Guteta *et al. BMC Microbiology* (2025) 25:6
https://doi.org/10.1186/s12866-024-03664-z

BMC Microbiology

RESEARCH

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Bacterial etiologies, antimicrobial susceptibility profiles and associated factors among patients with otitis media referred to Nekemte Public Health Research and Referral Laboratory Center, Western Ethiopia: A cross-sectional study

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Abstract

Background Otitis media is among the leading causes of illnesses responsible for causing hearing problems and adding significant costs to the public health system. Bacteria are the most common causative agents for otitis media. Currently, there is little information on the prevalence and antimicrobial susceptibility patterns of pathogenic bacterial isolates from patients with otitis media in Ethiopia.

Methodology A laboratory – based cross-sectional study was conducted from June to September 2023 among 242 patients with otitis media referred to Nekemte Public Health Research and Referral Laboratory Center. Sociodemographic and clinical data were obtained by trained nurses and/or health officers in face-to-face interviews using structured questionnaires. Middle ear discharge samples were collected by Ear, Nose and Throat (ENT) specialists (Otolaryngologist) following all aseptic techniques. Conventional culture, different biochemical tests and antimicrobial susceptibility testing were performed for all the isolated bacteria. Reference strains were used as a positive and negative controls. The data were checked for completeness and consistency, entered into EpiData version 4.6.06 and analyzed by SPSS version 25. Logistic regression analysis was performed to determine the associated factors of otitis media. Adjusted odds ratio was used to determine strength of association. Statistical significance was obtained at p-value of below 0.05. The data were interpreted using graphs, tables, and results statements.

Results A total of 242 middle ear discharge samples were collected and cultured from which 212 (87.6%) were culture positive. A total of 228 pathogenic bacterial isolates were recovered. The predominant bacterial isolates were

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S. aureus 92 (40.4%) followed by *P. aeruginosa* 33 (14.5%) and *E. coli* 24 (10.5%). One hundred and fifty-one (66.2%) bacterial pathogens were multidrug resistant. Piperacillin-tazobactam and tobramycin were relatively common drugs to which most of the isolates were susceptible while they were most resistant to ampicillin and tetracycline. Purulent discharge (p-value = 0.001), middle ear discharge ≥ 14 days (P-value = 0.000) and a history of active/passive smoking (P-value = 0.043) were significantly associated with otitis media.

Conclusion The prevalence of bacterial pathogens, most of which were multidrug-resistant, was high among patients with otitis media. A significant association was observed with purulent ear discharge, chronic otitis media, and passive or active smoking. Choosing the proper antibiotic for the treatment of bacterial infection is crucial.

Keywords Otitis media, Bacteria, Antimicrobial resistance

Background

Otitis media (OM) is a common inflammatory illness that can affect people of all ages and results in temporary or permanent hearing loss due to fluid effusion or pathological alterations in the tympanic membrane of the middle ear [1]. The spectrum of illnesses associated with OM ranges from acute to chronic and is clinically indicated by the presence of fluid accumulation in the middle ear that can cause temporary hearing loss [2].

Basically, OM is classified as acute, otitis media with effusion (OME), chronic (COM) and chronic suppurative (CSOM) based on duration, presence of infection and fluid type. Acute otitis media (AOM) is middle ear inflammation characterized by fluid accumulation with symptoms of otalgia, irritability, or fever lasting for two weeks. Chronic otitis media is long-term inflammation of the middle ear, which can include a variety of conditions characterized by recurring or persistent ear infections and inflammation. In OME, glue-like fluid is found in the middle ear behind an intact tympanic membrane and there is no signs and symptoms of an acute infection. In addition, persistent, chronic infection with ear discharge through a perforated tympanic membrane for more than six weeks is the characteristics of CSOM [3–5].

Chronic otitis media (COM) is further typed as mucosal and squamous diseases. In mucosal COM, pars tensa (eardrum) is permanently perforated. This is most likely the result of either recurrent acute otitis media in conjunction with or in isolation from chronic Eustachian tube dysfunction. On the other hand, chronic squamosal otitis media is distinguished by the existence of a retraction pocket in the tympanic membrane's pars flaccida (flaccid portion of tympanic membrane. This condition involves the prolonged presence of squamous epithelium in the middle ear and is associated with infection, inflammation, and potentially destructive changes in the ear structures [6, 7].

Young children are more vulnerable to OM than adults. Globally, over 80% of children aged below 3 years were affected by OM. Otitis media is primarily the cause of frequent antibiotic use and impaired quality of life among children. They place a heavy financial and social strain on families as well as the healthcare system. Approximately 21,000 deaths are attributed to complications from OM, and the combined yearly incidence of AOM and CSOM is estimated to be 740 million worldwide [8]. The peak prevalence of OM among children is mainly attributed to immature immune status, the anatomy of the Eustachian tube (shorter and horizontal), frequent exposure to URTIs and malnutrition [9, 10].

Otitis media is increasingly prevalent in Sub-Saharan Africa and other developing nations including Ethiopia. Due to limited microbiological laboratories, Sub-Saharan African nations rely solely on clinical data for therapy which could be responsible for more complicated OM [11]. Bacteria are the most common agents for OM. The most predominant pathogenic bacteria isolated from AOM included *M. catarrhalis*, *S. pneumoniae* and *H. influenzae*. Chronic suppurative otitis media (CSOM) is a permanent tympanic membrane rupture that causes recurring or persistent otorrhea over the course of two to three weeks and long-lasting middle ear inflammation. *P. aeruginosa*, *Klebsiella species*, *Proteus species*, *S. aureus* and *E. coli* are the predominant bacteria isolated from patients with CSOM [12].

The extensive application of antibiotics to treat OM has led to the growth of resistant bacteria, including strains that are resistant to many drugs [13]. Several studies have reported considerable resistance rates of bacteria isolated from middle ear. To ensure the best possible care and fight antibiotic resistance, it is essential to continuously and periodically evaluate the microbiological profile and antimicrobial susceptibility [14, 15].

In Ethiopia, few studies have investigated bacteriological profiles and antimicrobial susceptibility patterns of the middle ear discharge. The number of antibiotic-resistant bacteria is alarming and becoming a major public health problem in the management of patients with middle ear infection [16–18]. There are no data on the prevalence and antimicrobial susceptibility patterns of bacterial pathogens from middle ear infections in referred health facilities. This on the other hand might indicate that clinicians base their treatment on empirical evidence. Knowing the local antibiogram is important for cost-effective and appropriate treatment of otitis media and helps prevent complications that may arise due to the lack of treatment or improper treatment. Thus, the aim of this study was to acquire data on bacterial pathogens responsible for otitis media and their antibacterial susceptibility patterns among patients with otitis media referred to the Nekemte Public Health Research and Referral Laboratory Center for culture tests.

Methods

Study area, period and design

This study was conducted at Nekemte Public Health Research and Referral Laboratory Center among referred patients with otitis media from nearby health facilities from June to September 2023. A laboratory-based cross-sectional study was conducted in 242 patients with otitis media.

Study participants and sampling technique

Patients with middle ear discharge accompanied by laboratory-appropriate request form, those who were not receiving antibiotic and those who had evidence of discharging ears were included in the study. Participants were selected through convenience sampling. Middle ear discharges were collected ant transported to microbiology laboratory.

Data collection, sample processing and laboratory analysis

Sociodemographic and clinical data were collected in face-to-face interviews from study participants by nurses/health officers using structured questionnaires. Middle ear discharges were collected by ENT specialists. The collected discharge samples were inoculated on blood agar, chocolate agar and MacConkey agar following standard bacteriological techniques. Bacterial pathogens from positive culture results were identified by their characteristic appearance on the respective media, gram-staining reaction and pattern of biochemical profiles using standard procedures.

The antibiotic susceptibility all the isolated bacterial pathogens was tested via Kirby-Bauer disc diffusion method. Inocula were prepared by transferring 3–5 colonies from pure culture into 5 ml of normal saline and mixing thoroughly to make a homogenous suspension equivalent to the 0.5 McFarland standard. Using a sterile cotton swab, the bacterial suspension was distributed evenly over the entire surface of MHA (HIMEDIA, India) and left at room temperature for 15 min. The plates were then incubated at 37 °C for 16–18 h and observed for the zones of inhibition. The growth inhibition zone was measured by a ruler, and results were interpreted as whether the organism was sensitive, intermediate or resistant to the antimicrobial agents by comparison with standard guidelines based on the Clinical and Laboratory Standards Institute (CLSI, 2023) [19].

Data quality assurance

Different methods were used for assuring data quality. A standard and structured questionnaire prepared in English was used. The questionnaire was then translated to the local language (Afan Oromo) for data collection and then re-translated back into English for analysis. One day of training was given to the data collectors and supervisors on the data collection tool and procedures. To ensure validity, 5% of the questionnaire was pretested. The findings from the pretesting were utilized for modifying and adjusting of the instrument and interviewing technique. The data collectors were supervised closely by the supervisors and the principal investigator. The completeness of each questionnaire was checked daily by the principal investigator and the supervisors. To ensure consistency, coding, double entry and cleaning were performed. The entire data collection process was guided by the principal investigator.

The specimen containers were properly labeled with patient identifier, codes, collector initial and collection date and time. Then, the collected specimens were immediately transported to the clinical microbiology laboratory for processing according to existing SOPs. All patient information was checked for clarity and completeness. Media and all relevant reagents were carefully inspected and checked for expiration dates prior to use. The sterility of the prepared culture media was checked by incubating 5% of the batch at 35–37 °C overnight and evaluating it for possible contamination.

Trained laboratory experts performed the tests. All testing procedures were performed depending on the existing SOPs of the clinical microbiology laboratory. *S. aureus* (ATCC 25923) was used for checking the functionality of blood agar and chocolate agar, and *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used for MacConkey and biochemical tests. The performance of MHA was also checked by *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853).

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed using the Kirby–Bauer disk diffusion method. Antibiotic discs were selected based on the prescription pattern in the study area and recommendations from the Clinical Laboratory Standards Institute (CLSI, 2023). The grades of the susceptibility profile were read as sensitive (S), intermediate (I), or resistant (R) by comparison of the zone of inhibition with clinical and laboratory standards institute guidelines 33rd edition [19]. The following antibiotic disks were used for susceptibility testing: Ceftriaxone

Table 1 Age, sex and residence distribution of patients with otitis media referred to Nekemte Public Health Research and Referral Laboratory Center, Nekemte, June-September 2023

Variable		Frequency	Percentage
Age	< 5	91	37.6
	5–14	34	14.0
	15–24	44	18.2
	≥ 25	73	30.2
Sex	Male	129	53.3
	Female	113	46.7
Residence	Urban	165	68.2
	Rural	77	31.8

(30 μg), Cefoxitin (30 μg), Ceftazidime (30 μg), Penicillin G (10 μg), Ampicillin (10 μg), Amoxicillin-clavulanate (20/10μg), Piperacillin-tazobactam (100/10 μg), Meropenem (10 μg), Gentamicin (10 μg), Tobramycin (10 μg), Ciprofloxacin (5 μg), Azithromycin (15 μg), Clindamycin (30 μg), Vancomycin (30 μg), Tetracycline (30 μg), Chloramphenicol (30 μg) and Trimethoprim sulfamethoxazole (1.25/23.75).

Statistical analysis

The collected data were coded, entered into Epi-data version 4.6.0.6 software and then cleaned. From this software, they were exported to SPSS version 25 for analysis. Descriptive statistics were calculated to describe relevant variables. The data were presented in words, figures, and tables. Binary logistic regression analysis was used to select candidate variables for multivariable logistic regression analysis. Variables with P-values < 0.25 were candidates for multivariate analysis. The adjusted odds ratio (AOR) was used to determine the strength of the association. A P-value < 0.05 was considered to indicate statistical significance.

Results

Characteristics of the study participants

A total of 242 middle ear discharge samples were collected from the study participants and analyzed. Males and females accounted for 129 (53.3%) and 113 (46.7%) of the participants, respectively. The age of the participants ranged from 1 to 65 years, with mean and median ages of 17.1 and 14.0 years, respectively. Ninety-one (37.6%) of them were aged less than five years, while 73 (30.2%) were aged 25 years and older. One hundred sixty-five (68.2%) and 77 (31.8%) of the participants were from urban and rural areas, respectively (Table 1).

Prevalence of bacterial pathogens

Two hundred twelve middle ear discharge samples were positive for culture resulting in an overall 87.6% prevalence of bacterial isolates. Among cultured middle ear discharges, 196 (92.5%) yielded double growth while 16 (7.5%) showed single (mono) growth. A total of 228 bacterial pathogens were recovered from positive cultures constituting (107, 46.9%) Gram-positive and (121, 53.1%) Gram-negative bacteria. From the total bacterial isolates, *S. aureus* (92, 40.4%) and *P. aeruginosa* (33, 14.5%) were the predominant bacterial species followed by *E. coli* (24, 10.5%) and *K. pneumoniae* (16, 7.0%) (Table 2).

Table 2 Bacterial etiologic agents among patients with otitis media referred to Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, June-September 2023

Bacterial isolates		Frequency	Percentage
Gram – positive	*S. aureus*	92	40.4
	CONS	10	4.4
	S. pneumoniae	4	1.8
	Enterococcus spp.	1	0.4
Gram – negative	*E. coli*	24	10.5
	P. aeruginosa	33	14.5
	K. pneumonia	16	7.0
	Providencia spp.	13	5.7
	P. mirabilis	11	4.8
	P. vulgaris	11	4.8
	Acinetobacter spp.	6	2.6
	Citrobacter spp.	4	1.8
	K. oxytoca	3	1.3
Total		**228**	**100**

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed for both Gram-positive ($n=107$) and Gram-negative ($n=121$) bacterial pathogens isolated from the study participants. The predominant Gram-positive isolate, *S. aureus* showed the highest level of resistance to penicillin (86, 93.5%) followed by cefoxitin (81, 88.0%) but gentamicin (72, 78.3%) and clindamycin (74, 80.4%), the two antibiotics to which *S. aureus* is susceptible. It was found that 81 (88.0%) of the *S. aureus* isolates were MRSA (Table 3).

Twenty-three (95.8%), 32 (97%), 16 (100%), and 12 (92.3%) *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *Providencia species*, respectively, demonstrated susceptibility to piperacillin-tazobactam. Nine (27.3%) *P. aeruginosa* strains exhibited resistance to meropenem. The majority of 22 (91.7%) of *E. coli* isolates were resistant to ampicillin (Table 4).

Multidrug resistance

The overall prevalence of MDR bacteria in this study was 151 (66.2%). Among the Gram-positive and Gram-negative isolates, 91 (39.9%) and 60 (26.3%) were multidrug resistant, respectively. The predominant bacterial isolates were *S. aureus* (82, 54.3%), *E. coli* (20, 13.2%) and *K. pneumoniae* (11, 7.3%) (Table 5).

Table 3 Antimicrobial resistance patterns of Gram-positive bacterial isolates from patients with otitis media referred to Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, June- September 2023

Antibiotics tested	Number of resistant bacteria [*n* (%)]				
	S. aureus	CONS	S. pneumoniae	Enterococcusspp.	Total
	92	10	4	1	107
Ampicillin	NT	NT	NT	0 (0.0)	0 (0.0)
Azithromycin	41 (44.6)	7 (70.0)	0 (0.0)	NT	48 (44.9)
Cefoxitin	81 (88.0)	8 (80.0)	NT	NT	89 (83.2)
Chloramphenicol	29 (31.5)	5 (50.0)	NT	0 (0.0)	34 (31.8)
Ciprofloxacin	41 (44.6)	3 (30.0)	NT	NT	44 (41.1)
Clindamycin	17 (18.5)	3 (30.0)	0 (0.0)	NT	20 (18.7)
Cotrimoxazole	47 (51.1)	8 (80.0)	0 (0.0)	NT	55 (51.4)
Gentamicin	17 (18.5)	0 (0.0)	NT	NT	17 (15.9)
Penicillin	86 (93.5)	8 (80.0)	NT	0 (0.0)	94 (87.9)
Tetracycline	71 (77.2)	9 (90.0)	2 (50.0)	NT	82 (76.7)

CONS – coagulase negative staphylococci, NT – not tested

Possible risk factors for otitis media

Both bivariate and multivariate logistic regression analyses were performed to assess the possible risk factors for middle ear infection. Statistical significance was obtained for purulent middle ear discharge, duration of middle ear discharge ≥ 14 days and history of active/passive smoking. Study participants with purulent middle ear discharge were approximately six times more likely to have positive cultures for bacterial pathogens responsible for causing otitis media [AOR = 6.534 (95% CI: 2.112–20.208; p-value = 0.001)]. On the other hand, participants who experienced otorrhea of ≥14 days are approximately seven times more likely to have positive cultures than were those who experienced otorrhea of < 14 days [7.628 (95% CI = 3.135–18.558; p-value = 0.000)]. Those who had an active or passive smoking history were approximately eight times more likely to develop otitis media than to those without a history of active or passive smoking [8.817 (95% CI = 1.072–72.534; p-value = 0.043)] (Table 6).

Discussion

Otitis media is a major reason people seek medical attention globally, and its complications play a significant role in the development of preventable hearing loss, particularly in developing nations [20]. According to the present study OM was found to be a common health problem at all ages. According to the present study, OM was found a common health problem in all ages. However, a peak prevalence of 31.0% was observed among children under five years of age, which was similar to that reported in other studies in Ethiopia (28.1%) [21], higher than that reported in India (14.9%) [22] but lower than that study performed in Yemen (66.7%) [23]. This prevalence of OM in children is mainly attributed to immature immune status, the anatomy of the Eustachian tube (shorter and horizontal), frequent exposure to URTIs and malnutrition.

Gender-wise analysis of this study showed that males were more affected than females were. This finding, with male predominance, is in agreement with a study performed in Wollo, Ethiopia (50.4%) [16] and Pakistan (43.9%) [24] but lower than a study performed in India (56.7%) [25]. In contrast, other studies in Iran (57.8%) and Iraq (60.0%) [26, 27] showed that females were more affected by otitis media than males were. The differences in male and female predominance may be attributed to the nature of the sampling technique.

This study also provides insight into the prevalence of otitis media with respect to the residential location of study participants. The prevalence of otitis media among study participants from urban areas was 58.3%. This finding agrees with a study performed in Mekele, Ethiopia (52.0%) [28] but is lower than that in a study performed in Gondar, Ethiopia (76.5%) [29]. However, the present study disagrees with a study performed in China [30] in which 85.9% of rural areas were positive for OM. These disparities may come from the involvement of study participants from urban areas due to increased health seeking-behavior, proximity to health facilities and culture diagnostic services in the study areas.

In the present study, the overall middle ear discharge culture positivity rate was 87.6% (95% CI = 82.8–91.5). This finding is similar to that of study performed in Dessie, Ethiopia, which reported 89.4% [31] but it was higher than that of studies done in Gondar (76.7%) and Bahir Dar (80.4%) [17, 32]. In contrast, the current culture positivity rate was lower than that studies performed in Ghana (97%) and India (95.7%) [33, 34], which may be related to variations in the availability of isolation and identification media. This may be correlated with the fact that availability of relevant media and other supplies maximizes the frequency of culture positivity in middle ear discharge.

Table 4 Antimicrobial resistance patterns of Gram-negative bacterial isolates from patients with otitis media referred to Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, June-September 2023

Bacterial isolates	Antibiotics tested											
(*n* = 121)	AMC	AP	CAZ	CHL	CIP	COT	CRO	GEN	MEM	PTZ	TOB	TTC
P. aeruginosa *n* = 33	NT	NT	12 (36.4)	NT	13 (39.4)	NT	NT	NT	9 (27.3)	1 (3.0)	13 (9.1)	NT
E. coli *n* = 24	3 (12.5)	22 (91.7)	7 (29.2)	8 (33.3)	12 (50.0)	13 (54.2)	17 (70.8)	3 (12.5)	12 (50.0)	1 (4.2)	4 (16.7)	21 (87.5)
K. pneumoniae *n* = 16	2 (12.5)	NT	8 (50.0)	4 (25.0)	6 (37.5)	7 (43.8)	8 (50.0)	1 (6.3)	7 (43.8)	0 (0.0)	1 (6.3)	11 (68.8)
Providencia spp. *n* = 13	NT	NT	2 (15.4)	4 (30.8)	4 (30.8)	9 (69.2)	7 (53.8)	2 (15.4)	9 (69.2)	1 (7.7)	2 (15.4)	NT
P. mirabilis *n* = 11	1 (9.1)	11 (100)	4 (36.4)	5 (45.5)	3 (27.3)	4 (36.4)	6 (54.5)	3 (27.3)	6 (54.5)	0 (0.0)	3 (27.3	NT
P. vulgaris *n* = 11	1 (9.1)	NT	3 (27.3)	1 (9.1)	5 (45.5)	6 (54.5	8 (72.7)	0 (0.0)	7 (63.6)	0 (0.0)	0 (0.0)	NT
Acinetobacter spp. *n* = 6	NT	NT	1 (16.7)	NT	1 (16.7)	5 (83.3)	1 (16.7)	0 (0.0)	2 (33.3)	2 (33.3)	0 (0.0)	NT
Citrobacter spp. *n* = 4	NT	NT	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (50.0)	0 (0.0)	2 (50.0)	0 (0.0)	0 (0.0)	3 (75.0)
K. oxytoca *n* = 3	0 (0.0)	NT	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)	1 (33.3)	1 (33.3)

AMC = Amoxicillin – clavulanate AP = Ampicillin CAZ = Ceftazidime CHL = Chloramphenicol CIP = Ciprofloxacin COT = Cotrimoxazole CRO = Ceftriaxone GEN = Gentamicin MEM = Meropenem PTZ = Piperacillin-tazobactam TOB = Tobramycin TTC = Tetracycline NT = Not tested

Table 5 Multidrug resistance patterns of Gram-positive and Gram-negative bacterial isolates from patients with otitis media referred to Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, June-September 2023

Bacteria isolates	Antimicrobial susceptibility test results, No (%)				Total MDR
	R0	R1	R2	≥R3	
Gram-positive isolates					
S. aureus (*n* = 92)	0 (0.0)	5 (5.4)	5 (5.4)	82 (89.1)	82 (76.6)
CONS (*n* = 10)	0 (0.0)	1 (10.0)	0 (0.0)	9 (90.0)	9 (8.4)
S. pneumoniae (*n* = 4)	3 (75)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)
Enterococcus spp. (*n* = 1)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total (*n* = 107)	**4 (3.7)**	**7 (6.5)**	**5 (4.7)**	**91 (85.1)**	**91 (85.1)**
Gram-negative isolates					
P. aeruginosa (n = 33)	6 (18.2)	11 (33.3)	11 (33.3)	5 (15.2)	5 (4.1)
E. coli (n = 24)	0 (0.0)	2 (8.3)	2 (8.3)	20 (83.3)	20 (16.5)
K. pneumoniae (n = 16)	1 (6.3)	0 (0.0)	4 (25.0)	11 (68.8)	11 (9.1)
Providencia spp. *(n = 13)*	2 (15.4)	2 (15.4)	2 (15.4)	7 (53.8)	7 (5.8)
P. mirabilis (n = 11)	0 (0.0)	0 (0.0)	3 (27.3)	8 (72.7)	8 (6.6)
P. vulgaris (n = 11)	1 (9.1)	1 (9.1)	3 (27.3)	6 (54.5)	6 (5.0)
Acinetobacter spp. *(n = 6)*	1 (16.7)	1 (16.7)	2 (33.3)	2 (33.3)	2 (1.7)
Citrobacter spp. *(n = 4)*	4 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
K. oxytoca (n = 3)	1 (33.3)	1 (33.3)	0 (0.0)	1 (33.3)	1 (0.8)
Total (*n* = 121)	**16 (13.2)**	**18 (14.9)**	**27 (22.3)**	**60 (49.6)**	**60 (49.6)**

R0 - ≥R3 refers to the number of pathogenic bacterial isolates resistant to 0 - ≥3 different antibiotics

Table 6 Multivariate analyses to identify associated factors among study participants with otitis media referred to Nekemte Public Health Research and Referral Laboratory Center, Nekemte, June – September 2023

Variables		Culture Result, No (%)		COR (95% CI)	AOR (95% CI)	*P*-value
		Positive	Negative			
Age group	< 5	75 (31.0)	16 (6.6)	0.272 (0.087–0.853)	0.436 (0.116–1.641)	0.220
	5–14	28 (11.6)	6 (2.5)	0.271 (0.071–1.032)	0.524 (0.112–2.455)	0.412
	15–24	40 (16.5)	4 (1.7)	0.580 (0.137–2.446)	0.672 (0.132–3.408)	0.631
	≥ 25	69 (28.5)	4 (1.7)	**Reference**	**Reference**	
Residence	Rural	71 (29.3)	6 (2.5)	2.014 (0.788–5.151)	0.923 (0.305–2.791)	0.880
	Urban	141 (58.3)	24 (9.9)	**Reference**	**Reference**	
Ear involved	Right	114 (47.1)	20 (8.3)	**Reference**	**Reference**	
	Left	98 (40.5)	10 (4.1)	1.719 (0.768–3.848)	1.219 (0.470–3.162)	0.683
Appearance of middle ear discharge	Bloody	17 (7.0)	9 (3.7)	**Reference**	**Reference**	
	Purulent	157 (64.9)	10 (4.1)	8.312 (2.967–23.288)	**6.534 (2.112–20.208)**	**0.001***
	Mucoid	38 (15.7)	11 (4.5)	1.829 (0.640–5.228)	1.398 (0.427–4.580)	0.580
Duration of middle ear discharge	< 14 days	34 (14.0)	18 (7.4)	**Reference**	**Reference**	
	≥ 14 days	178 (73.6)	12 (5.0)	7.853 (3.468–17.783)	**7.628 (3.135–18.558)**	**0.000***
History of smoking	No	164 (67.8)	29 (12.0)	**Reference**	**Reference**	
	Yes	48 (19.8)	1 (0.4)	8.488 (1.127–63.936)	**8.817 (1.072–72.534)**	**0.043***
Treatment history for otitis media	No	122 (50.4)	21 (8.7)	**Reference**	**Reference**	
	Yes	90 (37.2)	9 (3.7)	1.721 (0.753–3.936)	0.895 (0.354–2.736)	0.976

*Associated factors for otitis media

CI = Confidence interval, COR = Crude odds ratio, AOR = Adjusted odds ratio

A total of 228 bacterial isolates were identified. Analysis of the Gram reactions of the isolates revealed that 53.1% [95% CI (46.6–59.5)] and 46.9% [95% CI (40.5–53.4%)] were Gram-negative and Gram-positive bacterial pathogens, respectively. Another study in Ethiopia reported 56.0% Gram-negative bacteria as the predominant species isolated from middle ear discharge, which was consistent with the results of the present study [28]. Reports from Somalia, Nigeria and Malaysia agree with the predominance of Gram-negative bacteria, with higher frequencies of 77.3%, 71.6% and 75.3%, respectively [35–37]. The reason for the higher prevalence could be the chronic nature of infection, where Gram-negative bacteria from external sources gain access to the auditory canal and eventually become predominant.

The predominant bacterial isolates in this study were *S. aureus* (40.5%) and *P. aeruginosa* (14.5%) which was similar to the findings of studies in Ethiopia [38], Pakistan [39] and China [40] but inconsistent with the findings of other studies in Ethiopia [16], India [41] and Turkey [42]. In addition, the isolation rates of Coagulase negative staphylococci, *S. pneumoniae* and *Enterococcus species* were 4.4%, 1.8% and 0.4%, respectively. At least one of these bacterial isolates has also been reported in other studies [28, 29, 32, 43–45]. This study revealed that Gram-negative bacterial isolates included *E. coli* (19.8%), *K. pneumoniae* (13.2%), *Providencia species* (10.7%), *P. mirabilis* (9.1%), *P. vulgaris* (9.1%), *Acinetobacter species* (5.0%), *Citrobacter species* (3.3%) and *K. oxytoca* (2.5%). Other studies from Ethiopia and other countries have also reported these bacteria at varying frequencies [11, 16, 21, 29, 36, 46]. Variations in climate and geography are possible reasons for the differences in the distribution of bacterial isolates [32].

In the present study, 93.5% of the *S. aureus* isolates resistant to penicillin whereas clindamycin and gentamicin were the two drugs to which 80.4% and 78.3% of isolates were susceptible, respectively. This finding is in agreement with other studies [32, 35]. On the other hand, *P. aeruginosa* demonstrated susceptibility patterns ranging from 33.3 to 97% with piperacillin-tazobactam being the most effective antibiotic. Studies from other parts of Ethiopia used different antibiotics and reported varying susceptibility patterns [28, 38]. Studies from other parts of the world tested different antibiotics with varying susceptibility patterns [33, 39, 47] with one of the studies [33] that tested piperacillin-tazobactam, ciprofloxacin and ceftazidime, which were also tested in this study. The use of different antibiotics in different studies is attributed to the occasional emergence of resistant strains from time to time, the availability of proposed antibiotics and local prescribing practices.

The overall MDR rate in this study was 66.2% [95% CI (59.7–72.3%)]. This finding is similar to those of studies performed in Ethiopia, which reported MDR rates of 67.0% [18] and 61.5% [48], but lower than those of another study that reported rates of 88.3% [29], however; these rates are higher than those of a study performed in India [46]. Variations in the reports of MDR rates might be attributed to differences in operational definitions of MDR strains, bacterial isolates and antibiotic discs tested.

There were statistically significant differences in culture positivity between those who were with purulent middle ear discharge compared to those who presented with other middle ear discharge appearances [p-value=0.001, AOR=6.534 (95% CI: 2.112–20.208)]. In contrast, a study done in Jimma reported that there was no significant association between purulent discharge and culture positivity [18], but one study from Iraq revealed a statistically significant association between middle ear discharge culture positivity and purulent discharge [47].

A significant difference was also observed between a middle ear discharge duration of ≥14 days and culture positivity [p-value=0.000, AOR=7.628 (95% CI: 3.135–18.558)], which was also supported by other studies [28, 29]. Chronic otitis is characterized by middle ear discharge that lasts for at least 14 days and a perforated tympanic membrane. Another risk factor for otitis media was a history of smoking in either an active or passive state. A significant association was observed between patients with history of smoking and the development of otitis media [p-value=0.043, AOR=8.817 (95% CI: 1.072–72.534)]. This association was also supported by other studies [18, 48, 49]. Smoking decreases the mucociliary activity of the respiratory epithelium, depresses local immune function, and enhances the adhesion of bacteria to the respiratory epithelium [49].

Strengths and limitations of the study

The strength of the study was that all the laboratory procedures were conducted following standard operating procedures. In addition, further studies can be built upon these findings, as there are no published data regarding the problem in the study area. The temporal relation between the exposure and outcome variables could not be established because the study design was cross-sectional in its nature. The small sample size and convenience sampling nature of this study prevented it from being representative of patients with otitis media in Ethiopia. On the other hand, anaerobic culture methods for fastidious bacteria and molecular techniques were not employed.

Conclusion

This study was conducted at Nekemte Public Health Research and Referral Laboratory Center. The present study indicated that bacterial middle ear infection has become an increasing health problem coupled with levels of multidrug resistance. *S. aureus* and *P. aeruginosa* were the leading causes for middle ear infection. There is an increase in the number of antibiotic-resistant bacteria recovered from patients with otitis media in the study area, and these bacteria are becoming a major public health problem in the management of patients with middle ear infection.

Abbreviations

AOM	Acute Otitis Media
AOR	Adjusted Odds Ratio
ATCC	American Type Culture Collection
CI	Confidence Interval
CLSI	Clinical Laboratory Standards Institute
CSOM	Chronic Suppurative Otitis Media
MDR	Multidrug Resistance
MHA	Muller Hinton Agar

OM	Otitis Media
SOP	Standard Operating Procedure
SPSS	Statistical Software for Social Sciences
URTI	Upper Respiratory Tract Infection

Acknowledgements
The authors are grateful to Salale University and the Oromia Health Bureau for providing opportunity to conduct this research. We also acknowledge Nekemte Public Health Research and Referral Laboratory Center for providing the laboratory setup, chemicals and reagents. Our deepest gratitude also goes to the study participants, staff members of the Clinical Microbiology Department of Nekemte Public Health Research and Referral Laboratory Center, referring health facilities and data collectors and supervisors. We would also sincerely thank the study participants for their participation in the study.

Author contributions
ET had a substantial contribution from conception to the acquisition of data. All the authors had a great contribution to study design, analysis, and interpretation of the findings. ET and BS were involved in laboratory analysis. ET and FA drafted the manuscript. All authors revised the paper carefully for important intellectual contents. All authors read and approved the final manuscript.

Funding
Partially funded by Oromia Health Bureau but does not have grant number. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability
All relevant data are within this paper.

Declarations

Ethics approval and consent to participate
The research proposal was ethically cleared by the Salale University Institutional Review Board (Reference Number: S/U-IRB/25/23). An official permission letter was obtained from Nekemte Public Health Research and Referral Laboratory Center. Participants were informed of the purpose of the study, risks associated with the study, confidentiality of personal data, and their right to take part in the study. After that, we obtained a written informed consent from adult study participants, whereas an assent was obtained from study participants less than 18 years of age. In addition, a consent was also obtained from their parents or legal guardians to participate in this study. Finally, specimens were collected from all study participants and analyzed accordingly. Laboratory results of study participants were communicated with their respective physicians for better management.

Competing interests
The authors declare no competing interests.

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Received: 21 May 2024 / Accepted: 19 November 2024
Published online: 07 January 2025

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