, MAC= macrolide, AM/CLA= Amoxicillin/clavulanic acid, AMX= Amoxicillin, n= number of isolates

3.3 Multi-drug resistant strain of aerobic isolates and plasmid curing

3.3.1 frequency distribution of multi-drug resistant isolates

Table 3.3.1 displayed the frequency of different isolates obtained during the course of investigation. The table showed that out of 77 isolates obtained, 18(23.4%) of these isolates were resistant to three or more classes of antimicrobial agents when tested using disc diffusion method. Among which, 80% of *Klebsiella spp* isolate were multi-drug resistant,. Interestingly, 26% of isolated *Staphylococcus aureus* happened to be multi-drug resistant. Conversely, no multidrug resistant isolate was encountered for *Staphylococcus epidermidis, Corynebacterium spp* and *Enterobacter aerogenes*

Table 3.3.1 frequency distribution of multi-drug resistant isolates

Isolates	Frequency	Percent	MDR Strain	percent
Staphylococcus aureus	27	35.1	7	26.0
Staphylococcus epidermidis	11	14.3	0	0
Cornybacterium spp	4	5.2	0	0
Bacillus spp	12	15.6	3	25
Klebsiella spp	5	6.5	4	80
Enterobacter aerogenes	2	2.6	0	0
Psueudomonas aeroginosa	16	20.8	4	25
	77	100.0	18	23.4

3.3.2 Plasmid curing of aerobic isolates using acridine orange

Table 3.3.2 showed the pattern of multi-drug resistance displayed by various isolates pre-curing and post curing using 0.1mg/mL and 0.4mg/mL Acridine orange. Notably, from the 7 multi-drug resistant Staphylococcus isolates, 85.7% (6 isolates) had a common resistance to penicillin antibiotics. After curing, which 50% of these resistant isolates lost their resistance and became susceptible to the killing action of Amoxicillin/clavulanic acid combination. The least Resistance displayed in *Staphylococcus aureus* was attributed to levofloxacin. This resistance was lost upon curing as all resistant Staphylococcus aureus became susceptible to the killing action of fluoroquinolones.

Pseudomonas aeruginosa recorded the highest resistance across all antimicrobial agents used with resistance to gentamycin, amoxicillin and azithromycin being the most prevalent. Post-curing, 0% susceptibility was attributed to aminoglycosides, macrolides and cephalosporins. All isolates initially resistant to levofloxacin became susceptible to its antimicrobial action post treatment with Acridine orange. Interestingly, resistance to penicillin which was persistent after supplementation with 0.1mg/mL Acridine orange was lost when the concentration was increased to 0.4mg/mL.

A 100% cure rate was noted for resistance to macrolide in Bacillus spp. However, resistance to fluoroquinolones was still conferred post curing as all isolate remained resistant to the lethal action of fluoroquinolones. Post curing, *Klebsiella spp* had a 100% susceptibility pattern to fluoroquinolones, macrolide and amoxicillin/clavulanic acid.

Table 3.3.2 Frequency distribution of uncured and cured aerobic isolates

ISOLATE	CODE		FQ	AMG	CEF	MAC	AM/CLA	AMOX
S. aureus N=7	1U		2	4	3	3	6	6
	1C	0.1AO	2	1	2	2	3	2
		0.4AO	2	1	2	2	3	2
		% CURED	100%	25%	66.7%	66.7%	50%	33.3%
P. aeruginosa N=4	2U		2	3	3	4	4	4
	2C	0.1AO	2	0	0	0	0	0
		0.4AO	2	0	0	0	1	1
		% CURED	100%	0%	0%	0%	25%	25%
Bacillus. spp N=3	3U		2	1	1	3	3	3
	3C	0.1AO	0	1	1	1	1	0
		0.4AO	0	1	1	1	1	0
		% CURED	0%	100%	100%	33.3%	33.3%	0%
Klebsiella spp N=4	4U		2	2	2	1	2	4
	4C	0.1AO	1	0	0	1	1	0
		0.4AO	1	0	0	1	1	0
		% CURED	50%	0%	0%	100%	50%	0%

Keyword; FQ= Fluoroquinolones, AMG= aminoglycoside, CEF= Cephalosporin, MAC= macrolide, AM/CLA= Amoxicillin/clavulanic acid, AMOX= Amoxicillin, AO= Acridine orange, n= number of isolates, U = uncured, C = cured

3.4 Result analysis of anaerobic isolates

3.4.1 Frequency distribution of facultative anaerobic isolates

The study isolated a total of 32 bacterial isolates under anaerobic condition from the nasal cavity of patients with sinusitis. As shown in Table 3.5.1, although no strict anaerobes were isolated, The frequency of *Pseudomonas aeruginosa* was the highest as it accounted for 31.3% of total population followed by *klebsiella spp* with a frequency of 28.1%. *Bacillus spp* had the least prevalence of colonization with a frequency of 18.8%.

3.4.2 Morphological and biochemical characteristics of anaerobic isolates

Table 3.4.2 descriptively showed the morphological and biochemical characteristics of isolates. In the morphological investigation of isolates, 16 isolates appeared elevated on the surface of agar used.16 isolates had a lawn appearance on agar surface. Notably, 28.1% of isolates presented as slimy when picked with a sterile wire loop. 22 isolates appeared mucoidal. Observation of hemolytic property showed that 7 isolates were α-hemolytic, 10 isolates were β-hemolytic, and 15 isolates were ɣ-hemolytic.

Biochemical characterization revealed that all 100% of isolates obtained were catalase and citrate positive, only *Staphylococcus aureus* isolates which accounted for 21.9% of total isolates were coagulase positive. 50% of isolates were oxidase and urease positive, no isolate was positive to indole test. The investigation showed that the highest frequency of isolates obtained were Gram Negative Bacilli accounting for 53.4%. The least encountered isolates were Gram Positive Cocci with a frequency of 21.9%.

Table 3.4.1Frequency distribution of facultative anaerobic isolates

Isolates	Frequency	Percent
Staphylococcus aureus	7	21.9
Bacillus spp	6	18.8
Klebsiella spp	9	28.1
Pseudomonas aeruginosa	10	31.3
Total	32	100.0

Table 3.4.2 Colonial and biochemical characteristics of facultative anaerobic isolates

MORPHOLOGICAL TEST		STAPH AUREUS	PSEUD AERO	KLEB SPP	BACIL SPP	TOTAL ISOLATE
Colonial characteristics	RAISED	7	0	9	0	16
	FLAT	0	10	0	6	16
	SLIMY	0	0	9	0	9
	MUCOID	6	10	0	6	22
	α-HAEMOLYSIS	3	4	0	0	7
	B HAEMOLYSIS	4	6	0	0	10
	Ɣ HAEMOLYSIS	0	0	9	6	15
Gram stain inference	GPC	7	0	0	0	7 GPC
	GNB	0	10	9	0	19 GNB
	GPB	0	0	0	6	6 GPB
Catalase test	POSITIVE	7	10	9	6	32 +VE
Coagulase test	POSITIVE	0	0	0	0	0 +VE
Oxidase test	POSITIVE	0	10	0	6	16 +VE
Citrate test	POSITIVE	7	10	9	6	32 +VE
Indole test	POSITIVE	0	0	0	0	0 +VE
Urease test	POSITIVE	7	0	9	0	16 +VE

Keyword; MSA= mannitol salt agar, STAPH AUREUS= *Staphylococcus aureus*, KLEB SPP=*klebsiella spp*, BACIL SPP= *Bacillus subtilis*, PSEUDO AERO =*Psueudomonas aeruginosa*

3.4.3 Association of facultative anaerobic isolates with patient demographics

Table 3.4.3 showed the association of Facultative anaerobic isolates and patient characteristics. More females had Facultative anaerobic isolates in their nasal cavity compared to males. The highest prevailing organism was *Pseudomonas aeruginosa* which was equally distributed among both genders. Same trend was observed in *Klebsiella pneumonia.* Interestingly, more males had a high prevalence of *Staphylococcus aureus* compared to female. Notably, the highest frequency of aerobic isolates was obtained from patients within the age of 21-25years with *Staphylococcus aureus* being the most encountered organism within this age range. Investigation revealed that the highest microbial load obtained were from students who participated in the study with *Staphylococcus aureus* being the most prevalent isolate while *Pseudomonas aeruginosa* was more prevalent among participants who were unemployed. Notably. Retired participants had the least number of isolates with a distribution *Pseudomonas aeruginosa.* Participants who never smoked or drank had the highest distribution of isolates with *Staphylococcus aureus* being the most prevalent for those who do not consume alcohol and *Pseudomonas aeruginosa* for participants who do not smoke. The least bioburden was recorded among participants who stopped smoking and those still drinking.

Table 3.4.3 Association of anaerobic isolates with patient demographics

		STAPH AUR	BACIL SPP	KLEB SPP	PSEUD AERO	TOTAL	P-Value Cl=95%
sex of patients	male	1	3	5	5	14	0.357
	female	6	3	4	5	18	
age of patients (years)	0-15	1	2	0	2	5	0.458
	16-25	4	0	3	3	10	
	26-35	1	3	2	2	8	
	36-45	1	1	1	0	3	
	46-55	0	0	1	1	2	
	56-60	0	0	2	0	2	
	>60	0	0	0	2	2	
Occupation	employed	0	1	1	2	4	0.025
	self employed	2	3	5	0	10	
	student	5	0	3	2	10	
	not employed	0	2	0	4	6	
	retired	0	0	0	2	2	
smoking history	never smoked	7	6	5	8	26	0.165
	stopped smoking	0	0	2	2	4	
	still smoking	0	0	2	0	2	
alcohol history	never drank	5	4	3	3	15	0.303
	still drinking	0	2	2	3	7	
	stopped drinking	2	0	4	4	10	

KEYWORDS; STAPH AUR= *Staphylococcus aureus*, KLEB SPP=*klebsiella pneumonia*, BACIL SPP= *Bacillus subtilis*, PSEUDO AERO =*Psueudomonas aeruginosa*

3.4.4 Association of anaerobic isolates with patient medical history

Table 3.4.4 showed the association of anaerobic isolates with disease characteristics in this study with statistically significant relationships between the variables measured. Investigation revealed that participants who had duration of symptoms longer than 14 days had the most bioburden in their nasal cavity with *Klebsiella spp* being the most prevalent isolate followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The least frequency of microbes was encountered among participants who suffered symptoms for between 11-14days with *Klebsiella spp* being the most prevalent isolate. Conversely, the most isolates were obtained from patients who had previous episodes of sinusitis symptoms with the highest distribution among participants who had symptoms more than four times in a year. Interestingly, the least number of isolates were obtained from participants who reported to have a family history of sinusitis.

Nasal discharge which was common among all participants recorded the highest number of isolates with *Pseudomonas aeruginosa* being the most predominant. Other symptoms recorded high numbers of isolates with facial and jaw swelling having the least number of isolates. Patients who reported cold weather as a trigger factor had the most abundance of isolates with *Pseudomonas aeruginosa* being the most frequent. The least trigger encountered was associated to pollen grains which recorded the least number of bioburden in the nasal cavity of patients with sinusitis. Notably, majority of participants who had no known co-morbidities had a higher burden of microbes compared to those who had other existing disease. Participants with asthma, ear infection, GERD recorded an even distribution of isolates with *Pseudomonas aeruginosa* being the most prevalent.

Table 3.4.4 Association of anaerobic isolates with patient medical history

		STAPH AUREUS	BACIL SPP	KLEB SPP	PSEUD AERO	TOTAL
Duration of symptoms	<5days	2	1	2	2	7
	6-10days	1	1	0	3	5
	11-14 days	0	1	2	1	4
	>14days	4	3	5	4	16
Previous episodes		6	4	4	6	20
Frequency of episodes	nil	1	2	5	4	12
	once a year	0	1	0	0	1
	twice a year	2	0	2	1	5
	three times a year	3	0	1	1	5
	> four times a year	1	3	1	4	9
Family history		2	1	3	3	9
SYMPTOMS						
nasal discharge		7	6	9	10	32
stuffiness		7	2	7	6	22
Headache		4	3	3	6	16
facial pain		3	1	7	6	17
Fever		0	4	1	4	9
facial and jaw swelling		1	1	2	2	4
mouth breathing		6	2	3	4	15
itchy throat		4	2	5	6	17

Keyword; STAPH AUREUS= *Staphylococcus aureus*, KLEB SPP=*klebsiella spp*, BACIL SPP= *Bacillus spp*,PSEUDO AERO =*Psueudomonas aeruginosa*

3.4.5 Antimicrobial susceptibility testing of facultative anaerobes

Table 3.4.5 shows the antimicrobial susceptibility pattern associated with these isolates to various antimicrobial agents used in the treatment of sinusitis. 78.1% of total isolates were susceptible to the antimicrobial action of levofloxacin with the highest susceptibility attributed to Pseudomonas aeruginosa (90% susceptibility). On the other hand, the least susceptibility pattern was noted to penicillin particularly amoxicillin as report showed a 25% susceptibility pattern across all isolates. The least susceptibility to penicillin was seen in *Klebsiella spp* as only 11.1% of *Klebsiella pneumonia* isolate were susceptible to amoxicillin.

Table 3.4.5 Antimicrobial susceptibility pattern of anaerobes

ISOLATE	FQ	AMG	CEF	MAC	AM/CLA	AMX
S. aureus	5	6	1	3	3	2
(n=7)	71.4%	85.7%	14.3%	42.8%	42.8%	28.6%
Bacillus spp	5	5	4	1	1	2
(n = 6)	83.3%	83.3%	66.7%	16.6%	16.7%	33.3%
Klebsiella spp	6	6	1	3	3	1
(n = 9)	66.7%	66.7%	11.1%	33.3%	33.3%	11.1%
P aeruginosa	9	7	7	4	6	3
(n = 10)	90%	70%	70%	40%	60%	30%
Total	25	24	13	11	13	8
(n=32)	78.1%	75%	40.6%	34.4%	40.6%	25%

Keyword; FQ= Fluoroquinolones, AMG= aminoglycoside, CEF= Cephalosporin, MAC= macrolide, AM/CLA= Amoxicillin/clavulanic acid, AMX= Amoxicillin, n= number of isolates

3.5 Multi-drug resistant strain of facultative isolates and plasmid curing

3.5.1 frequency distribution of multi-drug resistant facultative anaerobic isolates

Table 3.5.1 displayed the frequency of different isolates obtained during the course of investigation. The table showed that out of 32 isolates obtained, 25% of these isolates were resistant to three or more classes of antimicrobial agents when tested using disc diffusion method. Among which, 33.3% of isolated *Klebsiella spp* were multi-drug resistant, 30% of total *Staphylococcus aureus* were multi-drug resistant. Interestingly, no multi-drug resistant isolate was observed for *Bacillus subtilis.*

3.5.2 Plasmid curing of facultative anaerobic isolates using acridine orange

Table 3.5.2 showed the pattern of multi-drug resistance displayed by various isolates pre-curing and post curing using 0.1mg/mL and 0.4mg/mL Acridine orange. Notably, from the 3 multi-drug resistant *Pseudomonas aeruginosa* isolates, a common resistance to penicillin, cephalosporins, fluoroquinolone and aminoglycoside was encountered. Across these isolates, only resistance to fluoroquinolone was lost in one isolate. Similar trend was observed in *Klebsiella pneumonia* isolates. All isolates of *Staphylococcus aureus* remained resistant to all classes of antimicrobial agents before and after curing with acridine orange.

Table 3.5.1 frequency distribution of multi-drug resistant anaerobic isolates

isolates	**Frequency**	**Percent**	**MDR Strain**	**Percent**
Staphylococcus aureus	7	21.9	2	28.6
Bacillus subtilis	6	18.8	0	0
Klebsiella pneumonia	9	28.1	3	33.3
Pseudomonas aeroginosa	10	31.3	3	30
Total	32	100.0	8	25

Table 3.5.2 Plasmid curing of anaerobic isolates using acridine orange

ISOLATE		CODE	FQ	AMG	CEF	MAC	AM/CLA	AMX
P. aeruginosa N=4	1U		2	3	1	2	3	3
	1C	0.1AO	1	0	0	0	0	0
		0.4AO	1	0	0	0	0	0
		% CURED	50%	0%	0%	0%	0%	0%
K. pneumonia N=3	2U		2	2	2	3	3	3
	2C	0.1AO	0	1	1	1	1	1
		0.4AO	0	1	1	1	1	1
		% CURED	0%	50%	50%	33.3%	33.3%	33.3%
S. aureus N=2	3U		1	1	2	1	2	2
	3C	0.1AO	0	0	0	0	0	0
		0.4AO	0	0	0	0	0	0
		% CURED	0%	0%	0%	0%	0%	0%

KEYWORD; FQ= Fluoroquinolones, AMIGLY= aminoglycoside, CEF= Cephalosporin, MAC= macrolide, AMOX/CLAV= Amoxicillin/clavulanic acid, AMOX= Amoxicillin, AO= Acridine orange, n= number of isolates, U= uncured , C=cured

CHAPTER FOUR

DISCUSSION

4.1 Epidemiology of study participants

The study showed a higher frequency of sinusitis in female compared to male. This corresponded to an article by Brook,2023 which measured the distribution of sinusitis across gender and documented a 20.3% prevalence in women compared to an 11.5% in men. Also, in accordance to 2010 National Health Interview Survey age-adjusted data in the United States which revealed that female (15.5%) had a higher prevalence compared to male (9.8%). This was however in contrast to a research work by Ravanrara *et al.,* 2020 which reported that 65.7% of participants who underwent their study were male. Prevalence in female may be as a result of anatomical variation resulting in smaller diameter of sinus ostia which permits easy obstruction of sinus drainage. Another researcher attributed this prevalence to a closer contact between females and children who are prone to upper respiratory tract infection due to poorly developed immune system (Brook,2023). Also, a research by Ference *et al.,* 2015 related estrogen which is produced predominantly by the ovaries in large amount in female to the prevalence of sinusitis. The study discussed the role of estrogen in mediating inflammatory responses by causing elevated level of antibodies that are autoantigen specific in the tissues of patients with chronic rhinosinusitis. Another study by Luo *et al.,* 2021 buttressed these gender-based findings by relating the progression of otolaryngology diseases to the level of sex-linked hormones, particularly estrogens. This estrogen induces an up-regulation of the expression of C-C chemokine receptors which forms the basis of immune response. Estrogen signaling pathway causes the degranulation of eosinophil which results in the release of proinflammatory mediators which are major moderators in the progression and maintenance of disease state. Hence, this constant inflammation of the sinus epithelium

causes a distortion in the biological healing mechanism thus leading to further damage to the sinus and nasal epithelium.

From a sociological point of view, gender specific differences may be attributed to individual's urge and willingness to seek medical intervention when they come down with a disease as diagnosis of sinusitis is heavily dependent on symptoms reported during the course of medical care and interview. Sinusitis is common across all age group with differences emanating from symptoms, histopathology, and co-morbidities. However, research shows a higher prevalence in children below 13 years of age due to the anatomy of the sinuses and propensity to come down with Upper Respiratory Tract Infection due to poorly developed immunomodulators (Newton, 1996). This study however encountered more adults compared to children.

Investigation revealed that participants who never smoked were at higher risk of sinusitis(p-value<0.05). This was however contrary to a Research work by Reh *et al.,* 2012 which described social and environmental factors such as cigarette smoke as a major risk factor of upper respiratory tract infection. The impact of cigarette smoke on sinonasal mucosa results from toxic irritants that are released which causes alteration in mucocillary clearance in the sinuses. This deviant in findings may be attributed to small sample size and participant's unwillingness to provide sensitive information about their lifestyle. Majority of study participants reported never consuming alcohol. However, a study by Bendtsen *et al.,* 2008 showed that consumption of alcohol may worsen symptoms associated with sinusitis such as nasal congestion due to the dehydrating effect of alcohol.

4.2 Infectious disease characteristics

According to a study by Rosenfield,2016, the diagnosis of sinusitis is achieved upon the presence of running nasal discharge as well as nasal obstruction which persisted for over 10

days. All Study participants encountered reported nasal discharge as a striking symptom to the disease ranging from a variety of colour. This purulent discharge may be associated with the presence of bacteria in the sinuses, particularly the maxillary sinuses. Presence of these bioburden triggers an inflammatory response which is marked by the release of inflammatory mediators such as histamine. Histamine is known to cause dilation of blood vessels in the nasal lining as well as increased mucus production through production of prostaglandins (Lee *et al.,* 2020). A large proportion of study participants reported stuffiness which maybe be attributed to poor mucocillary drainage leading to a back log of fluid in the ostia and this results in increase pressure and the feeling of fullness in the nasal cavity. Other symptoms encountered during the course of investigation were consistent with previous research work done by Lindbaek and Hjortdahl, 2002 and also a more recent study by Sharma *et al.,* 2022. Result obtained showed a statistically relationship between past episodes and the prevalence of sinusitis. These recurrent episodes maybe be linked to anatomical anomaly, environmental factors, re-infection, misdiagnosis, poor therapy as well as antimicrobial resistance to clinically used antimicrobial agents. *Staphylococcus aureus* which is one of the most common microbiota of the nasal cavity and the most frequent organism isolated during the course of may continually colonize the sinuses and lead to recurrent infection (Sharma *et al.,* 2022). Although sinusitis often occur in people with no family background of the disease since disease progression is often dependent of individual factor, A study by Payne and Borish, 2011 attributed recessive gene and protein expression pattern to hypersensitivity to foreign invaders. Categorically making the disease genetically linked. The result from investigation was in concordance with this study as one-third of study participants had family history of sinusitis.

4.3. Distribution of microbial isolates

4.3.1. Frequency distribution of isolates

Organisms which require molecular oxygen as an electron acceptor in the production of energy in the Form of Adenosine Triphosphate (ATP) during respiration are classified as Aerobes (Regina,2019). Frequency distribution of isolates obtained from aerobic culture showed the highest prevalence of *Staphylococcus spp* which is in concordance with its role in the nasal cavity. A research by Krismer *et al.,* 2017 revealed that *Staphylococcus aureus* constitute a large portion of nasal bacterial density where they compete against other colonizers for attachment sites in the anterior nares. Other colonizer such as *S. epidermidis* results to secretion of molecules that hamper the adhesive characteristics of *S. aureus* all in the bid for survival. But *S. aureus* remains apex due to its ability to use limited available nutrient and resistance to secreted lysing agents. Attachment in these anterior nares form a reservoir for the spread of this pathogen. When conditions are favorable, these Facultative pathogens may rapidly proliferate and probe into farther distances and consequently cause infection of the sinuses (Sakr *et al.,* 2018).

The next predominant organism encountered was *Pseudomonas aeruginosa*, a gram-negative bacillus and also a known disease-causing opportunistic pathogen primary to the upper respiratory tract. Pseudomonas aeruginosa form a biofilm by attaching surface aggregate in a hydro-polymeric matrix which results in virulence of the organism as this biofilm is a major contributor to disease progression as well as confers resistance to antimicrobial agents resulting in persistent sinusal infection (Costerton *et al.,* 1999).

Klebsiella spp are rarely occurring opportunistic pathogens associated in patients with sinusitis (Lau *et al.*, 2008) which was evident in this study recorded low abundance of this organism. *Klebsiella spp* is one of the major pathogens linked to nosocomial infections pertaining to the upper respiratory tract (Dorman and Short, 2017). It mainly accounts for gut

colonization in humans where it plays an integral role in the development of mucosal immune system. However, there's still a dearth of knowledge about the role of this organism in upper respiratory tract infection. Further investigation would be necessary to determine whether these isolates have positive, negative, or neutral effects on nasal microbiota.

Copeland *et al.*, 2018 discussed that *Corynebacterium spp* accounts for one of the most prevalent causal agents associated with sinusitis. This was supported by a study carried out by Menberu *et a l.,* 2021 which recorded a prevalence of Corynebacterium spp across over 75% study participants. This was however contrary to this research work which recorded a mere distribution of this isolates among study participants. *Corynebacterium spp* is known to alter the bio-density of the nasal cavity due to its ability to release anti-staphylococcal molecules such as triacylglycerol lipase which disruptions the proliferation of *Staphylococcus spp* (Menberu *et al.,* 2021) and this results in Dysbiosis which is an imbalance between disease causing pathogens and commensal bacteria. Although *Bacillus spp* was isolated, limited findings are available to support the role of this organism to the prevalence, progression and maintenance of sinusitis.

Anaerobes uses any other inorganic acceptor other than oxygen such as sulphate, nitrate or molecular Sulphur for the production of energy in the form of ATP. This study however did not record any strict anaerobic isolate documented by various literature. However , Facultative anaerobes were isolated when specimen were cultured under anaerobic conditions which is consistent with a research work by Urbán *et al.,* 2020. among which, *Pseudomonas aeruginosa* was most abundant.

4.4. Morphological Characteristics of Aerobic and Facultative Anaerobic Isolates

The different forms that bacterial growth can take is highlighted by the division of bacterial colonies into elevated and flat colonies. Numerous factors, including bacterial species, isolates, growing circumstances, and genetic factors, can be responsible for the variability in colony morphology (Finkelstein *et al.,* 1992). Moreover, according to Talaiekhozani *et al.,* (2013). Different bacterial populations may exist in the same samples and they can be distinguished based on the observation of both elevated and flat isolates. According to Kassen *et al.,* (2013), these differences in colony morphology confers genetic variety and environmental adaptations made by bacterial species. Bacteria which produce capsule have a slimy (mucoid) appearance when examined by physical manipulation with a sterile wire loop. Upon examination, isolates which appeared mucoidal possess extracellular polysaccharide alginate which may confer resistance and enforce the survival of the organism (Grobe *et al.,* 2001). Hemolytic properties suggest the pathogenicity of bacterial cells. When cultured on blood agar, α-hemolysis observed resulted from partial break down of red blood cells and the colonies around appears greenish or brown. Unlike β-hemolysis which a clear zone due to complete destruction of red blood cells. Organisms that exhibit β-hemolysis are highly pathogenic as they exude toxic exotoxins which are detrimental to human health (Nizet,2002). Ɣ-hemolytic organisms do not destruct red blood cells hence the blood agar remains intact. Upon culture on a differential media, in this case mannitol salt agar, a golden yellow colour was observed for only *Staphylococcus aureus*. This is due to the fermentation of mannitol which causes phenol red pH indicator present in the media to turn yellow (Missiakas and Schneewind,2013). Other staphylococcal species do not ferment mannitol hence retain the colour of the agar plate. On nutrient agar, Pseudomonas aeruginosa is characterized by its blue-green appearance due to production of pyocyanin pigment (Abdelaziz *et al.,* 2023),

Staphylococcus aureus appeared slightly yellow while other isolates appeared between translucent to white.

Microscopic characterization using Gram Staining technique was used to classify isolates into Gram Positive and Gram Negative based on the colour of stain retained when viewed under a light microscope. Shape of cells was further used to classify isolates into cocci or bacilli. Isolates which retained the purple colour of the primary dye(crystal violet),were classified as Gram positive while those who lost the colour of the primary dye and picked the red colour of the counter stain(safranin red) where classified as Gram Negative. The difference in result is attributed to the amount of peptidoglycan present in the cell wall. Gram positive bacteria have more peptidoglycan in their cell wall which forms a complex with crystal violet. Upon decolorization, the complex still retains its integrity. Conversely, Gram negative bacteria have less of peptidoglycan and more of lipopolysaccharide in their cell wall resulting in loss of the primary stain upon decolorization. The cell wall hence picks up the colour of the counter stain which appears as pink under the compound microscope (Coico,2005). The investigation reported a variety of Gram-positive cocci, gram-positive bacilli and gram-negative bacilli. Differentiation based on gram staining is necessary as it gives an overview to how an isolate will be susceptible to the lethal actions of antimicrobial agent.

Biochemical tests are essential instruments for identifying and classifying different bacterial species according to their metabolic capacities. All isolates obtained where positive to catalase test. This infers that they contain the inducible enzyme, catalase which is released in-situ when the organism is placed in a reactive oxygen species such as hydrogen peroxide. Catalase enzyme converts this reactive species to water and oxygen. This trait is frequently linked to a variety of bacteria, including Gram-positive and Gram-negative species, making it a helpful tool for identifying these bacteria, as it was in this investigation (Reiner, 2010).

Isolates that are positive to coagulase test are particularly pathogenic and this characteristic is commonly associated with *Staphylococcus aureus* (Kateete *et al.,* 2010). Coagulase positive isolates produce inducible enzyme, coagulase which forms clot when it comes in contact with plasma. Isolates such as *Pseudomonas aeruginosa* were positive to oxidase test which confirms the presence of cytochrome oxidase enzyme. The principle of oxidase test relies on the use of tetramethyl-p-phenylenediamine dihydrochloride as an artificial electron donor for cytochrome C. A positive result is marked by a rapid colour change to purple and it infers that the isolate contains the enzyme cytochrome oxidase which catalyzes the oxidation of cytochrome C as well as reducing oxygen to form water in the electron transport chain(Shields and Cathcart, 2010). This test was used to differentiate between bacteria in this investigation. Isolates capable of utilizing 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. Urease test was used to differentiate between isolates with the ability to hydrolyze urea to produce ammonia and carbon dioxide of which 43 isolates tested positive to urease which was indicated by a colour change of the indicator(phenol red) from yellow to pink due to an increase in pH due to the presence of ammonia.

4.5 Antimicrobial susceptibility testing of isolates

Treatment option for bacterial sinusitis is usually empirical comprising of broad-spectrum antimicrobial agents as they cover a wide range of gram positive, gram negative, Aerobes and anaerobes. The excessive use of antimicrobial agent in general has resulted to the growing problem of antimicrobial resistance in the community and hospital settings (Carlet *et al.,* 2012). From the resulted obtained during the course of investigation, A large population of *Staphylococcus aureus* were susceptible to the bactericidal action of fluoroquinolones.

This maybe be attributed to inhibition of Deoxyribonucleic Acid (DNA) gyrase enzyme and Topoisomerase IV present in both gram positive and gram negative organisms respectively. This inhibition leads to a disruption in DNA replication ultimately leading to cell death. Resistance to fluoroquinolones is primarily due to production of multi-drug efflux pumps. Other mechanism of resistance may be due to impermeability of biocapsules to antimicrobial agents, spontaneous mutation and positive selection (Pantosti *et al.,*,2007). *Staphylococcus aureus* also showed significant susceptibility to aminoglycosides. This may be explained by the fact that aminoglycosides target the 30S ribosomal subunit of bacterial ribosome. They disrupt the bacterial process of protein synthesis by attaching themselves to the ribosome. Because of this interference, the genetic code cannot be read correctly, which results in the shortened polypeptide chains or defective proteins. Interestingly, aminoglycosides have a post-antibiotic action, which implies that they continue to be antibacterial long after the antibiotic has left the body. Their continued action adds to their effectiveness in combating Staphylococcus species. The bacteria are consequently unable to synthesis vital proteins required for their growth and survival. Resistance to aminoglycosides maybe be due to production of aminoglycoside-modifying enzymes which causes inactivation of the antimicrobial agents (Pantosti *et al.,* 2007). Appreciable *Staphylococcus aureus* were susceptible to cefotaxime which is a 3rd Generation Cephalosporin. It is a broad spectrum antibiotics which has cause the death of gram positive, gram negative and anaerobic bacteria (Padda and Nagalli, 2023). this bactericidal action is due to disruption of cell wall through inhibition of transpeptidation by transpeptidase enzyme. resistance to cefotaxime is due to the production of beta lactamase enzyme by *Staphylococcus aureus* which opens up the beta-lactam ring of cefotaxime and inherently cause the loss of antibacterial activity. Macrolide(azithromycin) resulted in the elimination of more than half of isolated *Staphylococcus aureus* . This was due to disruption of protein synthesis by binding to 50S

ribosomal subunit. Resistance to macrolides is linked to drug modification and active drug efflux pump. Resistance to macrolide may also be plasmid borne (Chiou *et al.,* 2023). Susceptibility of Staphylococcus aureus to penicillin antibiotics varied as there was appreciable significance between susceptibility to amoxicillin and amoxicillin/clavulanic acid. Staphylococcus spp produce beta lactamase enzyme as its intrinsic mechanism of resistance. Less than half of total isolates had varying susceptibility pattern to amoxicillin and amoxicillin/clavulanic acid. The difference in susceptibility pattern maybe be attributed to the presence of clavulanic acid, a beta lactamase inhibitor which works in synergy with amoxicillin to knock off beta lactamase enzyme there by allowing the bactericidal action of amoxicillin (Kapoor *et al.,* 2017) . Similar result trend was observed for *S. epidermidis* with all isolates being susceptibility to the bactericidal action of fluoroquinolones and aminoglycosides.

Klebsiella spp which is a gram-negative bacillus showed appreciable susceptibility to levofloxacin. Fluoroquinolones are known for their broad-spectrum activity against a wide range of bacteria, including both Gram-negative and Gram-positive species. The specific bacterial enzymes targeted by fluoroquinolones, including DNA gyrase, are critical for DNA supercoiling and maintaining DNA integrity during replication and repair. *Klebsiella spp* relies on these enzymes for its genetic processes, making it vulnerable to the disruption caused by fluoroquinolones. They also lack the ability to pump out macrolides, floroquinolones, aminoglycosides and cephalosporins from their system, and this accounts for susceptibility to these antimicrobial agents. This is evident as over half of isolated *Klebsiella spp* were susceptible to azithromycin, cefotaxime and gentamycin. However, all *Klebsiella spp* were resistant to penicillins. This maybe be as a result of enzymatic inactivation of penicillin as well as over expression of efflux pumps (Li *et al.,*,2012). Combination therapy via multiple mechanism of action potentiates the bactericidal activity

against *Klebsiella spp.* Bacillus spp are gram positive rods which showed highest susceptibility to gentamycin. Although aminoglycosides primarily inhibit gram negative pathogens, it is still considered a broad spectrum antibiotics as it has varying activities against a wide range of organisms (Vakulenko and Mobashery, 2003).

Pseudomonas aeruginosa were susceptible to the killing action of levofloxacin. Notably, poor susceptibility to macrolide was observed. This increase in resistance may be due to inability of the drug to penetrate the hydro polymeric matrix produced by *Pseudomonas aeruginosa*. This resistance infers that macrolides should not be considered as first line treatment in sinusitis patients infected with *Pseudomonas aeruginosa.*

Although similar isolates were obtained from aerobic and anaerobic culture, susceptibility pattern showed that majority of total isolates were susceptible to the killing action of fluoroquinolones. This is evident due to wide spectrum of activity against variety of organisms. Aminoglycosides eliminated majority of isolates during the course of investigation while less number of isolates were susceptible to macrolides. This prevalence in resistance may be as a result drug modification into inactive form or over expression of efflux pumps. Conversely, the least susceptibility pattern was attributed to penicillin. Resistance may be due to expression of beta lactamase enzymes against penicillin antibiotics.

4.6 Frequency of Multi-drug resistant Isolates and Plasmid Curing Analysis

The study recorded the prevalence multi-drug resistant across aerobic isolates obtained during the course of investigation. Highest prevalence of resistance was demonstrated by *Klebsiella spp* as all isolates were multi-drug resistant which resistance pattern cut across fluoroquinolones, cephalosporins, macrolides and penicillin. This suggests that resistance may not be due to plasmid but chromosomal resistance. *Klebsiella spp* resistance maybe be attributed to mutation in outer pore which prevents entry of antimicrobial agents to the

cytoplasm of the bacterial cell (Li *et al.,* 2023). Notably, *Klebsiella spp* lost its resistance to levofloxacin, azithromycin and amoxicillin/clavulanic acid. This may be due to the fact that fluoroquinolone inhibits the proliferation of plasmid RNA and this confers anti-plasmid activity. This activity in synergism with the intercalating activity of acridine orange covertly leads to the loss of plasmid function. This brings about susceptibility to the bactericidal action of fluoroquinolones on bacterial cells. Similar trend was significant across fluoroquinolone activity post curing to *S. aureus, P. aeruginosa* and *Bacillus spp.*

Interestingly, resistant *Bacillus spp* isolates remain resistant post curing and this shows that resistance demonstrated was not due to the presence of plasmid but may be linked to chromosomes expressed by production of efflux pumps (Athama *et al.,* 2004). Susceptibility to the action of aminoglycoside and cephalosporins indicates removal of multi-drug encoded plasmids. Variability in resistance pattern to penicillin after curing suggests the role of beta lactamase inhibitors in mitigating resistance to beta lactam antibiotics.

For *Staphylococcus aureus*, resistance to both penicillin antibiotics was evident. Post curing, half of these resistant isolate became susceptible to the antimicrobial activity of penicillin. This suggest that initial resistance conferred on the newly susceptible isolates were due to the presence of plasmids. The other half which remained resistant suggest chromosome-mediated resistance through alteration of penicillin binding protein as discussed by Ender *et al.,*. (2004).

For *Pseudomonas aeruginosa,* resistance to aminoglycoside, macrolide and Cephalosporin persisted post curing. This may be attributed to production of biofilms by this organism which confers innate resistance to the antimicrobial activities of these agent. Susceptibility to fluoroquinolones post curing connotes loss of plasmids coded for fluoroquinolone resistance.

A fraction of *Pseudomonas aeruginosa* lost resistance to penicillin. Resistance which persisted post curing may be due to mutagenesis (Bredenstein *et al.,* 2011).

Resistance across facultative anaerobic isolates was observed during the course of investigation. All *staphylococcus aureus* isolates remained resistant to all classes of antimicrobial agents. This suggest chromosomal resistance mediated by alteration in drug binding site, over expression of beta lactamase enzymes as well as production of multi-drug efflux pumps (Pantosti *et al.,*, 2007). similar trend was observed across *Pseudomonas aeruginosa* with only half of isolates becoming susceptible to fluoroquinolones. Persistent Resistance to fluoroquinolones post curing in *Klebsiella spp* suggest the role of chromosome mediated innate resistance towards antimicrobial resistance.

The variability in the maximum efficient concentration of a particular curing agent, as reported by Carlton and Brown (1981), spanning from 100 to 1000 times, as noted by Haque (2017), underscores the intricate nature of curing processes. Interestingly, resistance to penicillin in *Pseudomonas aeruginosa* which was persistent after supplementation with 0.1mg/mL Acridine orange was lost when the concentration was increased to 0.4mg/mL. This lays claim to a possibility of concentration dependent activity of plasmid curing agents. However limited research is available to support this claim. The particular species addressed and the curing agent's mode of action are two important variables that affect how successful a curative agent is. Together, these factors affect how the curing process turns out. Due to differences in their cell structures, metabolic pathways, and susceptibility to the agent's mode of action, various bacteria may react differently to the same curative agent. The mode of action of the curative agent is also very important. Certain curative agents exhibit differential efficacy against different microbes due to their ability to damage cell membranes, block key enzymes, or interfere with DNA replication. This event highlights the necessity of precise and customized curing procedures to reduce the likelihood of resistance development, underscoring the significance of having a thorough grasp of curing.

4.7 Limitations of the study

This research project, although offering valuable insights into the plasmid profiles of bacterial isolates and their correlation with participant demographics, encountered several limitations. Short time frame due to the institution's calendar hampered the initialization of the target sample size. Mutagenicity of Acridine, a chemical employed in the research process, is known for its mutagenic properties. This raised concerns about potential health risks associated with its use. Also, Unavailability of research reagent as some reagents such as nitrocefin disc could not be found in the country and this prevented a more elaborate research study. Finally, Inconsistent power supply further complicated the curing process. Preservation of specimen before curing increased the overall cost and complexity of the research.

CHAPTER FIVE

CONCLUSION

In conclusion, sinusitis is a prevalent condition that significantly impacted study participants across various demographics. Females were more predisposed to sinusitis compared to males and the major symptoms experienced were nasal discharge, stuffiness and cough. The role of bacteria towards recurrent episodes of sinusitis and the identification of plasmid-borne multi-drug resistance genes in study participants with sinusitis emphasizes how crucial it is to put policies like susceptibility testing and rational antibiotic use into place. These results demonstrate how critical it is to use antimicrobial stewardship techniques in the management of sinusitis. Healthcare professionals can optimize treatment efficacy and minimize the danger of antibiotic resistance development by carefully choosing antibiotics based on the susceptibility profiles of these resistant genes. This strategy is crucial for preserving the efficacy of antibiotics in the treatment of sinusitis and other upper respiratory tract infection and providing a halt to the development of antibiotic resistance, which is a global health challenge.

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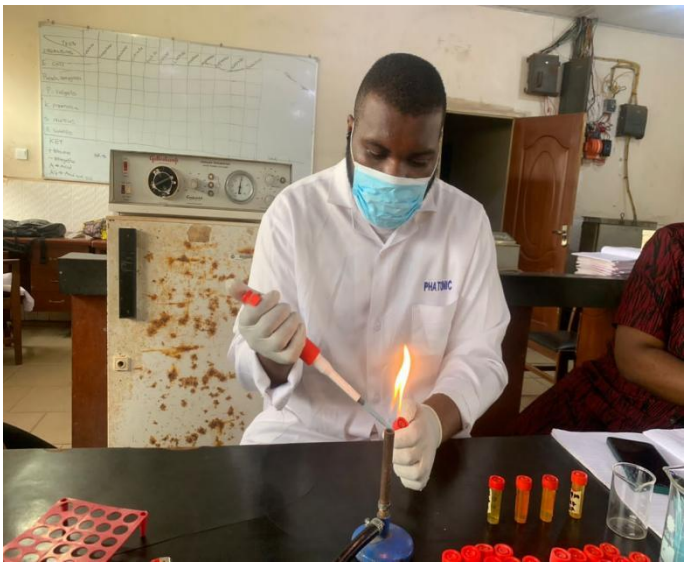
APPENDIX



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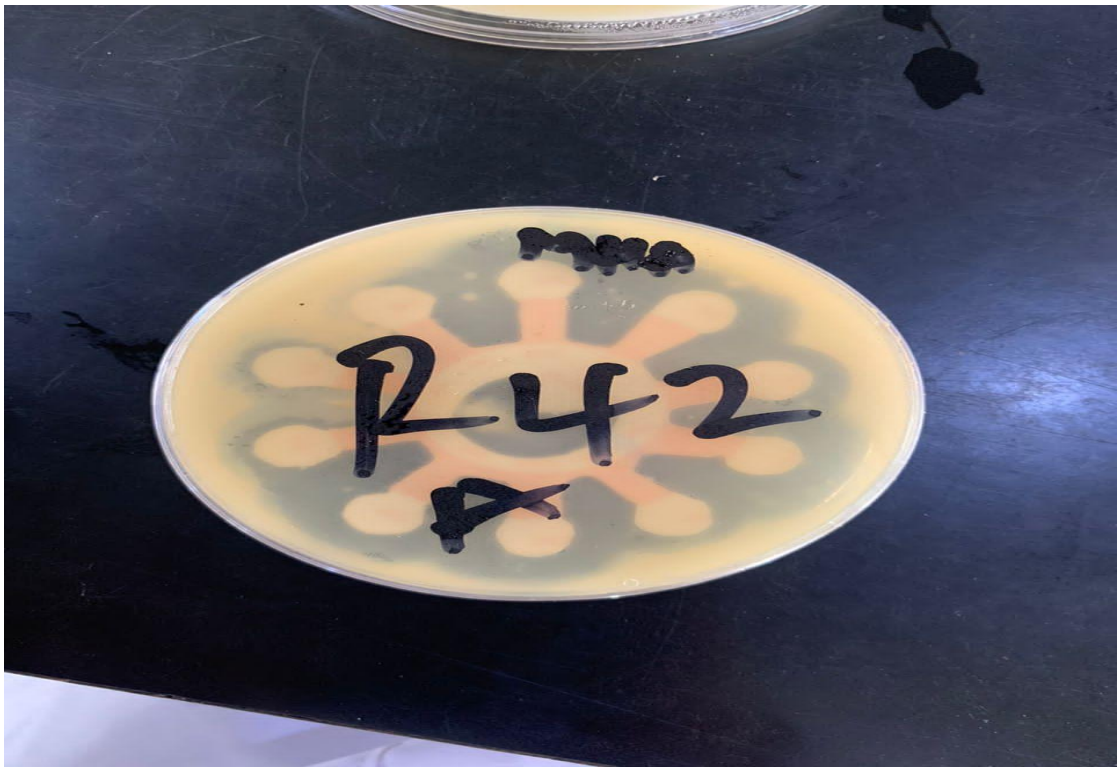
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APPENDIX 2

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
age of patients * sex of patients	43	100.0%	0	0.0%	43	100.0%
occupation * sex of patients	43	100.0%	0	0.0%	43	100.0%
smoking history * sex of patients	43	100.0%	0	0.0%	43	100.0%
alcohol history * sex of patients	43	100.0%	0	0.0%	43	100.0%

age of patients * sex of patients Crosstabulation

Count

		sex of patients		
		male	female	Total
age of patients	0-6 years	3	3	6
	7-15years	2	2	4
	16-20years	1	0	1
	21-25years	3	8	11
	26-30years	4	3	7
	31-35years	0	4	4
	36-45years	1	2	3
	46-55years	0	2	2
	56-60years	2	1	3
	>60years	2	0	2
Total		18	25	43

occupation * sex of patients Crosstabulation

Count

		sex of patients		
		male	female	Total
occupation	employed	0	5	5
	self employed	6	6	12
	student	4	9	13
	not employed	6	5	11
	retired	2	0	2
Total		18	25	43

smoking history * sex of patients Crosstabulation

Count

		sex of patients		
		male	female	Total
smoking history	never smoked	10	25	35
	stopped smoking	6	0	6
	still smoking	2	0	2
Total		18	25	43

alcohol history * sex of patients Crosstabulation

Count

		sex of patients		
		male	female	Total
alcohol history	never drank	8	15	23
	still drinking	3	4	7
	stopped drinking	7	6	13
Total		18	25	43

Statistics

		past medication history	present medication history
N	Valid	43	43
	Missing	0	0

isolates

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	staph aureus	27	35.1	35.1	35.1
	staph epidermidis	11	14.3	14.3	49.4
	cornybacterium	4	5.2	5.2	54.5
	bacillus cerus	5	6.5	6.5	61.0
	bacillus subtilis	7	9.1	9.1	70.1
	klebsiella pneumonia	3	3.9	3.9	74.0
	klebsiella oxytoca	2	2.6	2.6	76.6
	enterobacter aerogenes	2	2.6	2.6	79.2
	pseudomonas aeroginosa	16	20.8	20.8	100.0
	Total	77	100.0	100.0	

Gram staining

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	GPC	38	49.4	49.4	49.4
	GNB	23	29.9	29.9	79.2
	GPB	16	20.8	20.8	100.0
	Total	77	100.0	100.0	

oxidase test

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	23	29.9	29.9	29.9
	negative	54	70.1	70.1	100.0
	Total	77	100.0	100.0	

catalase

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	77	100.0	100.0	100.0

coagulase

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	27	35.1	35.1	35.1

	negative	50	64.9	64.9	100.0
	Total	77	100.0	100.0	

indole

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	2	2.6	2.6	2.6
	negative	75	97.4	97.4	100.0
	Total	77	100.0	100.0	

urease

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	43	55.8	55.8	55.8
	negative	34	44.2	44.2	100.0
	Total	77	100.0	100.0	

Gram staining

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	GPC	38	49.4	49.4	49.4
	GNB	23	29.9	29.9	79.2
	GPB	16	20.8	20.8	100.0
	Total	77	100.0	100.0	

oxidase test

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	23	29.9	29.9	29.9
	negative	54	70.1	70.1	100.0
	Total	77	100.0	100.0	

catalase

	Frequency	Percent	Valid Percent	Cumulative Percent

Valid	positive	77	100.0	100.0	100.0

coagulase

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	27	35.1	35.1	35.1
	negative	50	64.9	64.9	100.0
	Total	77	100.0	100.0	

indole

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	2	2.6	2.6	2.6
	negative	75	97.4	97.4	100.0
	Total	77	100.0	100.0	

urease

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	43	55.8	55.8	55.8
	negative	34	44.2	44.2	100.0
	Total	77	100.0	100.0	

Gram staining * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
Gram staining	GPC	27	11	0	0	0	0	0	0	0	38
	GNB	0	0	0	0	0	3	2	2	16	23
	GPB	0	0	4	5	7	0	0	0	0	16

Total		27	11	4	5	7	3	2	2	16	77

urease * isolates Crosstabulat

Count

colour of discharge * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
colour of discharge	clear	11	5	0	1	5	2	1	0	7	32
	yellow	15	5	3	4	2	1	1	2	8	41
	red	1	1	1	0	0	0	0	0	1	4
Total		27	11	4	5	7	3	2	2	16	77

other symptoms experienced * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
headache	selected	14	6	2	3	4	0	1	1	10	41
	not selected	13	5	2	2	3	3	1	1	6	36
Total		27	11	4	5	7	3	2	2	16	77

previous episodes of symptoms * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
previous episodes of symptoms	yes	18	7	1	4	6	1	1	2	12	52
	no	9	4	3	1	1	2	1	0	4	25
Total		27	11	4	5	7	3	2	2	16	77

stuffiness * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
stuffiness	selected	20	8	3	3	4	2	2	1	12	55
	not selected	7	3	1	2	3	1	0	1	4	22
Total		27	11	4	5	7	3	2	2	16	77

cold weather * isolates Crosstabulation

Count

	isolates	Tot

		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	al
cold weather	selected	14	6	3	3	4	0	0	1	10	41
	not selected	13	5	1	2	3	3	2	1	6	36
Total		27	11	4	5	7	3	2	2	16	77

pollen grain * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
pollen grain	selected	2	0	0	0	0	0	0	0	1	3
	not selected	24	11	4	5	7	3	2	2	14	72
	20	1	0	0	0	0	0	0	0	1	2
Total		27	11	4	5	7	3	2	2	16	77

ciprofloxacin * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
ciprofloxacin	sensitive	22	11	4	4	7	3	1	2	13	67
	intermediate	4	0	0	0	0	0	1	0	2	7
	resistant	1	0	0	1	0	0	0	0	1	3
Total		27	11	4	5	7	3	2	2	16	77

levofloxacin * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
levofloxacin	sensitive	24	11	4	4	5	2	1	2	13	66
	intermediate	1	0	0	1	0	0	0	0	1	3
	resistant	2	0	0	0	2	1	1	0	2	8
Total		27	11	4	5	7	3	2	2	16	77

azitthromycin * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
azitthromycin	Sensitive	14	10	4	3	5	2	1	1	3	43
	intermediate	7	1	0	0	0	0	1	0	3	12
	resistant	6	0	0	2	2	1	0	1	10	22
Total		27	11	4	5	7	3	2	2	16	77

gentamycin * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
gentamycin	Sensitive	21	11	3	4	7	2	1	1	12	62
	intermediate	0	0	0	0	0	0	0	1	0	1
	resistant	6	0	1	1	0	1	1	0	4	14

Total		27	11	4	5	7	3	2	2	16	77

ofloxacin * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
ofloxacin	Sensitive	21	10	4	2	6	3	1	2	10	59
	intermediate	3	1	0	2	1	0	1	0	4	12
	resistant	3	0	0	1	0	0	0	0	2	6
Total		27	11	4	5	7	3	2	2	16	77

amoxicillin * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
amoxicillin	Sensitive	12	6	2	2	5	0	0	1	8	36
	intermediate	2	2	0	1	0	0	0	0	0	5
	resistant	13	3	2	2	2	3	2	1	8	36
Total		27	11	4	5	7	3	2	2	16	77

augmentin * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
augmentin	sensitive	16	5	2	3	6	2	0	1	8	43
	intermediate	0	3	0	0	0	0	0	0	1	4

	resistant	11	3	2	2	1	1	2	1	7	30
Total		27	11	4	5	7	3	2	2	16	77

streptomycin * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
streptomycin	sensitive	16	11	4	3	6	1	0	2	13	56
	intermediate	3	0	0	0	1	0	0	0	1	5
	resistant	8	0	0	2	0	2	2	0	2	16
Total		27	11	4	5	7	3	2	2	16	77

pefloxacin * isolates Crosstabulation

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
pefloxacin	sensitive	23	11	4	3	6	1	1	1	12	62
	intermediate	3	0	0	1	0	0	0	1	2	7
	resistant	1	0	0	1	1	2	1	0	2	8
Total		27	11	4	5	7	3	2	2	16	77

Crosstab

Count

		isolates									
		staph aureus	staph epidermidis	cornybacterium	bacillus cerus	bacillus subtilis	klebsiella pneumonia	klebsiella oxytoca	enterobacter aerogenes	pseudomonas aeroginosa	Total
alcohol	never drank	15	7	2	4	4	2	2	1	5	42

history	still drinking	3	1	2	1	0	0	0	0	3	10
	stopped drinking	9	3	0	0	3	1	0	1	8	25
Total		27	11	4	5	7	3	2	2	16	77

APPENDIX 3

3.6 SEMI STRUCTURED QUESTIONNAIRE; PATIENTS WITH RHINITIS/SINUSITIS

CONSENT; Do you agree to participate in this research study?

Yes ☐ No ☐

Signature: _____ Date: _____

Section A: Personal Information
1 .PATIENT I.D: _____

1. Age: 0-6yrs [ ] 7-15yrs [ ] 16-20yrs [ ] 21-25yrs [ ] 26-30yrs [ ] 31-35yrs 36-45yrs [ ] 46-55yrs [ ] 56-60yrs [ ] >60yrs [ ]

3. Sex:M [ ] F [ ] Phone: _____ 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 [ ]

Section C: Medication History
1. Past Medication History: (Please provide details)
_____

2. Present Medication History: (Please provide details)
_____

Section D: Rhinosinusitis History
Clinical diagnosis; Rhinitis [ ] Sinusitis [ ] both [ ]

1. How long have you been experiencing symptoms of rhinitis/sinusitis?

<5 Days [ ] 6-10days [ ] 11-14 days [ ] >14days [ ]

2. Have you had previous episodes of rhinitis/sinusitis?

Yes [ ] No [ ]
If yes, how often in a year; Once [ ] twice [ ] three times [ ] >four times [ ]

3. Do any of your family members have a history of sinusitis or related respiratory conditions?

Yes [ ] No [ ]

Section E: Sinusitis/rhinitis Symptoms

1. What symptoms do you experience?

stuffiness [ ] Nasal discharge [ ] headache [ ] facial pain [ ] fever [ ] facial and jaw swelling [ ] cough [ ] itchy throat [ ] mouth breathing [ ]
others_____
2. what is the colour of discharge? Clear [ ] yellow [ ] red [ ]

3. Are there specific triggers or patterns associated with your symptoms?
Cold weather [ ] exercise [ ] dust [ ] smoke [ ] pollen grains [ ]
others_____

Section F: Medication History

1. Are you currently taking any medications for sinusitis/rhinitis?

Yes [ ] No [ ]
If yes, what medication?
Antibiotics [ ] nasal spray [ ] prednisolone [ ]
2. Have you used antibiotics or antibacterial agents for the treatment of sinusitis/rhinitis in the past?
Yes [ ] No [ ] if yes, specify_____

Section G; COMORBIDITIES Do you have any existing disease condition?

Asthma [ ] COPD [ ] Ear infection [ ] HIV [ ] GERD [ ]

others_____

Section H: Microbial Infection History
1. Have you had previous microbial infections that made you use antibiotics?
Yes [ ] No [ ]
If yes, what type of infection;_____

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 [ ]

Section I: Antimicrobial Susceptibility Testing

1. Have you done a lab test for any rhinosinusitis?
Yes [ ] No [ ]
2. How effective was the treatment based on the lab result? Please describe.
_____

Section J: 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?
_____