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Isolation and Antibiogram of some Bacterial Isolates from Patients with Ear Infection in a Tertiary Health Care Facility at Benin City, Edo State, Nigeria

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Abstract

Ear infection has been reported as a common health concern globally, impacting individuals across diverse demographic factors. Causative bacterial agents of this infection are constantly changing and require continuous surveillance to monitor emerging trends. This study investigated the bacterial etiologic agents and their susceptibility patterns among patients with ear infection at the study Centre. A total of 43 patients who visited the Ear, Nose and Throat clinic of the University of Benin Teaching Hospital that were diagnosed with ear infection were evaluated. Patient data and specimen were collected after which isolated bacteria were subjected to antimicrobial susceptibility testing against some antibiotics (Sparfloxacin, Azythromycin Amoxicillin-clavulanic acid and amoxicillin) using standard methods. Results obtained showed that patients aged 16-25 years were more predisposed to ear infection and substantial number of them (60.5%) reported previous history of the infection. Staphylococcus (48.70%) and Pseudomonas (26.30%) were the most predominant bacterial isolated from these patients. Other isolates included; Bacillus (14.5%), Corynebacterium (3.90%), Enterobacterium (1.30%), and Klebsiella (3.9%). Antibiogram revealed that 78.9% of the isolates were susceptible to the killing effects of Azithromycin and only 39.3% were susceptible to amoxicillin. However. 69.8% of isolates were susceptible to amoxicillin- clavulanic acid combination therapy. Conclusively, Staphylococcus and Pseudomonas, were the leading cause of ear infection at the study center. Azithromycin as well as amoxicillin- clavulanic acid combination were the most effective antibiotics against the encountered bacterial isolates and these antibiotics can be used as a first line drug in the management of ear infection at the study.

Keywords: Antibiogram, bacteria, ear, infections, susceptibility.

INTRODUCTION

An ear infection can affect different parts of the ear, leading to symptoms such as inflammation or irritation in the inner ear, swollen and blocked Eustachian tubes, and mucus build-up in the middle ear. Additionally, bacterial infections can cause inflammation of the external auditory canal, resulting in acute pain and discomfort. Globally, more than 360 million people suffer from disabling hearing loss, with infections accounting for up to 40% of

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preventable cases. Documented predisposing factors to ear infection include gender, economic status, and poor hygiene, smoking and bathing in rivers (Alenezi et al., 2017).

In many low- and middle-income countries, ear infections are primarily caused by a wide array of bacteria and fungi (Shangali et al., 2023). Treatment in these regions often relies on empirical treatment methods without laboratory investigation or antimicrobial susceptibility testing (AST), leading to treatment failures and recurrent infections. Additionally, this practice can result in the need for more expensive antibiotics, prolonged hospital stays, and increased healthcare costs, impacting the financial burden and quality of life for families (Getaneh et al., 2021).

In poor countries like Nigeria, where there is limited resources, the cost of carrying out antimicrobial susceptibility test prior to prescribing antibiotics is a major concern. Thus empirical therapy is the most employed therapeutic approach with little or no data available on the effectiveness of this treatments approach. Clinic observations suggest that many patients return with recurring infections, indicating that empirical methods alone may be insufficient (Mahwish et al., 2020). As a result of this concern there is a need for further research into the antimicrobial susceptibility patterns of bacteria causing ear infections as recommended by antimicrobial stewardship strategy to improve the appropriateness of antibiotic prescription for improved treatment outcome. This study aimed to identify the etiological bacterial pathogens associated with ear infections and their antimicrobial susceptibility pattern, with the goal of enhancing prevention, control measures, and updating management and treatment options for ear infections.

MATERIALS AND METHOD

Study Area

The study was carried out at the Ear, Nose and Throat (ENT) Clinics of the University of Benin Teaching Hospital, a tertiary health care centre that has an ENT clinic attached to it that attends to both in-patient and out-patient.

Specimen collection

Specimens were collected using sterile swab sticks, aided by a mirror to visualize the internal ear. The contaminated swab sticks were placed into holders containing 5ml of thioglycolate broth. All specimens were transported within 2 hours to the Department of Pharmaceutical Microbiology laboratory for further microbiological investigations.

Study Design

This was a prospective study of patients who visited the Ear, Nose, and Throat (E.N.T) unit of the University of Benin Teaching Hospital (UBTH) from January to February, 2024. The research team evaluated a convenient number of 43 patients due to time constraints and limited number of new patients. Since only previously evaluated patients returned for checkups, specimen collection adhered to ethical guidelines with approval from the Institutional Review Board and informed consent from all participants.

Ethical Considerations

In this study, informed consent was obtained from all participants, with assent from patients aged 17 and below provided through their parents. The Health Research Ethics Committee of the University of Benin Teaching Hospital approved the study protocol (PROTOCOL NUMBER: ADM/E22/A/VOL. VII/14838152180). The study adhered to the principles of voluntary participation, anonymity, and confidentiality. Patients were given the option to participate, withdraw at any time, or decline to answer specific questions. All information

provided was kept confidential, and patients' addresses were not requested in the questionnaire.

Data Collection

A semi-structured questionnaire was used to collect detailed information from each study patient. This included socio-demographic aspects such as coded names for confidentiality, gender, history of smoking and alcohol use, swimming history, medication history, and the presence of hereditary or underlying health conditions like diabetes, hypertension, and cardiovascular disease. The questionnaire also inquired about the frequency of antibiotic use within a year, presenting ear infection symptoms, ear cleaning habits, the use of earrings, and the location of the ear infection.

Population of Study, exclusion and inclusion criteria

The study was conducted among all eligible patients across all ages who presented to the clinics for washing and check-up within the designated study period (from January to February 2024). Only patients who had been evaluated and confirmed to have ear infection by the medical doctor were included in the study while patients who had been on antibiotics 2-4 weeks prior to specimen collection and patients visiting the clinic for procedures other than washing and ear check-up were excluded from the study.

Characterization and Identification of Isolates

Specimens were first incubated in a broth medium for 24 hours. Then, they were sub cultured onto different types of agar plates, including blood agar, Mannitol salt agar, and MacConkey agar. After incubation, colonies with distinct morphologies were selected for further testing. Gram stain was performed to differentiate between Gram-positive and Gram-negative bacteria based on their cell wall structure. Gram-positive bacteria stained purple, while Gram-negative bacteria stained pink. Additional biochemical tests, such as catalase, coagulase, indole, and oxidase tests, were also conducted to aid in the identification of the specific bacteria genera.

Antibiogram test

Antibiotic susceptibility test was carried out using the disk diffusion standard method as published by Bauer and Kirby, 1966 with some modifications. Standard inoculum adjusted to 0.5 McFarland standard of each isolate was spread evenly on the surface of already dried Mueller Hinton Agar. With the aid of a sterile forceps; single antibiotic disc (sparfloxacin, azithromycin, amoxicillin and amoxicillin clavulanic acid) were impinged onto the surface of each inoculated plate. All plates were incubated in an inverted position at 37°C for 24hours. Inhibition zone diameters were measured to the nearest millimeter and interpreted as susceptible, and or resistant by comparison with published guidelines for antimicrobial susceptibility testing for commonly occurring pathogens obtained from clinical isolates (EUCAST, 2015).

Method of Data Analysis

Data were coded and entered from patient data collection forms into statistical package SPSS (Statistical Package for the Social Sciences) version 27.0 software (SPSS Inc. Chicago IL USA). Descriptive statistics was used to report percentage frequency of patient demographics and antibiograms of bacterial isolates. Statistical significance between variables was calculated using the unpaired student's t-test. Difference between groups were considered significant at P value of < 0.05.

RESULTS AND DISCUSSION

Association of sex with Patient Demographics

Table 1; showed the demographic characteristics of the study population, covering age distribution, occupation, smoking history, alcohol history, and swimming habits. The age distribution of patients with otitis infection varied widely, with the majority (60.5%,) aged 16-25 years. A significant portion of the participants were unemployed. Regarding social behavior, most participants had never smoked (88.4%) or consumed alcohol (67.4%). Additionally, our findings revealed that a substantial number of patients (79.1 %,) had previously experienced ear infections.

TABLE 1; Association of sex with patient Demographics

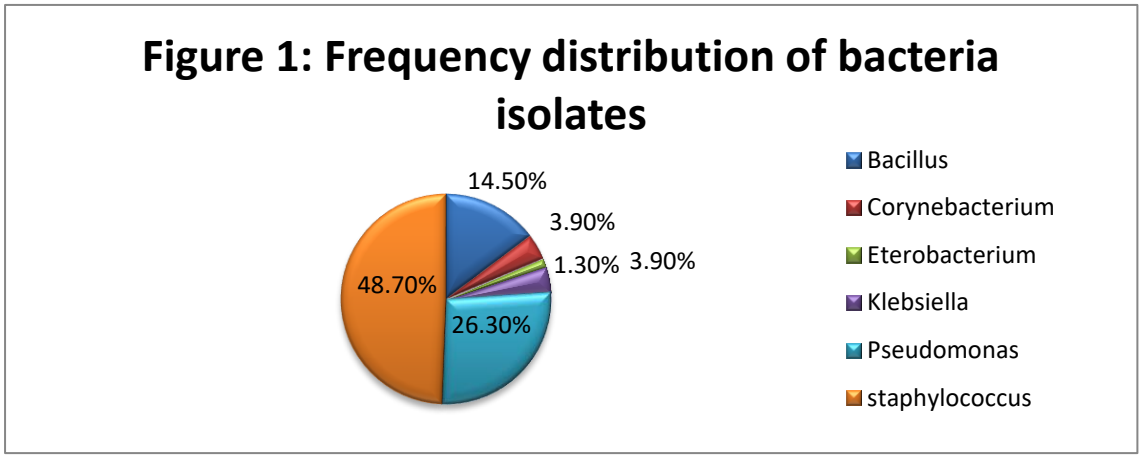
Characteristics	Values	Male (n=11)	Female(n=32)	Total	P-value
Age of patient	0-15yrs	1	4	5	0
	16-30yrs	5	21	26	
	30-45yrs	1	3	4	
	>45yrs	4	4	8	
Occupation	Unemployed	3	19	22	0.002
	Self-employed	1	2	3	
	Employed	5	5	10	
	Retired	1	0	1	
	Student	1	6	7	
Smoking history	Never	7	29	36	0.003
	Stopped	4	2	6	
	Ongoing	0	1	1	
Alcohol history	Never	4	25	29	0
	Stopped	4	1	5	
	Ongoing	3	6	9	
Swimming history	Never	8	30	38	0.099
	Stopped	1	1	2	
	Ongoing	2	1	3	
Earring wearing	Randomly	1	2	3	0
	Everyday	2	24	26	
	Monthly	1	2	3	
	Never	7	4	11	
Pus discharge	Yes	3	17	20	0.028
	No	8	15	23	
Previous microbial infection	Yes	10	24	34	0.002
	No	1	8	9	

The observed high rate of ear infection among older adolescent and young adults in this study may be associated with their life style and living conditions. Many individuals in this age range live in crowded environments at home and even in school dormitories. People living in close contact have been documented to be at greater risk for ear infections (Nick, 2023). Physical and social changes, unhealthy behaviors, and lack of education about proper ear

hygiene practices may be an additional contributory factor to the observed trend. Females have been reported to be genetically more susceptible to infections and autoimmune diseases compared with their male counterpart (Dias et al., 2022). This in agreement with the data obtained in this study as females accounted for 74.4% of our study participants suggestive of higher infection rate in this gender. A retrospective study in Ethiopia showed similar results with our study where patient's age 15-24years were more predisposed to ear infections. The same article however documented higher occurrence of ear infections in males (Getaneh et al., 2021). Patients who reported previous history of ear infection accounted for 79.1% of our study population. This may be due to therapeutic failure (bacteria resistance) weakening of the ear canal resulting in increased vulnerability to re-infection, exposure to harmful agents, and persistent fluid accumulation (Stinson, 2018). Patients who put on earrings everyday accounted for 60.5% of those who had ear infection. This may be attributed to getting their ear pierced in unhygienic environment, touching the ear with dirty hands, removing of earrings before piercing the ear, neglecting to clean the earring and frequent multiple piercing of the ears especially by females (Cleveland Clinic, 2023).

Frequency Distribution of Bacteria isolates

Figure 1; represents a bar chart showing the frequency of distribution of the isolated aerobic bacteria. From this study it is shown that majority of the isolated bacteria were either *Staphylococcus,* or *Pseudomonas,* as represented by a frequency distribution of 48.7% and 26.30% respectively. Other isolated bacteria and their respective percentages included; *Bacillus,* (14.5%), *Corynebacterium* (3.90%), *Klebsiella,* (3.90%) and *Enterobacterium* (1.30%), this is shown in figure 1 below.



Staphylococcus plays a significant role in the ear microbiome and may impact otic health, potentially leading to the development and progression of otic diseases. Also the high frequency of occurrence of this Gram positive cocci in this study may be due to its exceptional adhesion factor and ability to thrive in both aerobic and anaerobic conditions. *Psuedomonas* and *Klebsiella* are clinically important Gram negative opportunistic bacteria that may have found their way into the ear through inhalation. These enteric bacteria are associated with multidrug resistance phenomenon due to their ability to readily acquire mobile genetic materials that enhance horizontal gene transfer and consequently promoting the spread of antibiotic resistant genes within and between species (Garcia-Fernandez et al., 2019). Similarly, a retrospective study has reported *Staphylocci* and *Pseudomona* strains among the

most prevalent isolates involved in ear infections (Bahjat et al., 2023). *Enterobacteria* and *Corynbacterium*, are less prevalent and not typical otic microbiota.

Susceptibility pattern of bacteria isolates

Results obtained from susceptibility test of the isolates to the antibiotics used as presented in table 2 showed that that majority of the bacteria isolates were sensitive to azithromycin (78.9%) while 60.7% of the isolates were resistant to amoxicillin. However. 69.8% of isolates were susceptible to amoxicillin- clavulanic acid combination therapy.

TABLE 2; Susceptibility pattern of bacterial isolates from infected ear of patients at UBTH in percentage frequency (%)

Isolates	SP		AZ		AUG		AM	
	S	R	S	R	S	R	S	R
Bacillus spp (11)	5(46.6%)	6(53.4 %)	6(55%)	5(45%)	7(63.4%)	4(36.6%)	5(45%)	6(55%)
Corynebacterium (3)	1(33.3%)	2(66.7 %)	2(66.7%)	1(33.3%)	2(66.7%)	1(33.3%)	3(100%)	0(0%)
Eterobacterium (1)	0(0%)	1(100 %)	1(100%)	0(0%)	1(100%)	0(0%)	0(0%)	1(100%)
Klebsiella spp(3)	3(100%)	0(0%)	3(100%)	0(0%)	2(75%)	1(25%)	1(25%)	2(75%)
Pseudomonas (20)	15(75%)	5(25%)	14(70%)	6(30%)	9(45%)	11(55%)	3(15%)	17(75%)
Staphylococcus (37)	31(81.5 %)	6(19.5 %)	26(81.6 %)	11(19.4 %)	26(68.75 %)	11(31.3 %)	19(50.6 %)	18(49.4%)
Average %	55.9%	44.1%	78.9%	21.1%	69.8%	30.2%	39.3%	60.7%

KEY: SP=Sparfloxacin, AZ=Azithromycin,AUG=Amoxicillin-clavulinic acid , AM=Amoxicillin, S=Sensitive,R=Resistant

Azithromycin has been reported to be active against a wide range of Gram negative and positive bacteria even beta lactam resistant bacteria cells. It has fast penetration of the outer membrane of Gram negative bacteria and as an antibiofilm it has been documented to inhibit the formation and motility of biofilms in *Pseudomanas* (Heidary et al., 2022).This justifies the observed high susceptibility profile of over 70% of *Pseudomanas* cells to the killing effect of azithromycin in this study. Bacteria cells that do not possess beta lactamase enzymes are essentially susceptible to the killing effect of amoxicillin alone while amoxicillin-clavulanic acid target the beta lactamase producers (Huthner et al., 2020). In this study amoxicillin-clavulanic acid was active against 69.8% of bacterial isolates and this far out strips that of amoxicillin alone (39.4%); thus suggesting that majority of the bacteria isolates where beta lactamase producers.

CONCLUSION

This study explored the demographic factors of patients with ear infections as well as their bacteria etiologies and it associated antibiogram. Patients aged 16-25 years were more predisposed to ear infection. *Staphylococcus* (46.70%) and *Pseudomonas (*26.30%) were the leading cause of ear infection at the study center. Antibiogram revealed that Azithromycin as well as amoxicillin- clavulanic acid combination were the most effective antibiotics against the

encountered bacteria isolates and these antibiotics can be used as a first line drug in the management of ear infection at the study center.

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