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ORIGINAL ARTICLE

Frequency, Types and Antimicrobial Susceptibility Pattern of Bacteria in Culture Positive Ear Swabs of Patients Attending Referral Hospital in Southern Zone of Tanzania

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ABSTRACT

Background: Ear infections affect adults and children, and when left untreated, they may cause hearing loss and life-threatening complications. In our setting, ear infections are a common health problem. However, there is paucity of information on the etiology and antimicrobial susceptibility pattern.

Objective: This study aimed to determine the spectrum of bacteria causing ear infection and their antimicrobial susceptibility patterns at Secondary-level health facilities.

Methods: This cross sectional retrospective hospital-based study reviewed 288 data of patients diagnosed with ear infections from January 2021 to March 2023. Demographic, isolates and antimicrobial susceptibility information's were abstracted. Multidrug resistance (MDR) was regarded as resistance to at least one antibiotic in three or more categories/groups of antibiotics. Analysis was done using STATA software version 15. Descriptive analysis was summarized as frequency and proportion for categorical variables, and results presented using tables.

Results: We included 288 (50.4%) positive bacteria culture out of ear swab samples collected for culture in the analysis. Of the total bacterial isolates, majority were gram-negative, 178 (58.2%). The predominant isolate was Staphylococcus aureus 124 (40.5%), followed by Pseudomonas aeruginosa 80 (26.1%). Mixed bacterial infection was observed in 18 (6.2%) patients. Most enterobacterales were resistant to ampicillin, amoxicillin/clavulanic acid, tetracycline and trimethoprim-sulfamethoxazole. Pseudomonas aeruginosa was highly resistant to piperacillin. Multidrug resistance (MDR) was observed in 245 (80%) of the isolates. Gram positive isolates showed high resistance to erythromycin. Methicillin-resistant Staphylococcus aureus (MRSA) was observed in 67(59.3%) isolated Staphylococcus aureus.

Conclusion: Staphylococcus aureus and Pseudomonas aeruginosa were the predominant isolates in ear infections. Most of the bacteria were resistant to commonly used antibiotics. Multidrug resistance and MRSA were highly observed. Therefore, an antibiotic susceptibility test is important to guide antibiotic prescription practice.

BACKGROUND

An ear infection is a common problem for adults and children[1]. Ear infections include Acute Otitis Media (AOM), Chronic Suppurative Otitis Media (CSOM) and otitis externa (OE).[2] An ear infection is a major public health concern and when left untreated, may cause hearing loss, deafness, life-threatening complications and mortality.[3] In resource limited settings, most patients with ear infections delay seeking medical attention; hence, usually present with complications.[4] About 65-330 million people suffer from ear infection worldwide, and 60% report hearing impairment.[2] The highest rate of ear infection was reported from Sub-Saharan Africa and South Asia [5]. In Tanzania, the magnitude of ear infections was 53.3%.[6]

Gram positive and negative bacteria are more responsible for ear infections than viruses and fungi.[1,3] The most common bacteria causing ear infections are *Pseudomonas* species, *Staphylococcus aureus*, *Proteus* species, *Klebsiella* species, *Coagulase negative staphylococcus aureus* (CoNS), *Streptococcus* species and *Enterococcus* species.[5,7,8] In Africa, about 52.1% to 98.8% of ear discharge culture investigations revealed either one or mixed bacteria.[1,4,5,9–11] The most predominant bacterial isolates are reported to be *Pseudomonas aeruginosa* and *Staphylococcus aureus*.[4,9,10,12–14]

The emergence of resistant strains of gram positive and gram negative bacteria has led to ineffective antibiotics becoming a major global health problem.[8] High levels of resistance in enterobacterales have been reported to most antibiotics, including; third-generation cephalosporin, sulfamethoxazole/trimethoprim, amoxicillin/clavulanic acid, penicillin G, erythromycin, tetracycline and ampicillin.[1,4,10,14] Gram positive bacteria have been reported as highly resistant to erythromycin.[4] Antibiotics reported sensitive to bacteria were ciprofloxacin, meropenem,

amikacin, ceftriaxone and gentamicin.[4,14,15]

Drug resistant microorganisms are a growing global danger and have been increasing, challenging the management of ear infections.[4] Ear infections have a severe health and economic burden, especially in Africa and other developing countries with high disease prevalence.[1] The misuse of antibiotics by the public and empirical treatment of ear infections has contributed to the emergence and spread of antimicrobial resistance (AMR).[1] Resistance to two or more classes of antimicrobials was reported on *Pseudomonas aeruginosa* and *Proteus* species.[16] Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a serious concern in human medicine. The strain has developed resistance to many commonly used antimicrobials due to indiscriminate use, and treatment is becoming a challenge.[17] A high proportion of MRSA has been reported among patients with ear infections in Tanzania.[4]

Most health professionals treat ear infections empirically or adhere to the standard treatment guideline (STG) without considering culture and AST results. This has created a gap in managing most ear infections and raises the risk of acquiring multidrug resistant (MDR) bacteria resulting into more advanced disease and treatment complications. In our setting, approximately 2000 patients are diagnosed with ear infections yearly, and the return rate of patients after the initial treatment has been observed. This indicates that ear infection is a common health problem in our communities. However, the etiology and antimicrobial susceptibility of bacteria causing ear infections remains to be fully described. The findings from this study will provide insight into the management of ear infections in our setting. Therefore, this study aims to determine the spectrum of bacterial isolates and their antibiotic susceptibility pattern among patients diagnosed with ear infections.

MATERIALS AND METHODS

Study Design, Setting and Population

A cross sectional retrospective hospital based study was conducted to analyze data collected from January 2021 to March 2023 at St. Benedict Ndanda Referral Hospital (SBNRH), Mtwara, Tanzania. The hospital has a 300 bed capacity and, provides inpatient and outpatient services, including an Ear Nose and Throat (ENT) clinic. About 300 patients with ENT disease conditions are attended per month by ENT specialists and clinicians. The study involved patients diagnosed with ear infections; investigated for the etiology of infection through bacterial culture and antimicrobial susceptibility tests.

Sample Size Estimation

The sample size was estimated using Kish-Leslie (1965), considering the proportion (p) of culture-positive (75%) from a previous study conducted in Saudi Arabia,[12] a 95% confidence interval, z score (1.96) and a 5% margin of error (d). The estimated sample size was 288, as described below,

$$\text{Sample size} = \frac{Z_{1-\alpha/2}{}^2 p(1-p)}{d^2}$$

Sample Size = 1.96^2x 0.75(1-0.75)/ 0.05^2 = 288.12

Sampling Procedures

The ear swab samples were collected from the laboratory bacteriology register. Culture positive samples with complete information on demographic, isolate and antimicrobial susceptibility information were included. Moreover, patients were conveniently enrolled until the sample size was reached. Patients with negative ear swab culture results and incomplete data were excluded from the study.

Data Collection

We conducted a retrospective review of patients' records with ear infections whose ear swab samples were collected to diagnose ear infections from January 2021 to March 2023. Data were abstracted from the microbiology laboratory registers and worksheets using a data abstraction tool. We collected demographic information (age, sex), isolated organisms, and AST results.

Laboratory Procedures

According to the standard operating procedures at SBNRH, ear swab samples were collected aseptically by using sterile cotton swab techniques. Standard microbiological techniques such as colony morphology, microscopic features, standard phenotypic characters, and biochemical tests were used to identify the isolates. Antimicrobial susceptibility testing were done according to Clinical Laboratory Standard Institute (CLSI) guidelines.[18] The Microbiology laboratory adhered to quality control protocols guided by specific internal Standard Operating Procedures (SOPs) to enhance the quality of specimen processing and storage to minimize pre-analytical, analytical, and post-analytical errors.

Data Management and Analysis

The abstracted data were entered into Microsoft Excel and analyzed using STATA software version 15.1 (STATACorp, Texas, United States). Descriptive analyses were summarized as frequency and proportion for categorical variables, and results were presented using tables. Variables were summarized as frequencies, percentages, medians, and inter-quartile ranges. All intermediate AST results were categorized as resistant during analysis. Multidrug resistance (MDR) was regarded as resistance to at least one antibiotic in three or more categories/groups of antibiotics.

Ethical Consideration

We analysed data from routine services of patients seeking care and treatment in which ethical issues are strongly advocated. The St. Benedict Ndanda Referral Hospital Management provided ethical approval to review and analyze the data with Reference number SBNRH/706/1/42. All data obtained from patients' records were treated with complete confidentiality.

RESULTS

About 571 ear swab samples from patients with clinical features of ear infections subjected to culture and sensitivity from January 2021 to March 2023 were reviewed. Of all, 172 (30.1%) were culture-negative,

and 111(19.4%) did not meet the inclusion criteria. Therefore, the analysis included 288 (50.4%) positive bacteria cultures.

Demographic Characteristics of the Participants

A total of 288 subjects were included in the study; 145 (50.3%) were males. The median age was 27 (interquartile range (IQR) 16-42) years. The majority were aged between 20-29 (Table 1).

Distribution of Isolated Bacteria

There were 306 isolates from 288 culture positive samples. Of the 306 total bacterial isolates, the majority, 178 (58.2%), were gram negative bacteria. Two hundred and seventy patients (93.8%) had single bacterial infections, while 18 (6.2%) had mixed bacterial infections. The predominant bacterial isolates were *Staphylococcus aureus* 124 (40.5%), followed by *Pseudomonas aeruginosa* 80 (26.1%) (Table 2).

Antimicrobial Susceptibility Pattern

Most of the Enterobacterales and *Acinetobacter* species showed high resistance to trimethoprim-sulfamethoxazole 35 (83.3%), amoxicillin/clavulanic 36 (75%), ceftriaxone 24 (52.2%) and ceftazidime 17 (45.9%). *Pseudomonas aeruginosa* was highly resistant to piperacillin 38 (92.7%) and susceptible to ciprofloxacin (89.2%), meropenem 53 (89.8%), ceftazidime 48 (81.4%), gentamicin (76.7%) and imipenem 11 (73.3%),

Most *Staphylococcus aureus* isolates showed resistance to erythromycin 86(87.8%), gentamicin 26 (65.0%) and trimethoprim-sulfamethoxazole 27(54%), while *Streptococcus* species showed resistance to ceftriaxone 02 (66.7%) and cefotaxime 02 (66.7%). Out of 124 *Staphylococcus aureus* isolated, 113 (91.1%) were tested for Methicillin susceptibility, and 67 (59.3%) were MRSA (Table 3). Overall, bacterial isolates were 100% susceptible to meropenem, followed by amikacin 83.3%, ciprofloxacin (66.7%), and gentamicin (57.1%). Resistance to at least one antibiotic in three or more antibiotic categories (Multi-drug Resistance) was observed in 245 (80%) of the bacterial isolates (Table 3).

TABLE 1: Demographic Characteristics of the Participants (N=288)

Variable	Frequency/ Median	Percentage
Median Age	27(IQR:16-42)	
Age group (years)		
<10	57	19.8
10-19	38	13.2
20-29	64	22.2
30-39	44	15.3
40-49	29	10.1
≥50	56	19.4
Sex		
Male	145	50.3
Female	143	49.7

TABLE 2: Types and Frequency of Isolated Bacteria from Ear Swab Samples (N=288)

Identified organisms	Frequency	Percentage
Staphylococcus aureus	124	40.5
Pseudomonas aeruginosa	80	26.1
E. coli	24	7.8
Proteus mirabilis	18	5.9
Enterobacter cloacae	13	4.3
Klebsiella pneumoniae	10	3.3
Citrobacter freundii	7	2.3
Klebsiella oxytoca	7	2.3
Proteus vulgaris	6	2.0
Enterobacter aerogenes	4	1.3
Citrobacter koseri	4	1.3
Acinetobacter spp	3	1.0
Streptococcus pyogenes	3	1.0
Streptococcus pneumoniae	1	0.3
Serratia marcescens	1	0.3
unidentified gram-negative rods	1	0.3

TABLE 3: Antimicrobial Resistance Pattern of Bacterial Isolates from Ear Swab Samples

Bacterial Isolates	N	Antibiotic resistance (%) FOX	E	CD	AUG	CRO	CAZ	MRP	IMI	CN	CIP	COT	AK	PIP	CTX
Staphylococcus-aureus	124	59.3	87.8	41.7	NA	NA	NA	NA	NA	65.0	42.7	54.0	NA	NA	NA
Streptococcus spp	4	NA	0	0	50.0	66.7	NA	0	NA	NA	NA	NA	NA	NA	66.7
Pseudomonas-aeruginosa	80	NA	NA	NA	NA	NA	18.6	10.2	26.3	23.3	10.8	NA	19.6	92.7	NA
E. coli	24	NA	NA	NA	100.0	64.3	60.0	0	0	33.3	75.0	88.9	0	NA	85.7
Proteus spp	24	NA	NA	NA	33.3	30.0	36.4	21.1	NA	11.8	16.7	55.6	33.3	NA	33.3
Enterobacter spp	17	NA	NA	NA	90.9	71.4	50.0	15.4	NA	54.5	18.7	75.0	0	NA	50.0
Klebsiella spp	17	NA	NA	NA	88.9	40.0	40.0	21.4	NA	50.0	33.3	57.1	25.0	NA	NA
Citrobacter spp	11	NA	NA	NA	100.0	33.3	25.0	37.5	NA	0	33.3	100.0	0	NA	50.0
Acinetobacter spp	3	NA	NA	NA	0	100.0	100.0	33.3	NA	NA	66.7	50.0	50.0	NA	NA
Serratia spp	1	NA	NA	NA	0	100.0	100.0	0	NA	NA	100.0	NA	NA	NA	NA

NA-Not applicable, FOX-Cefoxitin, E-Erythromycin, CD-Clindamycin, AUG-Amoxicillin/clavulanic acid, CRO-Ceftriaxone, CAZ-Ceftazidime, MRP-Meropenem, IMI-Imipenem, CN-Gentamicin, CIP-Ciprofloxacin, COT- trimethoprim-sulfamethoxazole (sxt), AK-Amikacin, PIP-Piperacillin, CTX-Cefotaxime

DISCUSSION

An ear infection is a common clinical problem and the major cause of preventable hearing loss in children and adults. The study determined the spectrum of bacteria causing ear infection and their antibiotic susceptibility pattern to commonly used antibiotics. The study revealed that the predominant bacterial isolates were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Most Enterobacterales and *Acinetobacter* species were highly resistant to trimethoprim-sulfamethoxazole and amoxicillin/clavulanic, commonly used to treat various infections. *Staphylococcus aureus* and *Pseudomonas aureginosa* showed high resistance to erythromycin and piperacillin, respectively. On the other hand, we observed a high susceptibility of pathogens to meropenem, amikacin, and ciprofloxacin. Furthermore, Multi-Drug resistance (MDR) was reported in most of the isolates obtained.

The predominant bacterial isolates were *Staphylococcus aureus* and *Pseudomonas aeruginosa*, followed by *Escherichia coli* and *Proteus* species. This is consistent with studies conducted in Tanzania, Ethiopia, India, and Saudi Arabia.[4,11,12,14,19] However, this contradicts the findings of other studies that reported that *Proteus* species are the predominant bacterial isolates.[1,3,10] This variation in the distribution of isolates could be attributed to the differences in climate conditions and geographical areas[3,10] as well as levels of hygiene, education, and economic development in developed and developing countries.[2]

Staphylococcus aureus is considered one of the predominant bacterial causes of ear infections and shows a global concern in resistance to the most available treatment options.[20] Nowadays, the emergence of MRSA isolates is becoming a growing challenge as it can impair the management of *Staphylococcus aureus* infections.[20] World Health Organization (WHO) has reported that 64% of people with MRSA infections are more likely to die than people with drug-sensitive infections.[21] The study reveals more than fifty percent of *Staphylococcus aureus* isolates were MRSA. This finding is higher than that of similar studies conducted in Tanzania.[4,11] This is alarming as it indicates a rise in MRSA, which might be due to the widespread and uncontrolled use of antibiotics in our communities and empirically prescribing antibiotics without confirming with culture and sensitivity.[17] Moreover, frequent visits to hospitals due to chronic ear infections and the use of contaminated inanimate objects often found in the environment have been reported to raise the danger of exposure to MRSA.[4] The major concern revealed by this study is that there is an increase in MRSA, which is currently one of the greatest global public health challenges and thus, when not identified and treated early, may result in advanced disease and complications.

Most Enterobacterales and *Acinetobacter* species resisted trimethoprim-sulfamethoxazole, amoxicillin/clavulanic, ceftriaxone, and ceftazidime. Our finding is similar to the recent study conducted in Tanzania, which reported high resistance to amoxicillin/clavulanic, ceftazidime, and trimethoprim-sulfamethoxazole.[4] However, different bacterial isolates had high resistance patterns to different antibiotics. For instance, *Pseudomonas aeruginosa* was highly resistant to piperacillin, while other studies reported ceftazidime.[4] In the present study, gram-positive isolates were highly resistant to erythromycin. Similar resistance patterns were found in the study done in Muhimbili, Tanzania.[4] The frequent use of these antibiotics to treat various bacterial infections in our setting and the likelihood that most bacterial species have developed resistance to antimicrobial drugs over time may contribute to the observed resistance pattern.[4]

Resistance to at least one antibiotic in three or more antibiotic categories (multi-drug resistance) was highly observed in 80% of the bacterial isolates, contrary to a similar study conducted in Ethiopia.[22] The irrational use of antibiotics by the public and prescription of antibiotics without laboratory guidance may contribute to the emergence and spread of drug resistance patterns challenging the management of ear infections.[23,24] Therefore, drug prescriptions should be based on laboratory culture and susceptibility results whenever possible.

In the present study, most bacterial isolates were more sensitive to meropenem, followed by amikacin and ciprofloxacin. Similarly, this was reported in studies conducted in Ethiopia and Tanzania.[3,4,11]The standard treatment guideline in Tanzania recommends using ciprofloxacin as a drug of choice for treating ear infections.[25] Despite ciprofloxacin being prescribed more often in our setting, it remains effective for treating ear infections. The high sensitivity rate of meropenem in this study may be explained by the fact that this antibiotic is not frequently prescribed in our setting.[4]
The limitation of this study is that it was not possible to include data on the types of ear infections, whether it was Otitis Media (OM), Otitis Externa (OE) or Chronic Suppurative Otitis Media (CSOM). Hence, the study could not compare the association between bacterial isolates and the types of infection. The study could not obtain more information on other resistance patterns, such as Extended Spectrum Beta-Lactamase.

CONCLUSION

The study reports a high proportion of positive bacterial isolates, mostly gram-negative. The predominant isolates were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Furthermore, Multi-drug resistance was highly observed, with MRSA posing a great danger to managing ear infections. Most of the bacteria were resistant to commonly used antibiotics. Therefore, an antibiotic susceptibility test is important to guide the prescription of antibiotics.

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