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Etiology of ear infection and antimicrobial susceptibility pattern among patients attending otorhinolaryngology clinic at a tertiary hospital in Dar es Salaam, Tanzania

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Etiology of ear infection and antimicrobial susceptibility pattern among patients attending otorhinolaryngology clinic at a tertiary hospital in Dar es Salaam, Tanzania

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Keywords: Ear infection, resistance, antimicrobial susceptibility pattern.

ABSTRACT

OBJECTIVES: To determine the etiological pathogens causing ear infections and their antimicrobial susceptibility patterns among patients with ear complaints at a tertiary hospital in Dar es Salaam.

DESIGN: Hospital-based cross-sectional study.

SETTINGS: Otorhinolaryngology clinic at Muhimbili National Hospital, Dar es Salaam, Tanzania.

PARTICIPANTS: Patients presenting with signs and symptoms of ear infection.

MAIN OUTCOME MEASURE: Bacteria and fungi isolated from ear swab specimens of patients presenting with signs and symptoms of ear infecrion; and antimicrobial susceptibility patterns of isolated bacteria.

RESULTS: Two hundred fifty-five participants were enrolled, with a median age of 31 years and an interquartile range of 15- 49. Otitis externa was the predominant type of ear infection, accounting for 45.1%. We observed positive bacteria culture in 53.3% of study participants, where *S. aureus* (27.3%) and *P. aeruginosa* (24.2%) were the most frequently isolated bacteria, while *Candida* spp,12(63.8%), and *Aspergillus* spp, 9(36.2%) were the only isolated fungi. We report that 93% of isolated *Enterobacterales* were resistant to amoxicillin /clavulanic acid, and 73% were resistant to ceftazidime. In addition, we detected 34.4% extended-spectrum beta-lactamase-producing *Enterobacterales* (ESBL-PE) and 44.4% methicillin-resistance *Staphylococcus aureus* (MRSA).

CONCLUSIONS: The findings from this study reveal that the leading etiological agent of ear infection is bacteria. Furthermore, our findings show a significant proportion of ESBL-PE

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and MRSA-causing ear infections. Hence, detecting multidrug-resistant bacteria is crucial to improving ear infection management.

Strength and Limitation of the study

The present reports of the common bacterial and fungi etiology of ear infection; importantly the study has revealed the antimicrobial susceptibity patterns that is useful in guiding on the choice of empirical treatment in resource limited settings.

The present has some limitations, some fungal (moulds) isolates were not identified at specie level and anaerobic culure was not performed.

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1. Introduction

An ear infection is among the leading cause of deafness in many developing countries. Unfortunately, most patients with ear infections in resource-limited settings delay seeking medical attention; hence, usually present with complications (1). Bacteria are the leading pathogens of ear infection whereby, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Proteus mirabilis,* and *Klebsiella species* are the dominant bacteria species causing ear infection globally (1–6). In addition, *Candida spp* and *Aspergillus spp* are predominant fungal isolates responsible for ear infections (7–10). However, fungal ear infections are often undiagnosed due to limited diagnostic opportunities, especially in resource-limited countries, including Tanzania (5,6).

Most practitioners in our settings tend to treat ear infections empirically or adhere to the Standard Treatment Guideline (STG) without considering laboratory investigation and AST results. This has created a gap in managing most ear infections, which raises the risk of acquiring multidrug-resistant bacteria (11,12). When first-line antibiotics cannot treat infections, more costly antibiotics must be utilized. This consequently affects patients' treatment options, resulting in prolonged hospital stays and increased healthcare costs, which impacts families' financial burden and quality of life (13).

Etiological studies of ear infections are important to guide the choice of an effective antibiotic and monitoring bacterial patterns and their varying antimicrobial susceptibilities. This is crucial for risk analysis, mitigation measures, and logistical plans. Therefore, the present study aimed to determine the etiological pathogens and antimicrobial susceptibility patterns of bacteria causing ear infections. The data obtained will be used to strengthen the prevention and control measures and to update the management and treatment options for ear infections. Also, the information will serve as a baseline for countrywide surveillance of antibiotic resistance.

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2. Materials and Methods

Study design and settings

We conducted a hospital-based cross-sectional study from March to July 2021 in the otorhinolaryngology clinic at MNH, Dar es Salaam, Tanzania. The hospital serves as a National Referral Hospital, research center, and a university teaching hospital. MNH is the largest tertiary health care facility in Tanzania. The otorhinolaryngology department has inpatient and outpatient units; about 20 to 30 patients attend the outpatient clinic per day.

Study participants

The study included patients attending the otorhinolaryngology clinic with signs and symptoms of ear infection such as accumulation of fluid in the middle ear, bulging of the eardrum, ear pain, ear itching, perforation of the eardrum, and ear discharge (otorrhea). We excluded patients with other hearing disorders unrelated to infection (congenital malformations, physical head injury) and those on regular checkups.

Sample size, and sampling procedure

The study sample size was estimated using a Kish Leslie formula (1965) for a cross-sectional study considering the prevalence of 62.1% reported previously by Moshi et al in a study conducted in a teriary hospital in Mwanza city, Tanzania (3). The minimum sample size was 241 participants; considering the 5% non-response rate, we obtained a sample size of 255 participants.

Data collection

Data collection was conducted by two trained research assistants (RAs) and an ear, nose, and throat (ENT) surgeon; briefly, a structured questionnaire was administered to the participants by two RAs. RAs used the questionnaire to collect demographic data (age, sex, marital status,

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occupation, and education) and behavioral risk characteristics (swimming, frequent use of earphones, cotton buds, sharp objects, and cigarette smoking). In addition, the participants' clinical information, including the type of ear infection, use of antibiotics, nasal congestion or blockage, recurrent upper respiratory tract infection (URTI), and cerumen impaction, were also collected from the patient's medical records and during a physical examination by ENT surgeon.

Specimen collection

The ENT surgeon collected specimens with precaution to prevent contamination. The sterile swab was used to clear the oozing pus from the patient's ear; another sterile swab was then used to extract fresh pus from the ear. The collected specimen was stored at room temperature in Stuart transport media. All samples were transported to the Central Pathology Laboratory (CPL) at MNH for processing and testing.

Isolation and identification

Upon the arrival of the specimens at CPL, specimens were processed for culture and identification. Specimens were inoculated on selective and non-selective media; Chocolate agar (CA), Sheep-Blood agar (sBA), MacConkey agar (MCA), and Sabouraud dextrose agar (SDA). We used CA to isolate fastidious bacteria, such as *H. influenza* and *S. pneumoniae*, the frequent etiological agents of ear infection. MCA was used as a selective and differential medium for Gram-negative bacteria, and BA was used as a general-purpose medium. SDA was used for the isolation of fungal species. We incubated MCA in an aerobic environment and BA and CA in a 5% CO_2 environment at 37°C for 18 to 24 hours.

Bacterial isolates were identified by interpreting colonial morphologies, microscopic examination (Gram stain), and biochemical tests. The catalase and coagulase tests were performed for Gram-positive bacteria, while Kliger's Iron Agar, Sulfur Indole Motility

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(SIM), citrate, and urease tests were for gram-negative bacteria. Further, phenotypical identification and confirmation of Gram-negative bacterial isolates were performed by Analytical Profile Index tests, API 20E and API 20NE.

For fungal isolates, growth on the SDA plate was used preliminary to classify mold or yeast based on the colonial morphology and color. A germ tube test was used to identify *Candida albicans*. Additionally, Lactophenol cotton blue was used for molds to identify the conidial spore in *Aspergillus spp.*

Antimicrobial susceptibility testing

Antibiotic susceptibility test (AST) for bacterial isolates was performed using the Kirby Bauer disc diffusion method on Mueller-Hinton Agar (MHA), and MHA supplemented with 5% blood for *S. pneumonia* following the 2021 Clinical and Laboratory Standard Institute (CLSI) guidelines. Zones of inhibition were measured using a ruler in millimeters and interpreted as susceptible, resistant, or intermediate according to the 2021 CLSI guideline.

The antibiotic discs used were; ciprofloxacin (5µg), trimethoprim/ sulphamethoxazole (1.25/23.75µg), gentamycin (10µg), clindamycin (2 µg), erythromycin (15µg),) for gram-positive bacteria. Ciprofloxacin (5µg), trimethoprim/ sulphamethoxazole (1.25/23.75µg), gentamycin (10µg), meropenem (10µg), amoxicillin/ clavulanic acid (20µg), ceftriaxone (30µg) and ceftazidime (30µg) for *enterobacteriaceae* and *Acinetobacter spp*. Ciprofloxacin (5µg), gentamycin (10µg), meropenem (10µg) and ceftazidime (30µg) for *Pseudomonas spp*.

Standard methods were used to identify MRSA using cefoxitin (30µg) disc in which resistant isolates were considered MRSA positive. In addition, ESBL-PE screening was done using ceftazidime (30 µg) and cefotaxime (30µg) antibiotic discs, and if resistant, ESBL- PE confirmation was done by the double-disc synergy method (14).

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Quality control

The reference organisms and reagents were clearly and uniquely labeled, dated, and stored at optimal conditions. The room, incubator, and refrigerator temperature were monitored daily. The culture media were prepared following the manufacturer's guidelines and internal standard operating procedures and tested for performance and sterility.

Data Analysis

The data was analyzed using SPSS version 23 software. Continuous variables were summarized as the median and interquartile range (IQR), whereas percentages and proportions were used to describe categorical variables. The percentage of resistance was obtained by computing the number of bacteria species that resisted a specific drug over a total number of isolated bacterial species. AST intermediate results were regarded as resistant.

Patient and public involvement

Patients and the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

3. Results

Participants' demographic, clinical, and risk behavior characteristics

Two hundred fifty-five participants were recruited; 52.5% (134/255) were males. The median age was 31 years (IQR: 15- 49). The majority (30.2%) of participants were students, 32.9% had a college education, and 15.7% were from outside Dar es Salaam region (Table 1). The median duration of ear infections was 210 days (IQR: 21-1095). Otitis externa (OE) was the most common type of ear infection, accounting for 45.1% (115/255), followed by Chronic Suppurative Otitis Media (CSOM) (41.2%) (Figure 1).

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Around 49% of the participants with ear infections had a history of antibiotic use, whereby ciprofloxacin ear drop was the most prescribed topical antibiotic. Additionally, 33.3% of the study participants had nasal congestion/blockage/discharge, and 28.2% had recurrent URTI (Table 2).

Distribution of bacterial and fungal isolates causing ear infections

In this study, 136 out of 255 (53.3%) participants had a positive aerobic culture for either bacterial or fungal pathogen, whereby 10.3% (14/136) of participants had a polymicrobial infection (mixed growth of either two different bacteria or bacterial and fungal infection). A total of 150 isolates (bacteria and fungi) were identified, of which 87.3% (131/150) were bacteria. Of the isolated bacteria, Gram-negative, 71.0% (93/131) were predominant.

The predominant bacterial isolates were *S. aureus,* 27.5% (36/131), followed by *Pseudomonas aeruginosa,* 24.4% (32/131) (Figure 2A). On the other hand, *Candida spp* accounted for 63.2% (12/19) of the isolated fungi (data not shown). Further stratification of isolated pathogens by type of ear infection showed that *S. aureus* 16/131 (12.2%) was the most prevalent bacterium in OE patients, whereas *P. aeruginosa* 22/131 (16.8%) predominated in CSOM patients (Figure 2B).

In the present study 34.4% (21/61) of the enterobacterales, excluding *Pseudomonas aeruginosa*, were extended-spectrum beta-lactamase producing enterobacterales (ESBL-PE);and *Klebsiella spp* was predominant, accounting for 33.3% (7/21) of the ESBL-PE isolates (Figure 2C). On the other hand, 44.4% (16/36) of the *S.aureus* species were MRSA (data not shown).

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Antimicrobial susceptibility pattern of bacterial isolates

Almost all (93%) isolated *Enterobacterales* were resistant to amoxicillin/clavulanic acid, more so *E. coli* and *Acinetobacter spp* were 100% resistant. Also, 73% of isolated bacteria were resistant to ceftazidime, whereby *Pseudomonas aeruginosa* had the highest resistance rate of 75%. In addition, 43% of isolated bacteria were resistant to trimethoprim-sulfamethoxazole, whereby *E.coli* was leading with a 75% resistance rate. Resistance towards sulfamethoxazole-trimethoprim was higher among ESBL producers (57-100%) than non-ESBL producers (29-100%). At least 14% of the non-ESBL-PE bacteria were resistant to all the third-generation cephalosporins, and all non-ESBL-PE isolates were sensitive to meropenem. *S. aureus* had an 89% resistance rate to erythromycin. However, MRSA isolates were more resistant to sulfamethoxazole-trimethoprim (81%) and gentamicin (50%) than non-MRSA isolates 35% and 25% for sulfamethoxazole-trimethoprim and gentamicin, respectively. Most isolated bacteria had very low resistance rate against meropenem (4%) and ciprofloxacin (22%) (Table 3).

4. Discussion

Understanding the etiology of ear infections and resistance pattern is crucial in planning interventions and managing ear infections. The results indicate a substantial proportion of ear infections, with bacteria as the primary etiological agent. Most isolated bacteria were resistant to third-generation cephalosporins, sulfamethoxazole-trimethoprim, and amoxicillin/clavulanic acid. Gram-positive bacteria were highly resistant to erythromycin. The two antibiotics that worked the best were ciprofloxacin and meropenem. The results

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imply the need to review ear infection management and the selection of an efficient antibiotic.

The study found that many ear infections are of bacterial etiology. The finding is similar to studies done in Tanzania by Kennedy M et al. (2019) in Morogoro (4), Zephania A et al. (2019) in Dar es Salaam (15), Martha M et al. (2016) in Mwanza (3) and other studies in Kenya and India (16,17). We observed that *S. aureus* and *Pseudomonas aeruginosa* are ear infections' leading bacterial etiological agents, similar to previous studies in Tanzania, Nigeria, Angola, Kenya, and India (3,17–19). In addition, the present study found *Candida spp* and *Aspergillus spp* the fungal spp, causing ear infections consistent with previous findings in Tanzania and elsewhere (Nigeria, Iran, Ethiopia, Egypt, India) (3–5,20–22). Nonetheless, the contribution of fungi etiology in ear infections in the present study was expected because many individuals had risk behaviors for fungal ear infections, including excessive use of eardrops containing antibiotics, regular cleaning of ears, and swimming. Antibiotic overuse promotes the growth of fungi, and the regular ear cleaning habit removes cerumen and exposes ears to fungi colonization and, subsequently, infection (23,24).

The current study revealed a high proportion of MRSA (44.4%) and ESBL-PE (34.4%). In addition, our study showed *Klebsiella* spp (33.3%) as the dominant ESBL-PE. The higher proportion of MRSA and ESBL-PE coincide with studies done in Tanzania by Martha M et al among patients with chronic suppurative otitis media infection and another study in India (3,16). The greater inclination for self- and empirically prescribing antibiotics without considering laboratory culture and sensitivity may explain the higher proportion of ESBL and MRSA. The high incidence of ESBL and MRSA can also be explained by an increased inclination for people to visit hospital facilities due to chronic ear infection, which raises the danger of exposure to MDR bacteria. Additionally, the tendency to use inanimate objects to

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remove earwax can be attributed to the increased proportion of ESBL and MRSA, as these inanimate objects are often found in environments that may be contaminated with ESBL-producing bacteria and/or MRSA (25).

Almost all isolated bacteria (93%) were resistant to amoxicillin/clavulanic acid. Nearly three-quarters of gram-negative bacteria were resistant to ceftazidime, and about half were resistant to trimethoprim-sulfamethoxazole. On the other hand, 89% of isolate gram-positive were resistant to erythromycin. ESBL-PE and MRSA isolates were resistant to the most common antimicrobial agents compared to non-MRSA and non-ESBL-PE. The resistance patterns found in the current study are similar to those reported in other studies in Tanzania, Kenya, Ethiopia, India, Egypt, and Romania (3,4,17,18,26–29). 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.

In the present study, most isolated bacteria were sensitive to meropenem and ciprofloxacin. Ciprofloxacin is a drug of choice for ear infections as per standard treatment guidelines in our setting. The fact that meropenem is infrequently used to treat ear infections may explain the high sensitivity rate. Surprisingly, we observed that ciprofloxacin is still effective despite being prescribed often in our setting for treating ear infections. There is no clinical rationale as to why quinolones are still more effective in treating ear infections, but these results assure that quinolones are still beneficial as first-line topical antibiotics for ear infections.

5. CONCLUSION

The results of this study indicate that bacteria are the most common cause of ear infections in our context. Furthermore, we report that many multidrug-resistant bacteria (ESBL-PE and MRSA) are implicated in causing ear infections. Therefore, antimicrobial susceptibility

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testing is crucial to guide clinicians on the appropriate management of ear infections in our setting.

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Patient consent: In order to participate into the present study a written informed consent was obtained from adult participants (18 years and above), and assent and consent were requested from adolescents and the parents/ guardians of participants below 18 yrs. No personal participant's information is included in the results text, figures and tables.

Ethical approval: Ethical clearance was obtained from Muhimbili University of Health and Allied Sciences (MUHAS), Senate Research and Publication Committee, with reference

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Figure legends

Figure 1: Types of ear infection among study participants at MNH

The figure illustrates the distribution of ear infections among patients presenting with signs and symptoms of ear infection attending the otorhinolaryngology clinic at MNH (N=255). OM, OE, and CSOM stand for otitis media, otitis externa, and chronic suppurative otitis media, respectively.

Figure 2A-C: Distribution of bacterial isolates

The figure depicts the distribution of bacteria spp isolated among patients with ear infections attending the otorhinolaryngology clinic at MNH (n=131)(A). According to the type of ear infection (n=131), where OM (otitis media), OE(otitis externa), and CSOM (chronic suppurative otitis media) (B). Distribution of ESBL-producing bacteria among isolated gram-negative bacteria in patients attending the otorhinolaryngology clinic at MNH (n = 61) (C).

Tables

Table 1: Socio-demographic characteristics of the study participants (N=255)

Variables	Frequency (N) and Percentage (%)/Median(IQR)
Median age (years)	31 (15 - 49)
Sex	
Male	134 (52.5)
Female	121 (47.5)
Occupation	
Self-employed	56 (22.0)
Civil servants	62 (24.3)
Retired	49 (19.2)
Unemployed	88 (33.5)
Education	
Primary	75 (29.4)
Secondary	59 (23.1)
College	84 (32.9)
Illiterate	37 (14.5)

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Residence	
Within Dar es Salaam	215 (84.3)
Outside Dar es Salaam	40 (15.7)

Table 2: Baseline clinical and risk behavioral characteristics of the study participants (N=255)

Patients characteristics	**Frequency (N) and Percentage (%)/Median(IQR) (%)**
Median Duration of ear infection (days)	210 (21-1095)
Nasal discharge/blockage	
Yes	85 (33.3)
No	170 (66.7)
Recurrent URTI	
Yes	72 (28.2)
No	183 (71.8)
Use of hearing aid	
Yes	2 (0.8)
No	253 (99.2)
Earphone use	
Yes	41 (16.1)
No	214 (83.9)
Swimming	
Yes	8 (3.1)
No	247 (96.9)
Cotton bud use	
Yes	112 (43.9)
No	143 (56.1)
Sharp object use	
Yes	60 (23.5)
No	195 (76.5)
Ear cleaning habit	
Yes	119 (46.7)
No	136 (53.3)
Cerumen impaction	
Yes	45 (17.6)
No	210 (82.4)

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Table 3: Antimicrobial resistance pattern for isolated bacteria

	Bacteria isolates								
ANTIBIOTIC	*S.aureus* (N=36)	*Pseudomonas aeruginosa* (N=32)	*Klebsiella spp* (N=20)	*Acinetobacter spp* (N=10)	*Enterobcter spp* (N=6)	*E. coli* (N=8)	*Proteus spp* (N=12)	*Citrobacter spp* (N=5)	Over all (N=129)
Amikacin	NA	8(25)	4(20)	2(20)	3(50)	2(25)	1(8)	2(40)	22(24)
Sulfamethoxazole trimethoprim	20(56)	NA	8(40)	1(10)	4(66)	6(75)	3(25)	4(80)	46(47)
Gentamicin	13(36)	6(19)	9(45)	1(10)	1(17)	1(13)	7(58)	1(20)	39(30)
Ciprofloxacin	11(31)	11(34)	1(5)	0(0)	2(33)	2(25)	1(8)	0(0)	28(22)
Amoxicillin /clavulanic acid	NA	NA	18(90)	10(100)	5(83)	8(100)	11(92)	5(100)	57(93)
Ceftriaxone	NA	NA	9(45)	5(50)	3(50)	5(63)	6(50)	3(60)	31(51)
Ceftazidime	NA	24(75)	14(70)	7(70)	4(66)	5(63)	10(83)	4(80)	68(73)
Cefotaxime	NA	NA	9(45)	8(80)	3(50)	5(63)	6(50)	5(100)	36(73)
Meropenem	NA	2(6)	0(0)	1(10)	0(0)	0(0)	1(8)	0(0)	4(4.3)
Erythromycin	32(89)	NA	NA	NA	NA	NA	NA	NA	32(89)
Clindamycin	9(25)	NA	NA	NA	NA	NA	NA	NA	9(25)
Cefoxitin	16(44)	NA	NA	NA	NA	NA	NA	NA	16(44)

Footnote: NA: Indicates not applicable

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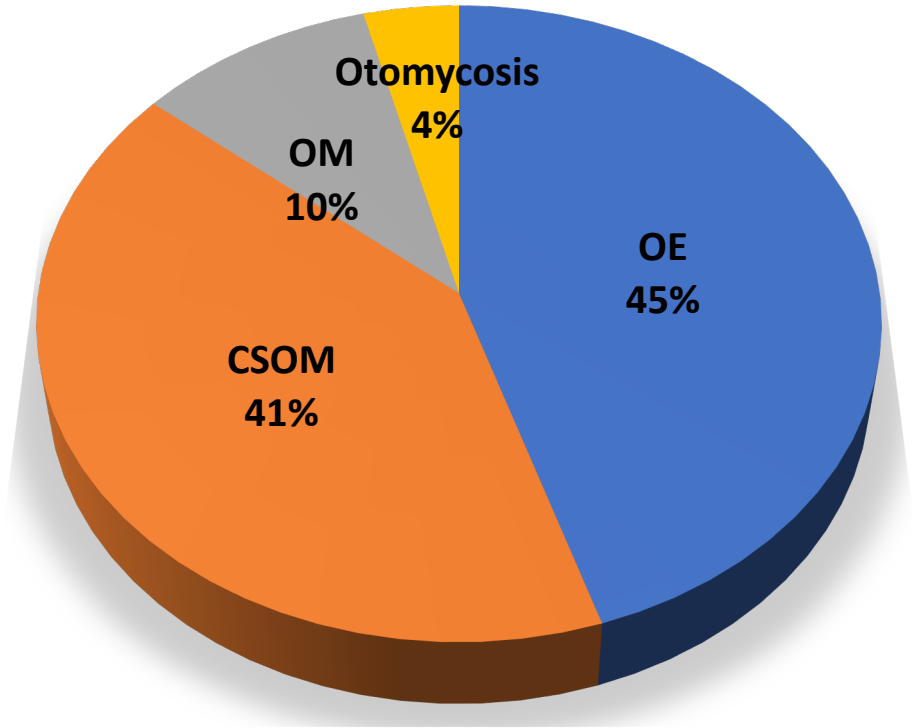
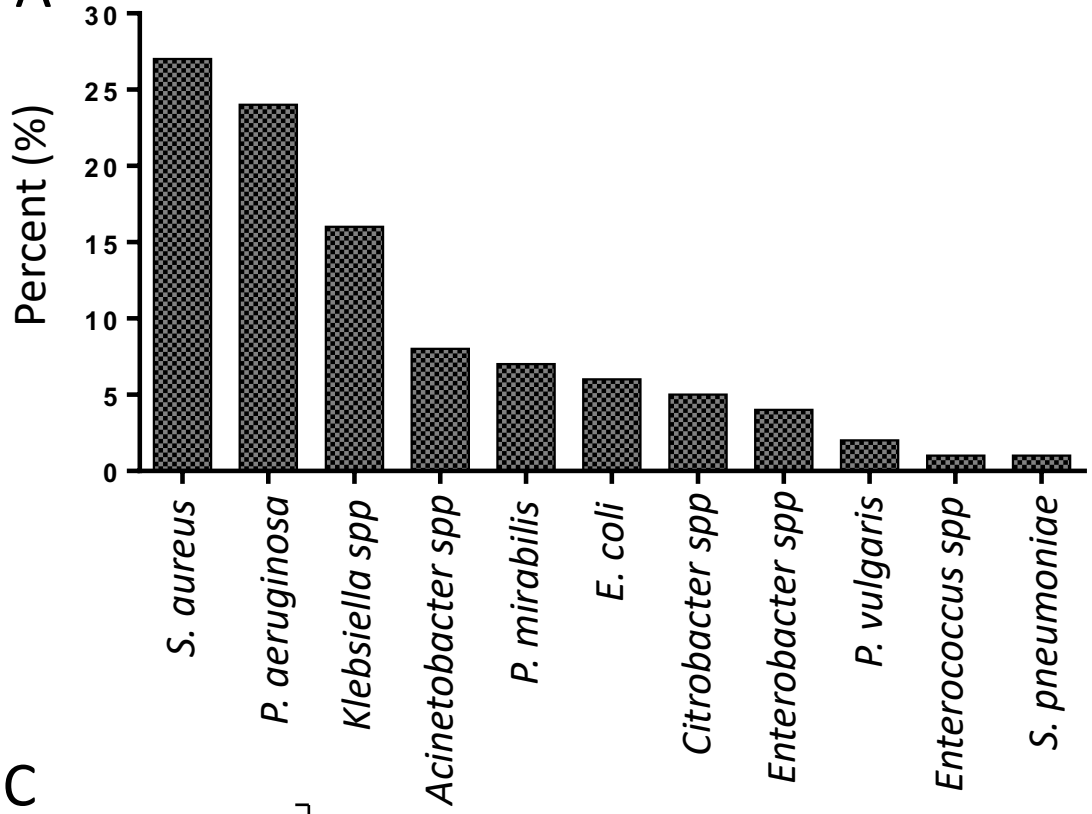
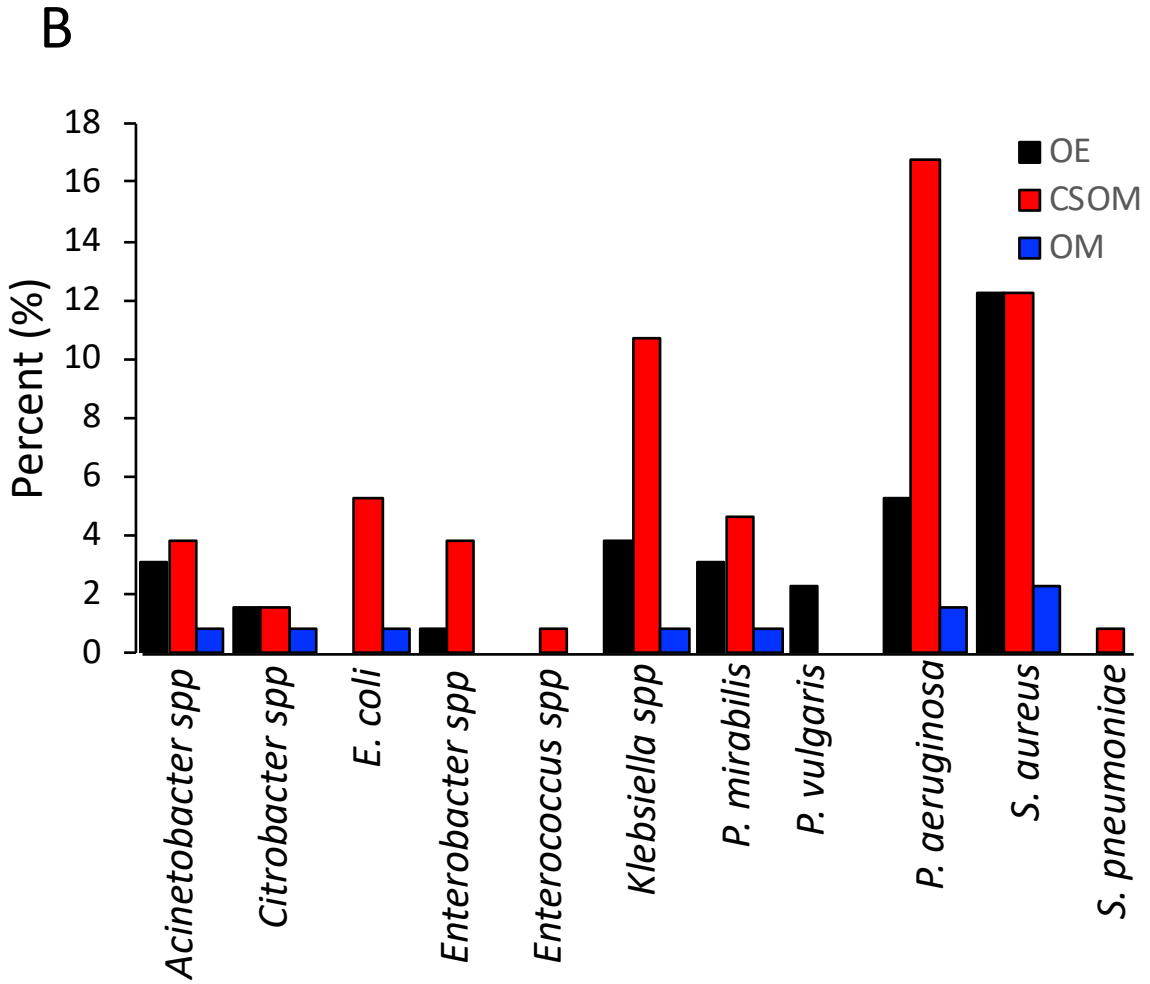
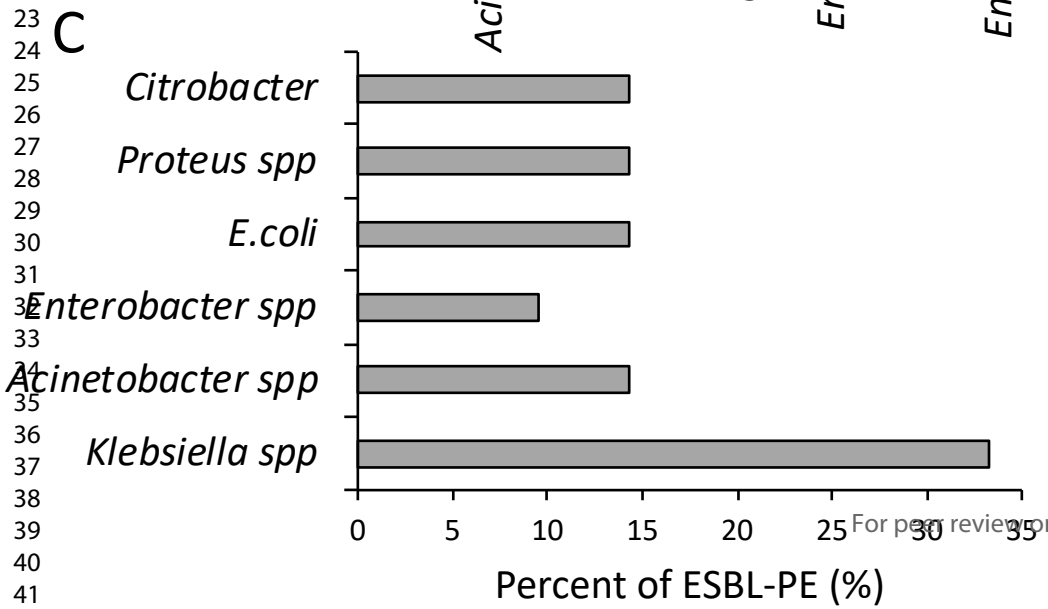

Otomycosis
4%
OM
10%
OE
45%
CSOM
41%







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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7
Bias	9	Describe any efforts to address potential sources of bias	*5-7*
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	5-8
		(*e*) Describe any sensitivity analyses	8
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	8-10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Etiology of ear infection and antimicrobial susceptibility pattern among patients attending otorhinolaryngology clinic at a tertiary hospital in Dar es Salaam, Tanzania: A hospital-based cross-sectional study

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Etiology of ear infection and antimicrobial susceptibility pattern among patients attending otorhinolaryngology clinic at a tertiary hospital in Dar es Salaam, Tanzania: A hospital-based cross-sectional study

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Keywords: Ear infection, resistance, antimicrobial susceptibility pattern.

ABSTRACT

OBJECTIVES: To determine the etiological pathogens causing ear infections and their antimicrobial susceptibility patterns among patients with ear complaints at a tertiary hospital in Dar es Salaam.

DESIGN: Hospital-based cross-sectional study.

SETTINGS: Otorhinolaryngology clinic at Muhimbili National Hospital, Dar es Salaam, Tanzania.

PARTICIPANTS: Patients presenting with signs and symptoms of ear infection.

MAIN OUTCOME MEASURE: Bacteria and fungi isolated from ear swab specimens of patients presenting with signs and symptoms of ear infection; and antimicrobial susceptibility patterns of isolated bacteria.

RESULTS: Two hundred fifty-five participants were enrolled, with a median age of 31 years and an interquartile range of 15- 49. Otitis externa was the predominant type of ear infection, accounting for 45.1%. We observed positive bacteria culture in 53.3% of study participants, in which 41% of isolates were obtained from patients with chronic suppurative otitis media (CSOM). Moreover, *S. aureus* (27.3%) and *P. aeruginosa* (24.2%) were the most frequently isolated bacteria, while *Candida* spp, 12(63.8%), and *Aspergillus* spp, 9(36.2%) were the only isolated fungi. Furthermore, we report that 93% of isolated *Enterobacterales* were resistant to amoxicillin /clavulanic acid, and 73% were resistant to ceftazidime. In addition, we detected 34.4% extended-spectrum beta-lactamase-producing *Enterobacterales* (ESBL-PE) and 44.4% methicillin-resistance *Staphylococcus aureus* (MRSA). We also found that 22% of the bacteria isolates were resistant to ciprofloxacin, a primary topical antibiotic used in managing ear infections.

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CONCLUSIONS: The findings from this study reveal that the leading etiological agent of ear infection is bacteria. Furthermore, our findings show a significant proportion of ESBL-PE and MRSA-causing ear infections. Hence, detecting multidrug-resistant bacteria is crucial to improving ear infection management.

Strength and Limitation of the study

- The present study has some strengths, we report the common bacterial and fungi etiology of ear infection in our study setting.
- Notably, the study has revealed the antimicrobial susceptibility patterns that are useful in guiding the choice of empirical treatment in similar settings with limited resources and comparable geographic, demographic, and social characteristics.
- The present study has some limitations; some fungal (molds) isolates were not identified to species level, and
- Anaerobic culture was not performed.

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1. Introduction

An ear infection is among the leading cause of deafness in many developing countries. Unfortunately, most patients with ear infections in resource-limited settings delay seeking medical attention; hence, usually present with complications (1). Bacteria are the leading pathogens of ear infection, whereby, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Proteus mirabilis,* and Klebsiella species are the dominant bacteria causing ear infection globally (1–6). In addition, *Candida* spp and *Aspergillus* spp are predominant fungal isolates responsible for ear infections (7–10). However, due to limited diagnostic opportunities, fungal ear infections are often undiagnosed, especially in resource-limited countries, including Tanzania (5,6).

Most practitioners in our settings tend to treat ear infections empirically or adhere to the Standard Treatment Guideline (STG) without considering laboratory investigation and antimicrobial susceptibility testing (AST) results. This has created a gap in managing most ear infections, which raises the risk of acquiring multidrug-resistant bacteria (11,12). When first-line antibiotics cannot treat diseases, more costly antibiotics must be utilized. This consequently affects patients' treatment options, resulting in prolonged hospital stays and increased healthcare costs, which impacts families' financial burden and quality of life (13). Furthermore, there needs to be more data on the effectiveness of empirical treatment in managing ear infections in Tanzania. However, experience based on the clinic's patient return rate after initial treatment for ear infections, it appears that a considerable number of patients return to the clinic with the same problem. This suggests that relying solely on empirical treatment methods may not be effective in treating ear infections. Hence this warrants further research to investigate the antimicrobial susceptibility patterns of bacteria isolated in ear infections to improve the outcome of ear infections following appropriate empirical treatment.

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Etiological studies of ear infections are essential to guide the choice of an effective antibiotic and monitor bacterial patterns and their varying antimicrobial susceptibilities. This is crucial for risk analysis, mitigation measures, and logistical plans. Therefore, the present study aimed to determine the etiological pathogens and antimicrobial susceptibility patterns of bacteria causing ear infections. The data obtained, if used, will strengthen the prevention and control measures and update the management and treatment options for ear infections. Also, the information will serve as a baseline for countrywide surveillance of antibiotic resistance.

2. Materials and Methods

Study design and settings

We conducted a hospital-based cross-sectional study from March to July 2021 in the otorhinolaryngology clinic at Muhimbili National Hospital (MNH), Dar es Salaam, Tanzania. MNH is the leading national referral hospital, research center, and a university teaching hospital. It is the largest tertiary healthcare facility in Tanzania. The hospital has a capacity of 1,500 beds, attending from 1,000 to 1,200 outpatients per week and admitting from 1,000 to 1,200 inpatients per week. The otorhinolaryngology department has inpatient and outpatient units; about 20 to 30 patients attend the outpatient clinic per day.

Study participants

The study included patients attending the otorhinolaryngology clinic with signs and symptoms of ear infection, such as accumulation of fluid in the middle ear, bulging of the eardrum, ear pain, ear itching, perforation of the eardrum, and ear discharge (otorrhea). We excluded patients with other hearing disorders unrelated to infection (congenital malformations, physical head injury) and those on regular checkups.

Sample size and sampling procedure

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The study sample size was estimated using a Kish Leslie formula (1965) for a cross-sectional study considering the prevalence of 62.1% reported previously by Moshi et al. in a study conducted in a tertiary hospital in Mwanza city, Tanzania (3). The minimum sample size was 241 participants; considering the 5% non-response rate, we obtained a sample size of 255 participants.

Data collection

Data collection was conducted by two trained research assistants (RAs) and an ear, nose, and throat (ENT) surgeon; briefly, a structured questionnaire was administered to the participants by two RAs. RAs used the questionnaire to collect demographic data (age, sex, marital status, occupation, and education) and behavioral risk characteristics (swimming, frequent use of earphones, cotton buds, sharp objects, and cigarette smoking). In addition, the participants' clinical information, including the type of ear infection, use of antibiotics, nasal congestion or blockage, recurrent upper respiratory tract infection (URTI), and cerumen impaction, were also collected from the patient's medical records and during a physical examination by ENT surgeon. In this study, CSOM was diagnosed when there is persistent otorrhea from the ear for at least 3-12 weeks despite appropriate medical treatment or when there is a persistent eardrum perforation with otorrhea for more than three months. This chronicity of otorrhea distinguishes CSOM from acute otitis media, a short-term middle ear infection with acute onset and rapid resolution.

Specimen collection

The ENT surgeon collected specimens with precaution to prevent contamination. The sterile swab was used to clear the oozing pus from the patient's ear; another sterile swab was then used to collect fresh pus. The collected specimens were kept at room temperature in Stuart transport media before processing at Central Pathology Laboratory (CPL).

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Isolation and identification

Upon arrival in the laboratory, specimens were processed for culture and identification. Each specimen was inoculated on selective and non-selective media; Chocolate agar (CA), Sheep-Blood agar (sBA), MacConkey agar (MCA), and Sabouraud dextrose agar (SDA). We used CA to isolate fastidious bacteria, such as *H. influenzae* and *S. pneumoniae*, the frequent etiological agents of ear infection. MCA was used as a selective and differential medium for Gram-negative bacteria, and BA was used as a general-purpose medium. SDA was used for the isolation of fungal species. We incubated MCA in an aerobic environment and BA and CA in a 5% CO_2 environment at 37°C for 18 to 24 hours.

Bacterial isolates were identified by interpreting colonial morphologies, microscopic examination (Gram stain), and biochemical tests. The catalase and coagulase tests were performed for Gram-positive bacteria, while Kligler Iron Agar, Sulfur Indole Motility (SIM), citrate, and urease tests were for gram-negative bacteria. Further, phenotypical identification and confirmation of Gram-negative bacterial isolates were performed by Analytical Profile Index tests, API 20E and API 20NE.

For fungal isolates, growth on the SDA plate was used preliminary to classify mold or yeast based on the colonial morphology and color. A germ tube test was used to identify *Candida albicans*. Additionally, Lactophenol cotton blue was used for molds to identify the conidial spore in *Aspergillus spp.*

Antimicrobial susceptibility testing

Antibiotic susceptibility test (AST) for bacterial isolates was performed using the Kirby Bauer disc diffusion method on Mueller-Hinton Agar (MHA), and MHA supplemented with 5% blood for *S. pneumonia* following the 2021 Clinical and Laboratory Standard Institute

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(CLSI) guidelines. Zones of inhibition were measured using a ruler in millimeters and interpreted as susceptible, resistant, or intermediate according to the 2021 CLSI guideline.

The antibiotic discs used were; ciprofloxacin (5µg), trimethoprim/ sulphamethoxazole (1.25/23.75µg), gentamycin (10µg), clindamycin (2 µg), erythromycin (15µg),) for gram-positive bacteria. Ciprofloxacin (5µg), trimethoprim/ sulphamethoxazole (1.25/23.75µg), gentamycin (10µg), meropenem (10µg), amoxicillin/ clavulanic acid (20µg), ceftriaxone (30µg) and ceftazidime (30µg) for *Enterobacterales* and *Acinetobacter spp*. Ciprofloxacin (5µg), gentamycin (10µg), meropenem (10µg) and ceftazidime (30µg) for *Pseudomonas spp*.

Standard methods were used to identify MRSA using cefoxitin (30µg) disc in which resistant isolates were considered MRSA positive. In addition, ESBL-PE screening was done using ceftazidime (30 µg) and cefotaxime (30µg) antibiotic discs, and if resistant, ESBL-PE confirmation was done by the double-disc synergy method (14).

Quality control

The reference organisms and reagents were clearly and uniquely labeled, dated, and stored at optimal conditions. The room, incubator, and refrigerator temperatures were monitored daily. The culture media were prepared following the manufacturer's guidelines and internal standard operating procedures and tested for performance and sterility.

Data Analysis

The data was analyzed using SPSS version 23 software. Continuous variables were summarized as the median and interquartile range (IQR), whereas percentages and proportions were used to describe categorical variables. The resistance rate was obtained by computing the number of bacteria that resisted a specific drug over a total number of isolated bacterial species. AST intermediate results were regarded as resistant.

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Patient and public involvement

Patients and the public were not involved in this research's design, conduct, reporting, or dissemination plans.

3. Results

Participants' demographic, clinical, and risk behavior characteristics

Two hundred fifty-five participants were recruited; 52.5% (134/255) were males. The median age was 31 years (IQR: 15- 49). Most participants (30.2%) were students, 32.9% had a college education, and 15.7% were from outside Dar es Salaam region (Table 1).

Table 1: Socio-demographic characteristics of the study participants (N=255)

Variables	Frequency (N) and Percentage (%)/Median (IQR)
Median age (years)	31 (15 - 49)
Sex	
Male	134 (52.5)
Female	121 (47.5)
Occupation	
Self-employed	56 (22.0)
Civil servants	62 (24.3)
Retired	49 (19.2)
Unemployed	88 (33.5)
Education	
Primary	75 (29.4)
Secondary	59 (23.1)
College	84 (32.9)
Illiterate	37 (14.5)
Residence	
Within Dar es Salaam	215 (84.3)
Outside Dar es Salaam	40 (15.7)

The median duration of ear infections was 210 days (IQR: 21-1095). Otitis externa (OE) was the most common type of ear infection, accounting for 45.1% (115/255), followed by Chronic Suppurative Otitis Media (CSOM) (41.2%) (Figure 1). Around 49% of the participants with

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ear infections had a history of antibiotic use, whereby ciprofloxacin ear drop was the most prescribed topical antibiotic. Additionally, 33.3% of the study participants had nasal congestion/blockage/discharge, and 28.2% had recurrent URTI (Table 2).

Table 2: Baseline clinical and risk behavioral characteristics of the study participants (N=255)

Patient characteristics	**Frequency (N) and Percentage (%)/Median (IQR) (%)**
Median Duration of ear infection (days)	210 (21-1095)
Nasal discharge/blockage	
Yes	85 (33.3)
No	170 (66.7)
Recurrent URTI	
Yes	72 (28.2)
No	183 (71.8)
Use of hearing aid	
Yes	2 (0.8)
No	253 (99.2)
Earphone use	
Yes	41 (16.1)
No	214 (83.9)
Swimming	
Yes	8 (3.1)
No	247 (96.9)
Cotton bud use	
Yes	112 (43.9)
No	143 (56.1)
Sharp object use	
Yes	60 (23.5)
No	195 (76.5)
Ear cleaning habit	
Yes	119 (46.7)
No	136 (53.3)
Cerumen impaction	
Yes	45 (17.6)
No	210 (82.4)

Distribution of bacterial and fungal isolates causing ear infections

In this study, 136 out of 255 (53.3%) participants had a positive aerobic culture for either bacterial or fungal pathogen, whereby 10.3% (14/136) of participants had a polymicrobial

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infection (mixed growth of either two different bacteria or bacterial and fungal infection). A total of 150 isolates (bacteria and fungi) were identified, of which 87.3% (131/150) were bacteria. Of the isolated bacteria, Gram-negative, 71.0% (93/131) were predominant.

The predominant bacterial isolates were *S. aureus,* 27.5% (36/131), followed by *Pseudomonas aeruginosa,* 24.4% (32/131) (Figure 2A). On the other hand, *Candida spp* accounted for 63.2% (12/19) of the isolated fungi (data not shown). Moreover, 41% of isolates were obtained from chronic suppurative otitis media (CSOM) patients. Further stratification of isolated pathogens by type of ear infection showed that *S. aureus* 16/131 (12.2%) was the most prevalent bacterium in OE patients, whereas *P. aeruginosa* 22/131 (16.8%) predominated in CSOM patients (Figure 2B).

In the present study, 34.4% (21/61) of the Enterobacterales, excluding *P. aeruginosa*, were extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-PE); and *Klebsiella* spp was predominant, accounting for 33.3% (7/21) of the ESBL-PE isolates (Figure 2C). On the other hand, 44.4% (16/36) of the *S. aureus* species were MRSA (data not shown).

Antimicrobial susceptibility pattern of bacterial isolates

Almost all (93%) isolated Enterobacterales were resistant to amoxicillin/clavulanic acid, more so *E. coli* and *Acinetobacter* spp were 100% resistant. Also, 73% of isolated bacteria were resistant to ceftazidime (data not shown), whereby *P. aeruginosa* had the highest resistance rate of 75%. In addition, 43% of isolated bacteria were resistant to trimethoprim-sulfamethoxazole (data not shown), whereby *E. coli* was leading with a 75% resistance rate. Sulfamethoxazole-trimethoprim resistance rates ranged from 57% to 100% among ESBL producers, higher than 29% to 100% among non-ESBL producers. Moreover, 14.6% (6/41) of the non-ESBL-PE bacteria were resistant to all the third-generation cephalosporins, and all

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non-ESBL-PE isolates were sensitive to meropenem. *S. aureus* had an 89% resistance rate to erythromycin. However, MRSA isolates were more resistant to sulfamethoxazole-trimethoprim (81%) and gentamicin (50%) than non-MRSA isolates 35% and 25% for sulfamethoxazole-trimethoprim and gentamicin, respectively. In the present study, we report that resistance to ciprofloxacin, a primary topical antibiotic used to manage ear infections, is 22%. Most isolated bacteria had a low resistance rate against meropenem (4%) (Table 3).

Table 3: Antimicrobial resistance pattern for isolated bacteria

	Bacteria isolates							
ANTIBIOTIC	*S.aureus* (N=36)	*Pseudomonas aeruginosa* (N=32)	*Klebsiella spp* (N=20)	*Acinetobacter spp* (N=10)	*Enterobcter spp* (N=6)	*E. coli* (N=8)	*Proteus spp* (N=12)	*Citrobacter spp* (N=5)
Amikacin	NA	8(25)	4(20)	2(20)	3(50)	2(25)	1(8)	2(40)
Sulfamethoxazole trimethoprim	20(56)	NA	8(40)	1(10)	4(66)	6(75)	3(25)	4(80)
Gentamicin	13(36)	6(19)	9(45)	1(10)	1(17)	1(13)	7(58)	1(20)
Ciprofloxacin	11(31)	11(34)	1(5)	0(0)	2(33)	2(25)	1(8)	0(0)
Amoxicillin /clavulanic acid	NA	NA	18(90)	10(100)	5(83)	8(100)	11(92)	5(100)
Ceftriaxone	NA	NA	9(45)	5(50)	3(50)	5(63)	6(50)	3(60)
Ceftazidime	NA	24(75)	14(70)	7(70)	4(66)	5(63)	10(83)	4(80)
Cefotaxime	NA	NA	9(45)	8(80)	3(50)	5(63)	6(50)	5(100)
Meropenem	NA	2(6)	0(0)	1(10)	0(0)	0(0)	1(8)	0(0)
Erythromycin	32(89)	NA	NA	NA	NA	NA	NA	NA
Clindamycin	9(25)	NA	NA	NA	NA	NA	NA	NA
Cefoxitin	16(44)	NA	NA	NA	NA	NA	NA	NA

Footnote: NA: Indicates not applicable

4. Discussion

Understanding the etiology of ear infections and resistance pattern is crucial in planning interventions and managing ear infections. The results indicate a substantial proportion of ear infections, with bacteria as the primary etiological agent. Most isolated bacteria were resistant to third-generation cephalosporins, sulfamethoxazole-trimethoprim, and amoxicillin/clavulanic acid. Gram-positive bacteria were highly resistant to erythromycin. The two antibiotics that worked the best were ciprofloxacin and meropenem. The results

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imply the need to review ear infection management and the selection of an efficient antibiotic.

The study found that many ear infections are of bacterial etiology. The finding is similar to studies done in Tanzania by Kennedy M et al. (2019) in Morogoro (4), Zephania A et al. (2019) in Dar es Salaam (15), Martha M et al. (2016) in Mwanza (3) and other studies in Kenya and India (16,17). Furthermore, we observed that *S. aureus* and *Pseudomonas aeruginosa* are ear infections' leading bacterial etiological agents, similar to previous studies in Tanzania, Nigeria, Angola, Kenya, and India (3,17–19). In addition, the present study found *Candida spp* and *Aspergillus spp* the fungal spp, causing ear infections consistent with previous findings in Tanzania and elsewhere (Nigeria, Iran, Ethiopia, Egypt, India) (3–5,20–22). Nonetheless, the contribution of fungi etiology in ear infections in the present study was expected because many individuals had risk behaviors for fungal ear infections, including excessive use of eardrops containing antibiotics, regular cleaning of ears, and swimming. Antibiotic overuse promotes the growth of fungi, and the regular ear cleaning habit removes cerumen and exposes ears to fungi colonization and, subsequently, infection (23,24).

The current study revealed a high proportion of MRSA (44.4%) and ESBL-PE (34.4%). In addition, our study showed *Klebsiella* spp (33.3%) as the dominant ESBL-PE. The higher proportion of MRSA and ESBL-PE coincides with studies done in Tanzania by Martha M et al. among patients with chronic suppurative otitis media infection and another study in India (3,16). The greater inclination for self- and empirically prescribing antibiotics without considering laboratory culture and sensitivity may explain the higher proportion of ESBL and MRSA. Furthermore, an increased tendency for people to visit hospital facilities due to chronic ear infections can also explain the high incidence of ESBL and MRSA, which raises the danger of exposure to MDR bacteria. Additionally, the tendency to use inanimate objects

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to remove earwax can be attributed to the increased proportion of ESBL and MRSA, as these inanimate objects are often found in environments that may be contaminated with ESBL-producing bacteria and MRSA (25).

Almost all isolated bacteria (93%) were resistant to amoxicillin/clavulanic acid. Nearly three-quarters of gram-negative bacteria were resistant to ceftazidime, and about half were resistant to trimethoprim-sulfamethoxazole. On the other hand, 89% of isolate gram-positive were resistant to erythromycin. ESBL-PE and MRSA isolates were resistant to the most common antimicrobial agents compared to non-MRSA and non-ESBL-PE. The resistance patterns found in the current study are similar to those reported in other studies in Tanzania, Kenya, Ethiopia, India, Egypt, and Romania (3,4,17,18,26–29). 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.

In the present study, most isolated bacteria were sensitive to meropenem and ciprofloxacin. Ciprofloxacin is a drug of choice for ear infections as per standard treatment guidelines in our setting. The fact that meropenem is infrequently used to treat ear infections may explain the high sensitivity rate. Surprisingly, we observed that ciprofloxacin is still effective despite being prescribed often in our setting for treating ear infections. There is no clinical rationale for why quinolones are still more effective in treating ear infections. However, these results assure that quinolones are still beneficial as first-line topical antibiotics for ear infections.

Study Limitations

- We could not identify the fungi isolates to species level. This is due to insufficient funding and the availability of resources. However, all fungi isolates were stored appropriately for future testing.

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- Due to financial constraints and lack of equipment, it was also impossible to isolate anaerobic bacteria from the collected pus specimen

5. CONCLUSION

The results of this study indicate that bacteria are the most common cause of ear infections in our context. Furthermore, we report that many multidrug-resistant bacteria (ESBL-PE and MRSA) are implicated in causing ear infections. Therefore, antimicrobial susceptibility testing is crucial to guide clinicians on appropriately managing ear infections in our setting.

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Contributors: AS, DK, and WM contributed to the conceptualization, data collection, and analysis study. AS, DK, WM, UK, AGM, AM, SM, AMM, MM, SEM, JM, and MM were involved in manuscript preparation. JM and MM profoundly reviewed the manuscript. AS is the guarantor of the study. The corresponding author attests that all listed authors meet authorship criteria and that no one meeting the criteria has been omitted. The lead author (the manuscript's guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Competing interests: The authors do not have any conflicts of interest.

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Patient consent: To participate in the present study, written informed consent was obtained from adult participants (18 years and above). Assent and consent were requested from adolescents and the parents/ guardians of participants below 18 yrs. The results text, figures, and tables include no personal participant information.

Ethical approval: Ethical clearance was obtained from Muhimbili University of Health and Allied Sciences (MUHAS), Senate Research and Publication Committee, with reference number DA.282/298/01.C/. The MNH administration provided permission to conduct the study.

Availability of data and materials: All relevant data generated and analyzed during this study are available from the corresponding author upon reasonable request.

Provenance and peer review: Not commissioned; externally peer-reviewed.

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Figure legends

Figure 1: Types of ear infection among study participants at MNH

The figure illustrates the distribution of ear infections among patients presenting with signs and symptoms of ear infection attending the otorhinolaryngology clinic at MNH (N=255). OM, OE, and CSOM stand for otitis media, otitis externa, and chronic suppurative otitis media, respectively.

Figure 2A-C: Distribution of bacterial isolates

The figure depicts the distribution of bacteria spp isolated among patients with ear infections attending the otorhinolaryngology clinic at MNH (n=131)(A). According to the type of ear infection (n=131), where OM (otitis media), OE(otitis externa), and CSOM (chronic suppurative otitis media) (B). Distribution of ESBL-producing bacteria among isolated gram-negative bacteria in patients attending the otorhinolaryngology clinic at MNH (n = 61) (C).

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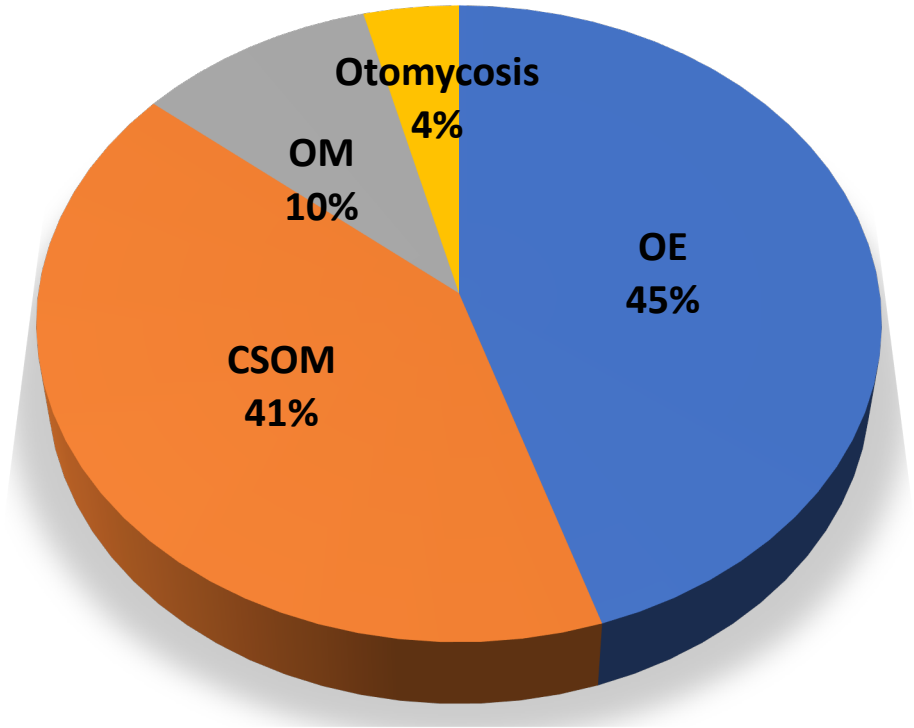
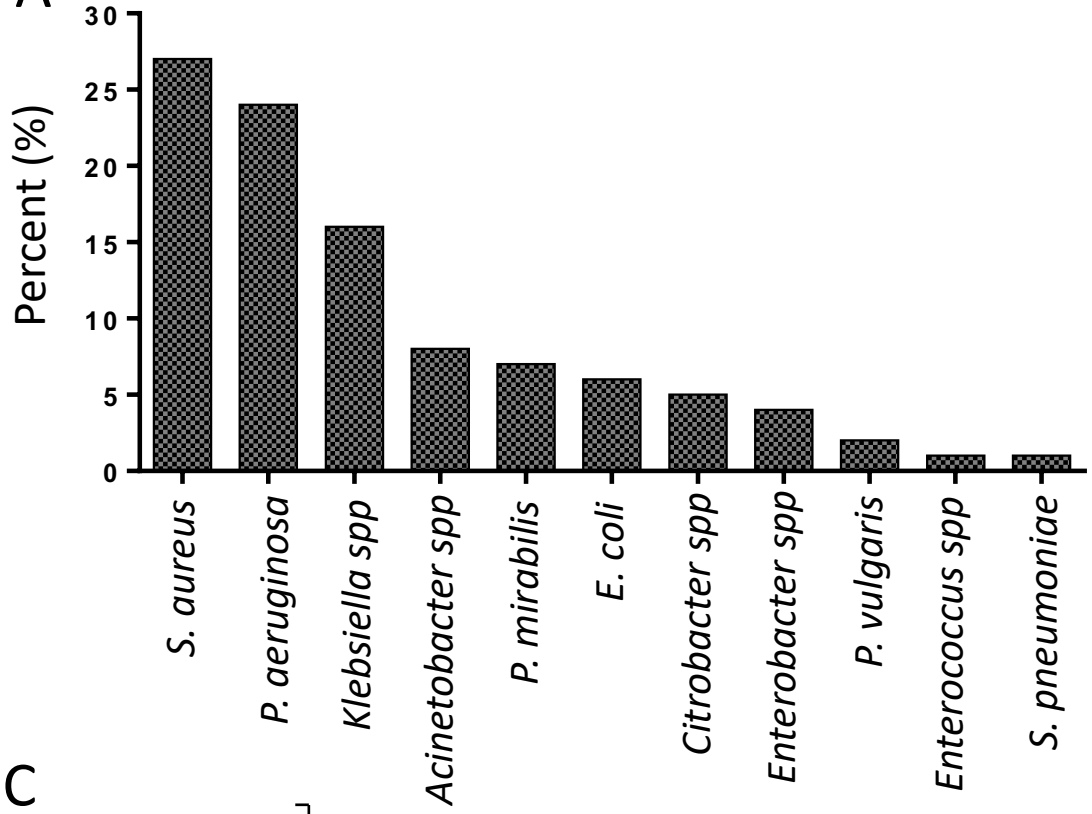
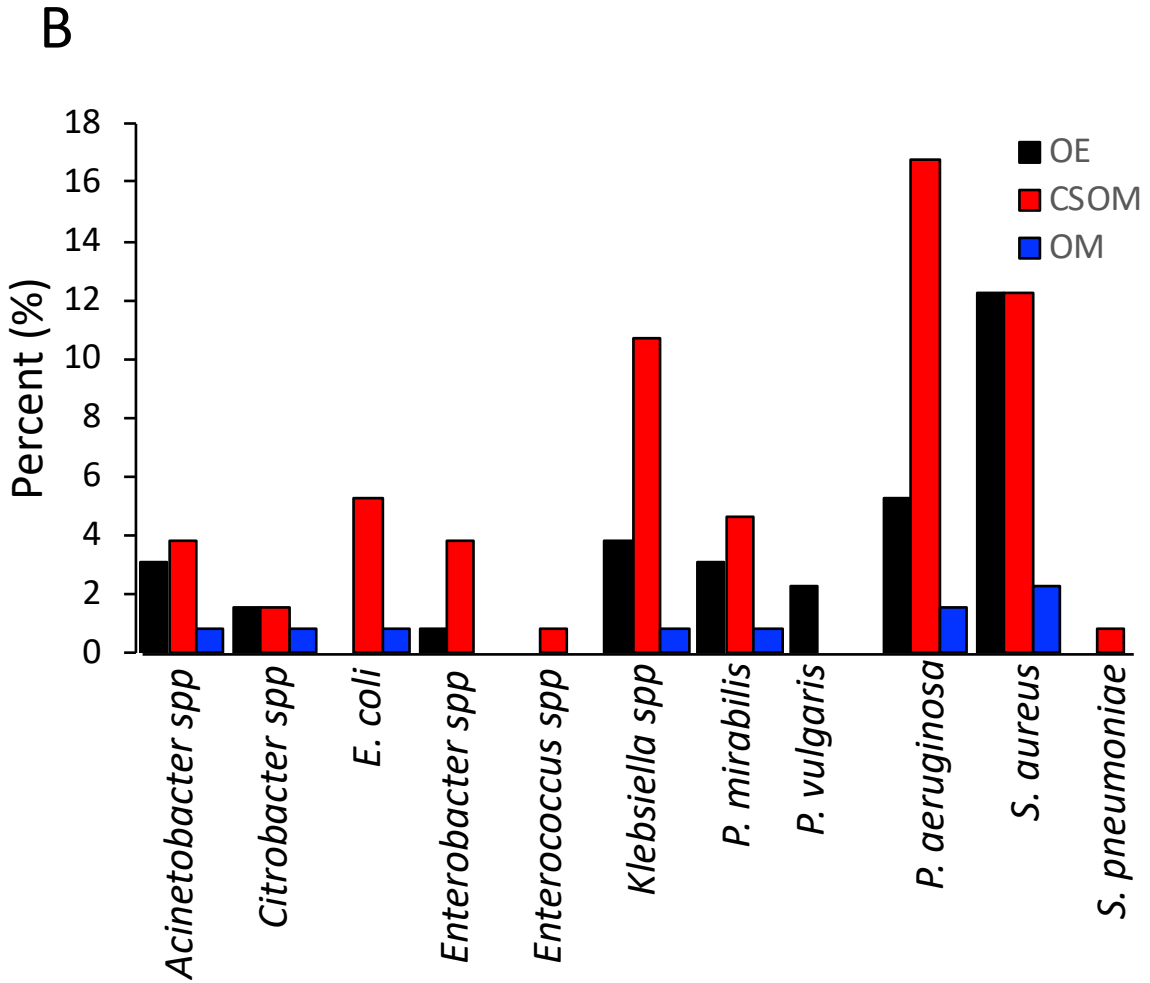
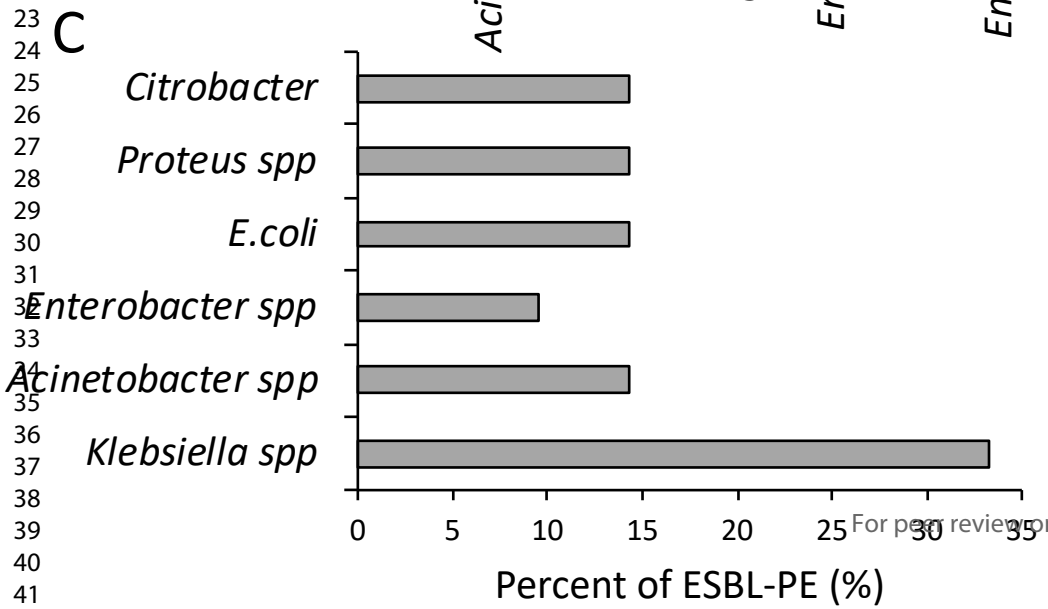

Otomycosis
4%
OM
10%
OE
45%
CSOM
41%







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STROBE Statement

	Page Number	Description
Title and abstract	1-3	(*a*) Etiology of ear infection and antimicrobial susceptibility pattern among patients attending otorhinolaryngology clinic at a tertiary hospital in Dar es Salaam, Tanzania (hospital-based cross-sectional study)
		(*b*) **OBJECTIVES:** To determine the etiological pathogens causing ear infections and their antimicrobial susceptibility patterns among patients with ear complaints at a tertiary hospital in Dar es Salaam. **DESIGN:** Hospital-based cross-sectional study. **SETTINGS:** Otorhinolaryngology clinic at Muhimbili National Hospital, Dar es Salaam, Tanzania. **PARTICIPANTS**: Patients presenting with signs and symptoms of ear infection. **MAIN OUTCOME MEASURE:** Bacteria and fungi isolated from ear swab specimens of patients presenting with signs and symptoms of ear infecrion; and antimicrobial susceptibility patterns of isolated bacteria. **RESULTS:** Two hundred fifty-five participants were enrolled, with a median age of 31 years and an interquartile range of 15- 49. Otitis externa was the predominant type of ear infection, accounting for 45.1%. We observed positive bacteria culture in 53.3% of study participants, in which 41% of isolates were obtained from patients with chronic superative otitis media (CSOM). Moreover, *S. aureus* (27.3%) and *P. aeruginosa* (24.2%) were the most frequently isolated bacteria, while *Candida spp,* 12(63.8%), and *Aspergillus spp*, 9(36.2%) were the only isolated fungi. We report that 93% of isolated Enterobacterales were resistant to amoxicillin /clavulanic acid, and 73% were resistant to ceftazidime. In addition, we detected 34.4% extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-PE) and 44.4% methicillin-resistance Staphylococcus aureus (MRSA). We also detected resistance to ciprofloxacin a primary topical antibiotic used in management of ear infection is 22%. **CONCLUSIONS**: The findings from this study reveal that the leading etiological agent of ear infection is bacteria. Furthermore, our findings show a significant proportion of ESBL-PE and MRSA-causing ear infections. Hence, detecting multidrug-resistant bacteria is crucial to improving ear infection management.
Introduction		
Background/rationale	4-5	An ear infection is among the leading cause of deafness in many developing countries. Unfortunately, most patients with ear infections in resource-limited settings delay seeking medical attention; hence, usually present with complications. Bacteria are the leading pathogens of ear infection whereby, *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis*, and *Klebsiella species* are the dominant bacteria species causing ear infection globally. In addition, *Candida spp* and *Aspergillus spp* are predominant fungal isolates

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		responsible for ear infections. However, fungal ear infections are often undiagnosed due to limited diagnostic opportunities, especially in resource-limited countries, including Tanzania. Most practitioners in our settings tend to treat ear infections empirically or adhere to the Standard Treatment Guideline (STG) without considering laboratory investigation and Antimicrobial susceptibility testing (AST) results. This has created a gap in managing most ear infections, which raises the risk of acquiring multidrug-resistant bacteria. When first-line antibiotics cannot treat infections, more costly antibiotics must be utilized. This consequently affects patients' treatment options, resulting in prolonged hospital stays and increased healthcare costs, which impacts families' financial burden and quality of life. Etiological studies of ear infections are important to guide the choice of an effective antibiotic and monitoring bacterial patterns and their varying antimicrobial susceptibilities. This is crucial for risk analysis, mitigation measures, and logistical plans. Therefore, the present study aimed to determine the etiological pathogens and antimicrobial susceptibility patterns of bacteria causing ear infections. The data obtained will be used to strengthen the prevention and control measures and to update the management and treatment options for ear infections. Also, the information will serve as a baseline for countrywide surveillance of antibiotic resistance.
Objectives	4	1. To determine bacterial species and proportion of fungi causing ear infection among patients attending otorhinolaryngology clinic at MNH. 2. To determine the antimicrobial susceptibility patterns of bacteria isolates causing ear infection among patients attending otorhinolaryngology clinic at MNH
Methods		
Study design	5	This is a hospital-based cross-sectional study
Setting	5	The study was carried out at the Muhimbili National Hospital (MNH), in Dar es Salaam, Tanzania, in the otorhinolaryngology clinic from March to July 2021. A standardized questionnaire and patient's medical records were used to obtain participant's social demographic, behavioural and clinical information. Ear swab was collected, then culture and antimicrobial susceptibility testing was performed for the isolated bacteria species.
Participants	5	The study included all the patients attending otorhinolaryngology clinic at MNH with ear complaints such as accumulation of fluid in the middle ear, bulging of the eardrum, ear pain, ear itching, perforation of the eardrum and ear discharge (otorrhea) and participants who consented or assented to participate in the study. We excluded patients with other hearing disorders (congenital malformations, physical head injury, etc.). And Patients who are on regular check-ups
Variables	6	**Outcome variables** -Ear infection **Independent variables** - demographic data (age, sex, marital status, occupation, and education) and behavioural risk characteristics (swimming, frequent use of earphones, cotton buds, sharp objects, and cigarette smoking). Clinical information, including the type of ear infection, use of antibiotics, nasal congestion or blockage, recurrent upper respiratory tract infection (URTI), and cerumen impaction
Data sources/	6	**Outcome variables** – Laboratory (Culture and sensitivity) was the source of data

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measurement		for outcome variables, where by percentage was used to summarize the bacteria and fungi species isolates and the magnitude of resistance for each isolated bacteria.
Bias	8	**Efforts to address potential sources of Bias** Results from this study depended on good practice in the pre-analytic stage including correct procedures during sample collection, proper labelling of the specimen and safe transportation of the sample including proper storage and temperature (2°C– 8°C) to the laboratory and this was guided by standard operating procedures. Clinical and demographic data for each study participant was obtained by using a standardized questionnaire and patients' medical records. The use of a standard questionnaire is said to be reliable because all participants were subjected to the same questionnaire when evaluating the factors associated with ear infection.
Study size	5	The sample size was estimated by using the Kish Leslie formula (1965) using the prevalence of 62% obtained from a study done in Tanzania. $N=\frac{Z^2P(1-P)}{D^2}$ Whereby Z=standard deviation of the normal distribution = 1.96 (confidence level at 95%) P=prevalence 62.1% (5) D=Margin of error 6% N=251 The minimum required sample size was 251 participants with ear infections.
Quantitative variables	8	Quantitative variables were summarized by calculating measures such as mean, median standard deviation, and quartiles to summarize the central tendency and dispersion of the data
Results		
Descriptive data	9-10	(a) Two hundred fifty-five participants were recruited; 52.5% (134/255) were males. The median age was 31 years (IQR: 15- 49). The majority (30.2%) of participants were students, 32.9% had a college education, and 15.7% were from outside Dar es Salaam region (Table 1). The median duration of ear infections was 210 days (IQR: 21-1095). Otitis externa (OE) was the most common type of ear infection, accounting for 45.1% (115/255), followed by Chronic Suppurative Otitis Media (CSOM) (41.2%). Around 49% of the participants with ear infections had a history of antibiotic use, whereby ciprofloxacin ear drop was the most prescribed topical antibiotic. Additionally, 33.3% of the study participants had nasal congestion/blockage/discharge, and 28.2% had recurrent URTI
		(b) No participant with missing variable
Outcome data	10-12	In this study, 136 out of 255 (53.3%) participants had a positive aerobic culture for either bacterial or fungal pathogen. A total of 150 isolates (bacteria and fungi) were identified, of which 87.3% (131/150) were bacteria. Of the isolated bacteria, Gram-negative, 71.0% (93/131) were predominant. The predominant bacterial isolates were *S. aureus,* 27.5% (36/131), followed by *Pseudomonas aeruginosa*, 24.4% (32/131). On the other hand, *Candida spp* accounted for 63.2% (12/19) of the isolated fungi (data not shown). Further stratification of isolated pathogens by type of ear infection showed that *S. aureus* 16/131 (12.2%) was the most prevalent bacterium in OE patients, whereas *P.*

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		aeruginosa 22/131 (16.8%) predominated in CSOM patients In the present study 34.4% (21/61) of the *Enterobacterales*, excluding *Pseudomonas aeruginosa*, were extended-spectrum beta-lactamase producing *Enterobacterales* (ESBL-PE); and *Klebsiella spp* was predominant, accounting for 33.3% (7/21) of the ESBL-PE isolates. On the other hand, 44.4% (16/36) of the *S.aureus* species were MRSA Almost all (93%) isolated Enterobacterales were resistant to amoxicillin/clavulanic acid, more so *E. coli* and *Acinetobacter spp* were 100% resistant. Also, 73% of isolated bacteria were resistant to ceftazidime, whereby *Pseudomonas aeruginosa* had the highest resistance rate of 75%. In addition, 43% of isolated bacteria were resistant to trimethoprim-sulfamethoxazole, whereby *E.coli* was leading with a 75% resistance rate.
Discussion		
Key results		53.3% of research participants reported positive bacteria cultures. *S. aureus* (27.3%) and *P.aeruginosa* (24.2%) were the most frequently isolated bacteria, whereas *Candida spp*. 12(63.8%), and *Aspergillus spp*. 9(36.2%) were the only isolated fungi. 73% of the identified Enterobacterales were resistant to ceftazidime, while 93% were resistant to amoxicillin/clavulanic acid. Furthermore, we identified 44.4% methicillin-resistant *Staphylococcus aureus* (MRSA) and 34.4% extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-PE)
Limitations	14-15	1. The present study was not able to identify the fungi isolates to species level. This is due to insufficient funding and availability of resources. To mitigate this all fungi isolates were stored appropriately for future testing to specie level. 2. Due to financial constraints and lack of equipment, it was also not possible to isolate anaerobic bacteria from the collected pus specimen
Interpretation	12-14	According to the study many ear infections are of bacterial etiology, *S. aureus* and *Pseudomonas aeruginosa* are the most commonly isolated bacteria. The majority of isolated bacteria were resistant to amoxicillin/clavulanic acid, sulfamethoxazole-trimethoprim, Erythromycin and third-generation cephalosporins. The most effective antibiotics were ciprofloxacin and meropenem. The findings suggest that ear infection management procedures and the choice of an effective antibiotic need to be reviewed. The results are consistent with research conducted in Tanzania by Kennedy M. et al. (2019) in Morogoro, Zephania A. et al. (2019) in Dar es Salaam, Martha M. et al. (2016) in Mwanza, as well as research from Kenya and India. The current study revealed a high proportion of MRSA (44.4%) and ESBL-PE (34.4%). ESBL-PE and MRSA isolates were resistant to the most common antimicrobial agents compared to non-MRSA and non-ESBL-PE. In addition, our study showed Klebsiella spp (33.3%) as the dominant ESBL-PE. The higher proportion of MRSA and ESBL-PE coincide with other studies in Tanzania, Kenya, Ethiopia, India, Egypt, and Romania. The greater inclination for self- and empirically prescribing antibiotics without considering laboratory culture and sensitivity may explain the higher proportion of ESBL and MRSA. The high incidence of ESBL and MRSA can also be explained by an increased inclination for people to visit hospital facilities due to chronic ear infection, which raises the danger of exposure to MDR bacteria. Additionally, the tendency to use inanimate

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		objects to remove earwax can be attributed to the increased proportion of ESBL and MRSA, as these inanimate objects are often found in environments that may be contaminated with ESBL-producing bacteria and/or MRSA Ciprofloxacin is a drug of choice for ear infections as per standard treatment guidelines in our setting. The fact that meropenem is infrequently used to treat ear infections may explain the high sensitivity rate. Surprisingly, we observed that ciprofloxacin is still effective despite being prescribed often in our setting for treating ear infections. There is no clinical rationale as to why quinolones are still more effective in treating ear infections, but these results assure that quinolones are still beneficial as first-line topical antibiotics for ear infections.
Generalisability		According to the study's findings, bacteria are the most common etiological factor in ear infections. Additionally, our results indicate that a significant percentage of ear infections are caused by ESBL-PE and MRSA. Therefore, identifying multidrug-resistant bacteria is essential to enhancing the management of ear infections. The study has also identified patterns of antimicrobial susceptibility that are helpful in guiding the selection of empirical treatment in environments with limited resources and comparable geographic, demographic, and social characteristics.
Other information		
Funding	15	No funding was received for this study.

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Etiology of ear infection and antimicrobial susceptibility pattern among patients attending otorhinolaryngology clinic at a tertiary hospital in Dar es Salaam, Tanzania: A hospital-based cross-sectional study

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Etiology of ear infection and antimicrobial susceptibility pattern among patients attending otorhinolaryngology clinic at a tertiary hospital in Dar es Salaam, Tanzania: A hospital-based cross-sectional study

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Keywords: Ear infection, resistance, antimicrobial susceptibility pattern.

ABSTRACT

OBJECTIVES: To determine the etiological pathogens causing ear infections and their antimicrobial susceptibility patterns among patients with ear complaints at a tertiary hospital in Dar es Salaam.

DESIGN: Hospital-based cross-sectional study.

SETTINGS: Otorhinolaryngology clinic at Muhimbili National Hospital, Dar es Salaam, Tanzania.

PARTICIPANTS: Patients presenting with signs and symptoms of ear infection.

MAIN OUTCOME MEASURE: Bacteria and fungi isolated from ear swab specimens of patients presenting with signs and symptoms of ear infection; and antimicrobial susceptibility patterns of isolated bacteria.

RESULTS: Two hundred fifty-five participants were enrolled, with a median age of 31 years and an interquartile range of 15- 49. Otitis externa was the predominant type of ear infection, accounting for 45.1%. We observed positive bacteria culture in 53.3% of study participants, in which 41% of isolates were obtained from patients with chronic suppurative otitis media (CSOM). Moreover, *S. aureus* (27.3%) and *P. aeruginosa* (24.2%) were the most frequently isolated bacteria, while *Candida* spp, 12(63.8%), and *Aspergillus* spp, 9(36.2%) were the only isolated fungi. Furthermore, we report that 93% of isolated *Enterobacterales* were resistant to amoxicillin /clavulanic acid, and 73% were resistant to ceftazidime. In addition, we detected 34.4% extended-spectrum beta-lactamase-producing *Enterobacterales* (ESBL-PE) and 44.4% methicillin-resistance *Staphylococcus aureus* (MRSA). We also found that 22% of the bacteria isolates were resistant to ciprofloxacin, a primary topical antibiotic used in managing ear infections.

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CONCLUSIONS: The findings from this study reveal that the leading etiological agent of ear infection is bacteria. Furthermore, our findings show a significant proportion of ESBL-PE and MRSA-causing ear infections. Hence, detecting multidrug-resistant bacteria is crucial to improving ear infection management.

Strength and Limitation of the study

- The present study has some strengths, we report the common bacterial and fungi etiology of ear infection in our study setting.
- Notably, the study has revealed the antimicrobial susceptibility patterns that are useful in guiding the choice of empirical treatment in similar settings with limited resources and comparable geographic, demographic, and social characteristics.
- The present study has some limitations; some fungal (molds) isolates were not identified to species level, and
- Anaerobic culture was not performed.

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1. Introduction

An ear infection is among the leading cause of deafness in many developing countries. Unfortunately, most patients with ear infections in resource-limited settings delay seeking medical attention; hence, usually present with complications (1). Bacteria are the leading pathogens of ear infection, whereby, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Proteus mirabilis,* and Klebsiella species are the dominant bacteria causing ear infection globally (1–6). In addition, *Candida* spp and *Aspergillus* spp are predominant fungal isolates responsible for ear infections (7–10). However, due to limited diagnostic opportunities, fungal ear infections are often undiagnosed, especially in resource-limited countries, including Tanzania (5,6).

Most practitioners in our settings tend to treat ear infections empirically or adhere to the Standard Treatment Guideline (STG) without considering laboratory investigation and antimicrobial susceptibility testing (AST) results. This has created a gap in managing most ear infections, which raises the risk of acquiring multidrug-resistant bacteria (11,12). When first-line antibiotics cannot treat diseases, more costly antibiotics must be utilized. This consequently affects patients' treatment options, resulting in prolonged hospital stays and increased healthcare costs, which impacts families' financial burden and quality of life (13). Furthermore, there needs to be more data on the effectiveness of empirical treatment in managing ear infections in Tanzania. However, experience based on the clinic's patient return rate after initial treatment for ear infections, it appears that a considerable number of patients return to the clinic with the same problem. This suggests that relying solely on empirical treatment methods may not be effective in treating ear infections. Hence this warrants further research to investigate the antimicrobial susceptibility patterns of bacteria isolated in ear infections to improve the outcome of ear infections following appropriate empirical treatment.

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Etiological studies of ear infections are essential to guide the choice of an effective antibiotic and monitor bacterial patterns and their varying antimicrobial susceptibilities. This is crucial for risk analysis, mitigation measures, and logistical plans. Therefore, the present study aimed to determine the etiological pathogens and antimicrobial susceptibility patterns of bacteria causing ear infections. The data obtained, if used, will strengthen the prevention and control measures and update the management and treatment options for ear infections. Also, the information will serve as a baseline for countrywide surveillance of antibiotic resistance.

2. Materials and Methods

Study design and settings

We conducted a hospital-based cross-sectional study from March to July 2021 in the otorhinolaryngology clinic at Muhimbili National Hospital (MNH), Dar es Salaam, Tanzania. MNH is the leading national referral hospital, research center, and a university teaching hospital. It is the largest tertiary healthcare facility in Tanzania. The hospital has a capacity of 1,500 beds, attending from 1,000 to 1,200 outpatients per week and admitting from 1,000 to 1,200 inpatients per week. The otorhinolaryngology department has inpatient and outpatientunits; about 20 to 30 patients attend the outpatient clinic per day.

Study participants

The study included patients attending the otorhinolaryngology clinic with signs and symptoms of ear infection, such as accumulation of fluid in the middle ear, bulging of the eardrum, ear pain, ear itching, perforation of the eardrum, and ear discharge (otorrhea). We excluded patients with other hearing disorders unrelated to infection (congenital malformations, physical head injury) and those on regular checkups.

Sample size and sampling procedure

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The study sample size was estimated using a Kish Leslie formula (1965) for a cross-sectional study considering the prevalence of 62.1% reported previously by Moshi et al. in a study conducted in a tertiary hospital in Mwanza city, Tanzania (3). The minimum sample size was 241 participants; considering the 5% non-response rate, we obtained a sample size of 255 participants.

Data collection

Data collection was conducted by two trained research assistants (RAs) and an ear, nose, and throat (ENT) surgeon; briefly, a structured questionnaire was administered to the participants by two RAs. RAs used the questionnaire to collect demographic data (age, sex, marital status, occupation, and education) and behavioral risk characteristics (swimming, frequent use of earphones, cotton buds, sharp objects, and cigarette smoking). In addition, the participants' clinical information, including the type of ear infection, use of antibiotics, nasal congestion or blockage, recurrent upper respiratory tract infection (URTI), and cerumen impaction, were also collected from the patient's medical records and during a physical examination by ENT surgeon. In this study, CSOM was diagnosed when there is persistent otorrhea from the ear for at least 3-12 weeks despite appropriate medical treatment or when there is a persistent eardrum perforation with otorrhea for more than three months. This chronicity of otorrhea distinguishes CSOM from acute otitis media, a short-term middle ear infection with acute onset and rapid resolution.

Specimen collection

The ENT surgeon collected specimens with precaution to prevent contamination. The sterile swab was used to clear the oozing pus from the patient's ear; another sterile swab was then used to collect fresh pus. The collected specimens were kept at room temperature in Stuart transport media before processing at Central Pathology Laboratory (CPL).

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Isolation and identification

Upon arrival in the laboratory, specimens were processed for culture and identification. Each specimen was inoculated on selective and non-selective media; Chocolate agar (CA), Sheep-Blood agar (sBA), MacConkey agar (MCA), and Sabouraud dextrose agar (SDA). We used CA to isolate fastidious bacteria, such as *H. influenzae* and *S. pneumoniae*, the frequent etiological agents of ear infection. MCA was used as a selective and differential medium for Gram-negative bacteria, and BA was used as a general-purpose medium. SDA was used for the isolation of fungal species. We incubated MCA in an aerobic environment and BA and CA in a 5% CO_2 environment at 37°C for 18 to 24 hours.

Bacterial isolates were identified by interpreting colonial morphologies, microscopic examination (Gram stain), and biochemical tests. The catalase and coagulase tests were performed for Gram-positive bacteria, while Kligler Iron Agar, Sulfur Indole Motility (SIM), citrate, and urease tests were for gram-negative bacteria. Further, phenotypical identification and confirmation of Gram-negative bacterial isolates were performed by Analytical Profile Index tests, API 20E and API 20NE.

For fungal isolates, growth on the SDA plate was used preliminary to classify mold or yeast based on the colonial morphology and color. A germ tube test was used to identify *Candida albicans*. Additionally, Lactophenol cotton blue was used for molds to identify the conidial spore in *Aspergillus spp.*

Antimicrobial susceptibility testing

Antibiotic susceptibility test (AST) for bacterial isolates was performed using the Kirby Bauer disc diffusion method on Mueller-Hinton Agar (MHA), and MHA supplemented with 5% blood for *S. pneumonia* following the 2021 Clinical and Laboratory Standard Institute

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(CLSI) guidelines. Zones of inhibition were measured using a ruler in millimeters and interpreted as susceptible, resistant, or intermediate according to the 2021 CLSI guideline.

The antibiotic discs used were; ciprofloxacin (5µg), trimethoprim/ sulphamethoxazole (1.25/23.75µg), gentamycin (10µg), clindamycin (2 µg), erythromycin (15µg),) for gram-positive bacteria. Ciprofloxacin (5µg), trimethoprim/ sulphamethoxazole (1.25/23.75µg), gentamycin (10µg), meropenem (10µg), amoxicillin/ clavulanic acid (20µg), ceftriaxone (30µg) and ceftazidime (30µg) for *Enterobacterales* and *Acinetobacter spp*. Ciprofloxacin (5µg), gentamycin (10µg), meropenem (10µg) and ceftazidime (30µg) for *Pseudomonas spp*.

Standard methods were used to identify MRSA using cefoxitin (30µg) disc in which resistant isolates were considered MRSA positive. In addition, ESBL-PE screening was done using ceftazidime (30 µg) and cefotaxime (30µg) antibiotic discs, and if resistant, ESBL-PE confirmation was done by the double-disc synergy method (14).

Quality control

The reference organisms and reagents were clearly and uniquely labeled, dated, and stored at optimal conditions. The room, incubator, and refrigerator temperatures were monitored daily. The culture media were prepared following the manufacturer's guidelines and internal standard operating procedures and tested for performance and sterility.

Data Analysis

The data was analyzed using SPSS version 23 software. Continuous variables were summarized as the median and interquartile range (IQR), whereas percentages and proportions were used to describe categorical variables. The resistance rate was obtained by computing the number of bacteria that resisted a specific drug over a total number of isolated bacterial species. AST intermediate results were regarded as resistant.

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Reporting Guideline

This study adhered to the STROBE guidelines for cross-sectional studies, which provide a checklist for reporting observational studies. The checklist includes crucial elements that should be included in the report, such as the study design, participant selection, data collection, and statistical analysis. The authors have carefully reviewed the checklist to ensure that they incorporated each relevant item into the study design and analysis. The authors utilized a standardized data collection tool to collect information on all study participants and employed appropriate statistical methods to analyze the data and draw conclusions.

Patient and public involvement

Patients and the public were not involved in this research's design, conduct, reporting, or dissemination plans.

3. Results

Participants' demographic, clinical, and risk behavior characteristics

Two hundred fifty-five participants were recruited; 52.5% (134/255) were males. The median age was 31 years (IQR: 15- 49). Most participants (30.2%) were students, 32.9% had a college education, and 15.7% were from outside Dar es Salaam region (Table 1).

Table 1: Socio-demographic characteristics of the study participants (N=255)

Variables	Frequency (N) and Percentage (%)/Median (IQR)
Median age (years)	31 (15 - 49)
Sex	
Male	134 (52.5)
Female	121 (47.5)
Occupation	
Self-employed	56 (22.0)
Civil servants	62 (24.3)

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Retired	49 (19.2)
Unemployed	88 (33.5)
Education	
Primary	75 (29.4)
Secondary	59 (23.1)
College	84 (32.9)
Illiterate	37 (14.5)
Residence	
Within Dar es Salaam	215 (84.3)
Outside Dar es Salaam	40 (15.7)

The median duration of ear infections was 210 days (IQR: 21-1095). Otitis externa (OE) was the most common type of ear infection, accounting for 45.1% (115/255), followed by Chronic Suppurative Otitis Media (CSOM) (41.2%) (Figure 1). Around 49% of the participants with ear infections had a history of antibiotic use, whereby ciprofloxacin ear drop was the most prescribed topical antibiotic. Additionally, 33.3% of the study participants had nasal congestion/blockage/discharge, and 28.2% had recurrent URTI (Table 2).

Table 2: Baseline clinical and risk behavioral characteristics of the study participants (N=255)

Patient characteristics	**Frequency (N) and Percentage (%)/Median (IQR) (%)**
Median Duration of ear infection (days)	210 (21-1095)
Nasal discharge/blockage	
Yes	85 (33.3)
No	170 (66.7)
Recurrent URTI	
Yes	72 (28.2)
No	183 (71.8)
Use of hearing aid	
Yes	2 (0.8)
No	253 (99.2)
Earphone use	
Yes	41 (16.1)
No	214 (83.9)
Swimming	
Yes	8 (3.1)
No	247 (96.9)
Cotton bud use	
Yes	112 (43.9)

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No	143 (56.1)
Sharp object use	
Yes	60 (23.5)
No	195 (76.5)
Ear cleaning habit	
Yes	119 (46.7)
No	136 (53.3)
Cerumen impaction	
Yes	45 (17.6)
No	210 (82.4)

Distribution of bacterial and fungal isolates causing ear infections

In this study, 136 out of 255 (53.3%) participants had a positive aerobic culture for either bacterial or fungal pathogen, whereby 10.3% (14/136) of participants had a polymicrobial infection (mixed growth of either two different bacteria or bacterial and fungal infection). A total of 150 isolates (bacteria and fungi) were identified, of which 87.3% (131/150) were bacteria. Of the isolated bacteria, Gram-negative, 71.0% (93/131) were predominant.

The predominant bacterial isolates were *S. aureus,* 27.5% (36/131), followed by *Pseudomonas aeruginosa,* 24.4% (32/131) (Figure 2A). On the other hand, *Candida spp* accounted for 63.2% (12/19) of the isolated fungi (data not shown). Moreover, 41% of isolates were obtained from chronic suppurative otitis media (CSOM) patients. Further stratification of isolated pathogens by type of ear infection showed that *S. aureus* 16/131 (12.2%) was the most prevalent bacterium in OE patients, whereas *P. aeruginosa* 22/131 (16.8%) predominated in CSOM patients (Figure 2B).

In the present study, 34.4% (21/61) of the Enterobacterales, excluding *P. aeruginosa*, were extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-PE); and *Klebsiella* spp was predominant, accounting for 33.3% (7/21) of the ESBL-PE isolates (Figure 2C). On the other hand, 44.4% (16/36) of the *S. aureus* species were MRSA (data not shown).

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Antimicrobial susceptibility pattern of bacterial isolates

Almost all (93%) isolated Enterobacterales were resistant to amoxicillin/clavulanic acid, more so *E. coli* and *Acinetobacter* spp were 100% resistant. Also, 73% of isolated bacteria were resistant to ceftazidime (data not shown), whereby *P. aeruginosa* had the highest resistance rate of 75%. In addition, 43% of isolated bacteria were resistant to trimethoprim-sulfamethoxazole (data not shown), whereby *E. coli* was leading with a 75% resistance rate. Sulfamethoxazole-trimethoprim resistance rates ranged from 57% to 100% among ESBL producers, higher than 29% to 100% among non-ESBL producers. Moreover, 14.6% (6/41) of the non-ESBL-PE bacteria were resistant to all the third-generation cephalosporins, and all non-ESBL-PE isolates were sensitive to meropenem. *S. aureus* had an 89% resistance rate to erythromycin. However, MRSA isolates were more resistant to sulfamethoxazole-trimethoprim (81%) and gentamicin (50%) than non-MRSA isolates 35% and 25% for sulfamethoxazole-trimethoprim and gentamicin, respectively. In the present study, we report that resistance to ciprofloxacin, a primary topical antibiotic used to manage ear infections, is 22%. Most isolated bacteria had a low resistance rate against meropenem (4%) (Table 3).

Table 3: Antimicrobial resistance pattern for isolated bacteria

	Bacteria isolates							
ANTIBIOTIC	*S.aureus* (N=36)	*Pseudomonas aeruginosa* (N=32)	*Klebsiella spp* (N=20)	*Acinetobacter spp* (N=10)	*Enterobcter spp* (N=6)	*E. coli* (N=8)	*Proteus spp* (N=12)	*Citrobacter spp* (N=5)
Amikacin	NA	8(25)	4(20)	2(20)	3(50)	2(25)	1(8)	2(40)
Sulfamethoxazole trimethoprim	20(56)	NA	8(40)	1(10)	4(66)	6(75)	3(25)	4(80)
Gentamicin	13(36)	6(19)	9(45)	1(10)	1(17)	1(13)	7(58)	1(20)
Ciprofloxacin	11(31)	11(34)	1(5)	0(0)	2(33)	2(25)	1(8)	0(0)
Amoxicillin /clavulanic acid	NA	NA	18(90)	10(100)	5(83)	8(100)	11(92)	5(100)
Ceftriaxone	NA	NA	9(45)	5(50)	3(50)	5(63)	6(50)	3(60)
Ceftazidime	NA	24(75)	14(70)	7(70)	4(66)	5(63)	10(83)	4(80)
Cefotaxime	NA	NA	9(45)	8(80)	3(50)	5(63)	6(50)	5(100)
Meropenem	NA	2(6)	0(0)	1(10)	0(0)	0(0)	1(8)	0(0)
Erythromycin	32(89)	NA	NA	NA	NA	NA	NA	NA
Clindamycin	9(25)	NA	NA	NA	NA	NA	NA	NA
Cefoxitin	16(44)	NA	NA	NA	NA	NA	NA	NA

Footnote: NA: Indicates not applicable

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4. Discussion

Understanding the etiology of ear infections and resistance pattern is crucial in planning interventions and managing ear infections. The results indicate a substantial proportion of ear infections, with bacteria as the primary etiological agent. Most isolated bacteria were resistant to third-generation cephalosporins, sulfamethoxazole-trimethoprim, and amoxicillin/clavulanic acid. Gram-positive bacteria were highly resistant to erythromycin. The two antibiotics that worked the best were ciprofloxacin and meropenem. The results imply the need to review ear infection management and the selection of an efficient antibiotic.

The study found that many ear infections are of bacterial etiology. The finding is similar to studies done in Tanzania by Kennedy M et al. (2019) in Morogoro (4), Zephania A et al. (2019) in Dar es Salaam (15), Martha M et al. (2016) in Mwanza (3) and other studies in Kenya and India (16,17). Furthermore, we observed that *S. aureus* and *Pseudomonas aeruginosa* are ear infections' leading bacterial etiological agents, similar to previous studies in Tanzania, Nigeria, Angola, Kenya, and India (3,17–19). In addition, the present study found *Candida spp* and *Aspergillus spp* the fungal spp, causing ear infections consistent with previous findings in Tanzania and elsewhere (Nigeria, Iran, Ethiopia, Egypt, India) (3–5,20–22). Nonetheless, the contribution of fungi etiology in ear infections in the present study was expected because many individuals had risk behaviors for fungal ear infections, including excessive use of eardrops containing antibiotics, regular cleaning of ears, and swimming. Antibiotic overuse promotes the growth of fungi, and the regular ear cleaning habit removes cerumen and exposes ears to fungi colonization and, subsequently, infection (23,24).

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The current study revealed a high proportion of MRSA (44.4%) and ESBL-PE (34.4%). In addition, our study showed *Klebsiella* spp (33.3%) as the dominant ESBL-PE. The higher proportion of MRSA and ESBL-PE coincides with studies done in Tanzania by Martha M et al. among patients with chronic suppurative otitis media infection and another study in India (3,16). The greater inclination for self- and empirically prescribing antibiotics without considering laboratory culture and sensitivity may explain the higher proportion of ESBL and MRSA. Furthermore, an increased tendency for people to visit hospital facilities due to chronic ear infections can also explain the high incidence of ESBL and MRSA, which raises the danger of exposure to MDR bacteria. Additionally, the tendency to use inanimate objects to remove earwax can be attributed to the increased proportion of ESBL and MRSA, as these inanimate objects are often found in environments that may be contaminated with ESBL-producing bacteria and MRSA (25).

Almost all isolated bacteria (93%) were resistant to amoxicillin/clavulanic acid. Nearly three-quarters of gram-negative bacteria were resistant to ceftazidime, and about half were resistant to trimethoprim-sulfamethoxazole. On the other hand, 89% of isolate gram-positive were resistant to erythromycin. ESBL-PE and MRSA isolates were resistant to the most common antimicrobial agents compared to non-MRSA and non-ESBL-PE. The resistance patterns found in the current study are similar to those reported in other studies in Tanzania, Kenya, Ethiopia, India, Egypt, and Romania (3,4,17,18,26–29). 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.

In the present study, most isolated bacteria were sensitive to meropenem and ciprofloxacin. Ciprofloxacin is a drug of choice for ear infections as per standard treatment guidelines in our setting. The fact that meropenem is infrequently used to treat ear infections may explain the

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high sensitivity rate. Surprisingly, we observed that ciprofloxacin is still effective despite being prescribed often in our setting for treating ear infections. There is no clinical rationale for why quinolones are still more effective in treating ear infections. However, these results assure that quinolones are still beneficial as first-line topical antibiotics for ear infections. The present study has some limitations. We were not able to identify the fungi isolates to species level. This is due to insufficient funding and the availability of resources. To mitigate this all fungi isolates were stored appropriately for future testing. In addition, due to financial constraints and lack of equipment, it was impossible to isolate anaerobic bacteria from the collected pus specimen

5. CONCLUSION

The results of this study indicate that bacteria are the most common cause of ear infections in our context. Furthermore, we report that many multidrug-resistant bacteria (ESBL-PE and MRSA) are implicated in causing ear infections. Therefore, antimicrobial susceptibility testing is crucial to guide clinicians on appropriately managing ear infections in our setting.

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Contributors: AS, DK, and WM contributed to the conceptualization, data collection, and analysis study. AS, DK, WM, UK, AGM, AM, SM, AMM, MM, SEM, JM, and MM were involved in manuscript preparation. JM and MM profoundly reviewed the manuscript. AS is the guarantor of the study. The corresponding author attests that all listed authors meet authorship criteria and that no one meeting the criteria has been omitted. The lead author (the manuscript's guarantor) affirms that the manuscript is an honest, accurate, and transparent

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account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Competing interests: The authors do not have any conflicts of interest.

Patient consent: To participate in the present study, written informed consent was obtained from adult participants (18 years and above). Assent and consent were requested from adolescents and the parents/ guardians of participants below 18 yrs. The results text, figures, and tables include no personal participant information.

Ethical approval: Ethical clearance was obtained from Muhimbili University of Health and Allied Sciences (MUHAS), Senate Research and Publication Committee, with reference number DA.282/298/01.C/. The MNH administration provided permission to conduct the study.

Availability of data and materials: All relevant data generated and analyzed during this study are available from the corresponding author upon reasonable request.

Provenance and peer review: Not commissioned; externally peer-reviewed.

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Figure legends

Figure 1: Types of ear infection among study participants at MNH

The figure illustrates the distribution of ear infections among patients presenting with signs and symptoms of ear infection attending the otorhinolaryngology clinic at MNH (N=255). OM, OE, and CSOM stand for otitis media, otitis externa, and chronic suppurative otitis media, respectively.

Figure 2A-C: Distribution of bacterial isolates

The figure depicts the distribution of bacteria spp isolated among patients with ear infections attending the otorhinolaryngology clinic at MNH (n=131)(A). According to the type of ear infection (n=131), where OM (otitis media), OE(otitis externa), and CSOM (chronic suppurative otitis media) (B). Distribution of ESBL-producing bacteria among isolated gram-negative bacteria in patients attending the otorhinolaryngology clinic at MNH (n = 61) (C).

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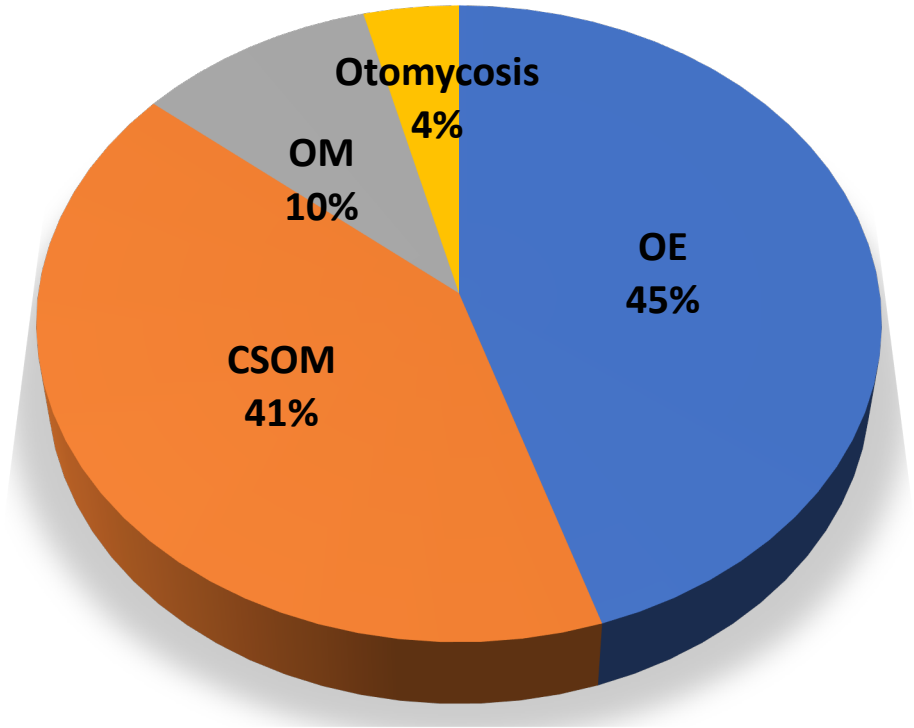
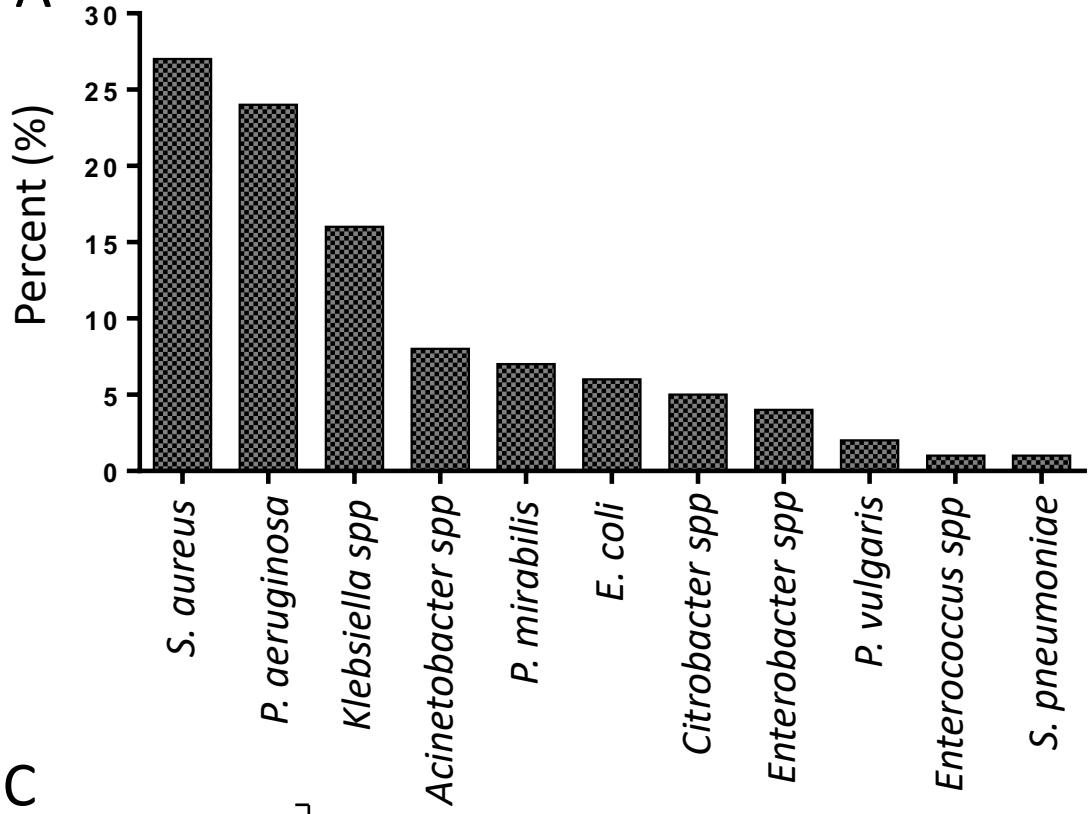
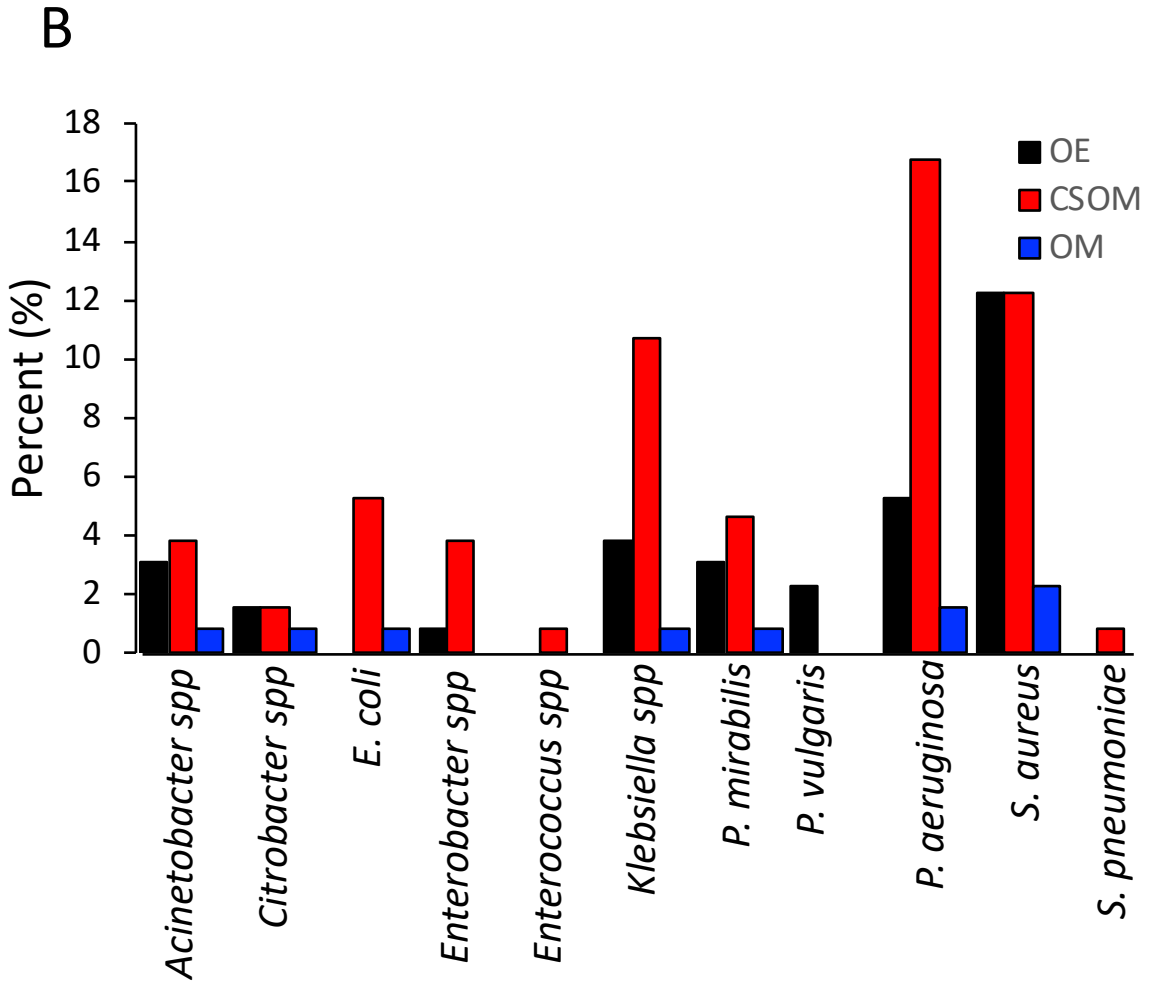
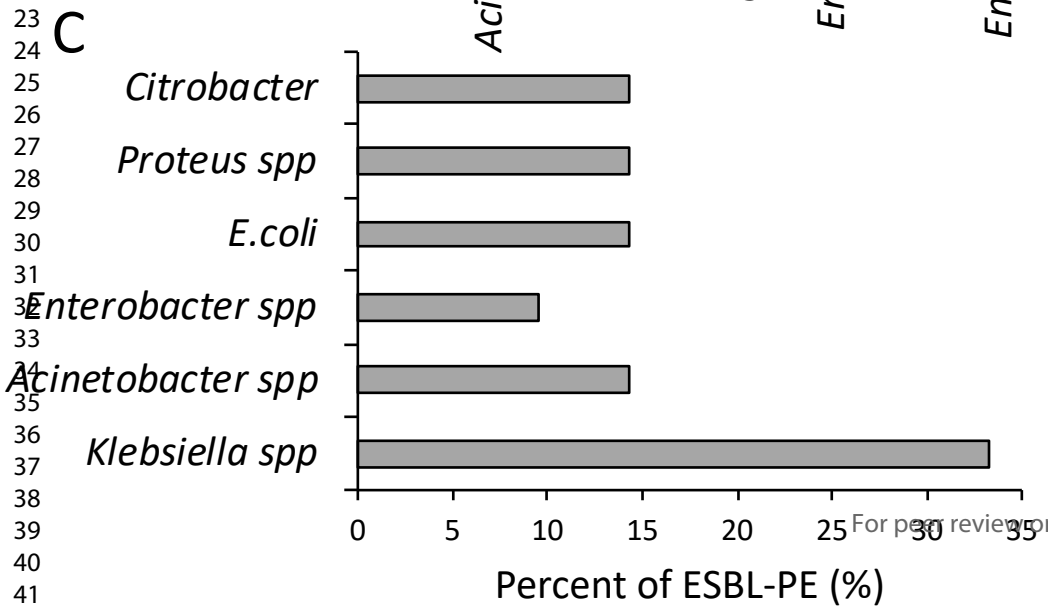

Otomycosis
4%
OM
10%
OE
45%
CSOM
41%







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STROBE Statement

	Page Number	Description
Title and abstract	1-3	(*a*) Etiology of ear infection and antimicrobial susceptibility pattern among patients attending otorhinolaryngology clinic at a tertiary hospital in Dar es Salaam, Tanzania (hospital-based cross-sectional study)
		(*b*) **OBJECTIVES:** To determine the etiological pathogens causing ear infections and their antimicrobial susceptibility patterns among patients with ear complaints at a tertiary hospital in Dar es Salaam. **DESIGN:** Hospital-based cross-sectional study. **SETTINGS:** Otorhinolaryngology clinic at Muhimbili National Hospital, Dar es Salaam, Tanzania. **PARTICIPANTS**: Patients presenting with signs and symptoms of ear infection. **MAIN OUTCOME MEASURE:** Bacteria and fungi isolated from ear swab specimens of patients presenting with signs and symptoms of ear infecrion; and antimicrobial susceptibility patterns of isolated bacteria. **RESULTS:** Two hundred fifty-five participants were enrolled, with a median age of 31 years and an interquartile range of 15- 49. Otitis externa was the predominant type of ear infection, accounting for 45.1%. We observed positive bacteria culture in 53.3% of study participants, in which 41% of isolates were obtained from patients with chronic superative otitis media (CSOM). Moreover, *S. aureus* (27.3%) and *P. aeruginosa* (24.2%) were the most frequently isolated bacteria, while *Candida spp,* 12(63.8%), and *Aspergillus spp*, 9(36.2%) were the only isolated fungi. We report that 93% of isolated Enterobacterales were resistant to amoxicillin /clavulanic acid, and 73% were resistant to ceftazidime. In addition, we detected 34.4% extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-PE) and 44.4% methicillin-resistance Staphylococcus aureus (MRSA). We also detected resistance to ciprofloxacin a primary topical antibiotic used in management of ear infection is 22%. **CONCLUSIONS**: The findings from this study reveal that the leading etiological agent of ear infection is bacteria. Furthermore, our findings show a significant proportion of ESBL-PE and MRSA-causing ear infections. Hence, detecting multidrug-resistant bacteria is crucial to improving ear infection management.
Introduction		
Background/rationale	4-5	An ear infection is among the leading cause of deafness in many developing countries. Unfortunately, most patients with ear infections in resource-limited settings delay seeking medical attention; hence, usually present with complications. Bacteria are the leading pathogens of ear infection whereby, *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis*, and *Klebsiella species* are the dominant bacteria species causing ear infection globally. In addition, *Candida spp* and *Aspergillus spp* are predominant fungal isolates

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		responsible for ear infections. However, fungal ear infections are often undiagnosed due to limited diagnostic opportunities, especially in resource-limited countries, including Tanzania. Most practitioners in our settings tend to treat ear infections empirically or adhere to the Standard Treatment Guideline (STG) without considering laboratory investigation and Antimicrobial susceptibility testing (AST) results. This has created a gap in managing most ear infections, which raises the risk of acquiring multidrug-resistant bacteria. When first-line antibiotics cannot treat infections, more costly antibiotics must be utilized. This consequently affects patients' treatment options, resulting in prolonged hospital stays and increased healthcare costs, which impacts families' financial burden and quality of life. Etiological studies of ear infections are important to guide the choice of an effective antibiotic and monitoring bacterial patterns and their varying antimicrobial susceptibilities. This is crucial for risk analysis, mitigation measures, and logistical plans. Therefore, the present study aimed to determine the etiological pathogens and antimicrobial susceptibility patterns of bacteria causing ear infections. The data obtained will be used to strengthen the prevention and control measures and to update the management and treatment options for ear infections. Also, the information will serve as a baseline for countrywide surveillance of antibiotic resistance.
Objectives	4	1. To determine bacterial species and proportion of fungi causing ear infection among patients attending otorhinolaryngology clinic at MNH. 2. To determine the antimicrobial susceptibility patterns of bacteria isolates causing ear infection among patients attending otorhinolaryngology clinic at MNH
Methods		
Study design	5	This is a hospital-based cross-sectional study
Setting	5	The study was carried out at the Muhimbili National Hospital (MNH), in Dar es Salaam, Tanzania, in the otorhinolaryngology clinic from March to July 2021. A standardized questionnaire and patient's medical records were used to obtain participant's social demographic, behavioural and clinical information. Ear swab was collected, then culture and antimicrobial susceptibility testing was performed for the isolated bacteria species.
Participants	5	The study included all the patients attending otorhinolaryngology clinic at MNH with ear complaints such as accumulation of fluid in the middle ear, bulging of the eardrum, ear pain, ear itching, perforation of the eardrum and ear discharge (otorrhea) and participants who consented or assented to participate in the study. We excluded patients with other hearing disorders (congenital malformations, physical head injury, etc.). And Patients who are on regular check-ups
Variables	6	**Outcome variables** -Ear infection **Independent variables** - demographic data (age, sex, marital status, occupation, and education) and behavioural risk characteristics (swimming, frequent use of earphones, cotton buds, sharp objects, and cigarette smoking). Clinical information, including the type of ear infection, use of antibiotics, nasal congestion or blockage, recurrent upper respiratory tract infection (URTI), and cerumen impaction
Data sources/	6	**Outcome variables** – Laboratory (Culture and sensitivity) was the source of data

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measurement		for outcome variables, where by percentage was used to summarize the bacteria and fungi species isolates and the magnitude of resistance for each isolated bacteria.
Bias	8	**Efforts to address potential sources of Bias** Results from this study depended on good practice in the pre-analytic stage including correct procedures during sample collection, proper labelling of the specimen and safe transportation of the sample including proper storage and temperature (2°C– 8°C) to the laboratory and this was guided by standard operating procedures. Clinical and demographic data for each study participant was obtained by using a standardized questionnaire and patients' medical records. The use of a standard questionnaire is said to be reliable because all participants were subjected to the same questionnaire when evaluating the factors associated with ear infection.
Study size	5	The sample size was estimated by using the Kish Leslie formula (1965) using the prevalence of 62% obtained from a study done in Tanzania. $N=\frac{Z^2P(1-P)}{D^2}$ Whereby Z=standard deviation of the normal distribution = 1.96 (confidence level at 95%) P=prevalence 62.1% (5) D=Margin of error 6% N=251 The minimum required sample size was 251 participants with ear infections.
Quantitative variables	8	Quantitative variables were summarized by calculating measures such as mean, median standard deviation, and quartiles to summarize the central tendency and dispersion of the data
Results		
Descriptive data	9-10	(a) Two hundred fifty-five participants were recruited; 52.5% (134/255) were males. The median age was 31 years (IQR: 15- 49). The majority (30.2%) of participants were students, 32.9% had a college education, and 15.7% were from outside Dar es Salaam region (Table 1). The median duration of ear infections was 210 days (IQR: 21-1095). Otitis externa (OE) was the most common type of ear infection, accounting for 45.1% (115/255), followed by Chronic Suppurative Otitis Media (CSOM) (41.2%). Around 49% of the participants with ear infections had a history of antibiotic use, whereby ciprofloxacin ear drop was the most prescribed topical antibiotic. Additionally, 33.3% of the study participants had nasal congestion/blockage/discharge, and 28.2% had recurrent URTI
		(b) No participant with missing variable
Outcome data	10-12	In this study, 136 out of 255 (53.3%) participants had a positive aerobic culture for either bacterial or fungal pathogen. A total of 150 isolates (bacteria and fungi) were identified, of which 87.3% (131/150) were bacteria. Of the isolated bacteria, Gram-negative, 71.0% (93/131) were predominant. The predominant bacterial isolates were *S. aureus,* 27.5% (36/131), followed by *Pseudomonas aeruginosa*, 24.4% (32/131). On the other hand, *Candida spp* accounted for 63.2% (12/19) of the isolated fungi (data not shown). Further stratification of isolated pathogens by type of ear infection showed that *S. aureus* 16/131 (12.2%) was the most prevalent bacterium in OE patients, whereas *P.*

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		aeruginosa 22/131 (16.8%) predominated in CSOM patients In the present study 34.4% (21/61) of the *Enterobacterales*, excluding *Pseudomonas aeruginosa*, were extended-spectrum beta-lactamase producing *Enterobacterales* (ESBL-PE); and *Klebsiella spp* was predominant, accounting for 33.3% (7/21) of the ESBL-PE isolates. On the other hand, 44.4% (16/36) of the *S.aureus* species were MRSA Almost all (93%) isolated Enterobacterales were resistant to amoxicillin/clavulanic acid, more so *E. coli* and *Acinetobacter spp* were 100% resistant. Also, 73% of isolated bacteria were resistant to ceftazidime, whereby *Pseudomonas aeruginosa* had the highest resistance rate of 75%. In addition, 43% of isolated bacteria were resistant to trimethoprim-sulfamethoxazole, whereby *E.coli* was leading with a 75% resistance rate.
Discussion		
Key results		53.3% of research participants reported positive bacteria cultures. *S. aureus* (27.3%) and *P.aeruginosa* (24.2%) were the most frequently isolated bacteria, whereas *Candida spp*. 12(63.8%), and *Aspergillus spp*. 9(36.2%) were the only isolated fungi. 73% of the identified Enterobacterales were resistant to ceftazidime, while 93% were resistant to amoxicillin/clavulanic acid. Furthermore, we identified 44.4% methicillin-resistant *Staphylococcus aureus* (MRSA) and 34.4% extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-PE)
Limitations	14-15	1. The present study was not able to identify the fungi isolates to species level. This is due to insufficient funding and availability of resources. To mitigate this all fungi isolates were stored appropriately for future testing to specie level. 2. Due to financial constraints and lack of equipment, it was also not possible to isolate anaerobic bacteria from the collected pus specimen
Interpretation	12-14	According to the study many ear infections are of bacterial etiology, *S. aureus* and *Pseudomonas aeruginosa* are the most commonly isolated bacteria. The majority of isolated bacteria were resistant to amoxicillin/clavulanic acid, sulfamethoxazole-trimethoprim, Erythromycin and third-generation cephalosporins. The most effective antibiotics were ciprofloxacin and meropenem. The findings suggest that ear infection management procedures and the choice of an effective antibiotic need to be reviewed. The results are consistent with research conducted in Tanzania by Kennedy M. et al. (2019) in Morogoro, Zephania A. et al. (2019) in Dar es Salaam, Martha M. et al. (2016) in Mwanza, as well as research from Kenya and India. The current study revealed a high proportion of MRSA (44.4%) and ESBL-PE (34.4%). ESBL-PE and MRSA isolates were resistant to the most common antimicrobial agents compared to non-MRSA and non-ESBL-PE. In addition, our study showed Klebsiella spp (33.3%) as the dominant ESBL-PE. The higher proportion of MRSA and ESBL-PE coincide with other studies in Tanzania, Kenya, Ethiopia, India, Egypt, and Romania. The greater inclination for self- and empirically prescribing antibiotics without considering laboratory culture and sensitivity may explain the higher proportion of ESBL and MRSA. The high incidence of ESBL and MRSA can also be explained by an increased inclination for people to visit hospital facilities due to chronic ear infection, which raises the danger of exposure to MDR bacteria. Additionally, the tendency to use inanimate

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		objects to remove earwax can be attributed to the increased proportion of ESBL and MRSA, as these inanimate objects are often found in environments that may be contaminated with ESBL-producing bacteria and/or MRSA Ciprofloxacin is a drug of choice for ear infections as per standard treatment guidelines in our setting. The fact that meropenem is infrequently used to treat ear infections may explain the high sensitivity rate. Surprisingly, we observed that ciprofloxacin is still effective despite being prescribed often in our setting for treating ear infections. There is no clinical rationale as to why quinolones are still more effective in treating ear infections, but these results assure that quinolones are still beneficial as first-line topical antibiotics for ear infections.
Generalisability		According to the study's findings, bacteria are the most common etiological factor in ear infections. Additionally, our results indicate that a significant percentage of ear infections are caused by ESBL-PE and MRSA. Therefore, identifying multidrug-resistant bacteria is essential to enhancing the management of ear infections. The study has also identified patterns of antimicrobial susceptibility that are helpful in guiding the selection of empirical treatment in environments with limited resources and comparable geographic, demographic, and social characteristics.
Other information		
Funding	15	No funding was received for this study.

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