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ORIGINAL RESEARCH

Bacterial Etiologies of Ear Infection and Their Antimicrobial Susceptibility Pattern at the University of Gondar Comprehensive Specialized Hospital, Gondar, Northwest Ethiopia: A Six-Year Retrospective Study

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Background: An ear infection is responsible for up to 40% of preventable hearing impairment; one of the reasons for frequent and unwise antibiotic usage, especially in the developing world. Since the incidence of antibiotic resistance is increasing, especially in resource-limited countries, up-to-date knowledge on the susceptibility of ear-discharge isolates to antibiotic is important for better patient treatment. Therefore, this study aimed at determining the bacterial etiologies and their antibiotic susceptibility profiles among patients suspected with ear infections.

Methods: We collected retrospective data from bacteriological results of ear discharge samples from 2013 to 2018. Sample collection, culture preparation, and bacterial identification were performed using standard microbiological techniques. Antimicrobial susceptibility testing was performed following Clinical and Laboratory Standard Institute (CLSI) guidelines. We extracted and inputted the data using Epi-info version 7 and exported it to SPSS version 20 for analysis.

Results: The overall ear-discharge culture positivity rate was 283/369 (76.7%) (95% CI = 72.4–81.3), with 14/283 (4.95%) mixed infections. *Staphylococcus aureus* (27.9%), *Proteus* spps (20.8%), *Streptococcus* spps (10%), and *Pseudomonas* spps (8.92%) were the main isolates. High-level resistance rates for tetracycline (77.6%), penicillins (67.2%), erythromycin (52.6%), and co-trimoxazole (52%), and low-level resistance rates for fluoroquinolones (23.3%), aminoglycosides (23.7%), and cephalosporins (29.8%) were observed. More than 45% of isolates, with 50.9% of Gram-negative and 37.3% of Gram-positive, were multidrug-resistant.

Conclusion: *Staphylococcus aureus, Proteus mirabilis, Proteus vulgaris, Escherichia coli*, and *Pseudomonas aeruginosa* were the leading cause of ear infections. The presence of high number of multidrug-resistant strains calls for the need for periodic and continuous follow-up of antibiotic usage in the study area. Further studies are recommended to explore the types of ear infections, with their etiologic agents and possible risk factors.

Keywords: ear infection, bacterial etiologies, antibiotic susceptibility, Gondar, Ethiopia

Background

Worldwide, there are more than 360 million people with disabling hearing loss. Over 60% of this hearing loss could be preventable, and infection is responsible for up to 40% of this preventable hearing loss.[1–6] Ear infection can occur in the outer (Otitis Externa (OE)), middle (Otitis Media (OM)), or inner (Otitis Interna (OI)) parts of the ear.[7–9] Otitis

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media or inflammation of the middle ear is the most common and significant disease in the world; it can be suppurative, acute OM (AOM) and chronic OM (COM), or non-suppurative, OM with effusion (OME).[10–12] Acute otitis media (AOM), COM, and OME are common problems affecting many peoples, especially young children.[13–15] Acute otitis media (AOM) is the presence of fluid along with a rapid onset of signs and symptoms of inflammation in the middle ear.[13,16] Otitis media with effusion (OME) is the presence of ear effusion in the absence of acute infection[14] whereas, COM is defined when ear effusion (fluid) remains in the middle ear for a long time.[15] The etiologies of ear infections can be bacteria, viruses, or fungi. However, bacteria such as *Pseudomonas aeruginosa* (*P. aeruginosa), Staphylococcus aureus* (*S. aureus), Streptococcus pneumoniae* (*S. pneumoniae), Streptococcus pyogenes (S. pyogenes), Haemophilus influenzae* (*H. influenzae*), and *Proteus* species are predominantly responsible for the infection.[8,17,18]

Upper respiratory tract infection, previous history of AOM, low social status, passive smoker, poor nutrition, and others can be risk factors for OM.[19] Untreated AOM can also be a risk factor for COM, eardrum perforation, facial nerve paralysis, meningitis, or mastoiditis.[20]

Antibiotics can help treat bilateral AOM and both AOM and discharging ear among children aged < 2 years of age. Even though health care practitioners use antibiotics to treat children with AOM, they are not usually the answer to treat the condition.[21,22] In addition, in low-income countries, most antibiotics are not ordered based on results from culture and antibiotic susceptibility testing (AST). As a result, misuse or overuse of antimicrobial agents, which facilitates the emergence of antimicrobial resistance, is prevalent in these areas.[23,24] Furthermore, most health practitioners in these areas are not aware of the antibiotic-resistant pattern in their health facilities.[25] Thus, ensuring proper patient care is impossible if clinicians do not practice appropriate antibiotic use based on the AST results and halt the continuous emergence of resistant bacteria due to antibiotic misuse or overuse.[26]

In Ethiopia, prospective and retrospective studies on the bacterial etiologies of ear infection and their antibiotic susceptibility pattern (ASP) are present.[27–31] Those studies reported that *S. aureus, P. aeruginosa, Proteus* species, and other lactose fermenter Gram-negative bacteria (GNB) are the predominant ear discharge isolates. Moreover, a significant number of isolates reported by these studies were multidrug resistant (MDR), which is defined as acquired non-susceptibility to at least one antibiotic in three or more antimicrobial classes.[32] Despite these studies in different parts of Ethiopia, there is still limited published data on the epidemiology of bacterial ear infections and their ASP in the study area.

Materials and Methods

Study Design, Area, and Period

A hospital-based retrospective study was conducted from 1st Jan 2013 to 30th Dec 2018 at the University of Gondar Comprehensive Specialized Hospital (UoGCSH), Northwest Ethiopia. This hospital is the only specialized hospitals in Gondar town and one of the biggest teaching hospitals in the Amhara region. It provides inpatient and outpatient services for more than 5 million populations.

Study Participants

The study participants were all patients suspected of ear infections and whose ear-discharge samples were provided and analyzed in the bacteriology laboratory of the UoGCSH during the study period.

Data Collection

We did a retrospective review of six-year laboratory records of all ear swabs taken from patients suspected of having ear infections from all departments and units of the UoGCSH. We collected demographic characteristics of patients (age and sex), type of patient, diagnosis year, isolated organisms, and AST results from the laboratory record books using data abstraction form.

Laboratory Method

Ear discharge was collected under a strict aseptic technique using a sterile cotton swab from each patient. Within 2 hrs of collection, swabs were transported to the microbiology laboratory of UoGCSH for culture and AST. The specimens were directly inoculated on Blood agar, Chocolate agar, and MacConkey agar (OXOID, UK). MacConkey and Blood agar plates were incubated in aerobic conditions and Chocolate agar plates in a candle jar. All the inoculated media were incubated at 37°C for 18–24 hr. The identification of bacterial isolates by Gram staining, colony morphology, pigment production, or type of haemolysis on Blood agar follows a standard procedure.[33] Moreover, the UoGCSH bacteriology laboratory used Catalase production, Coagulase production, Optochin sensitivity, and Bacitracin sensitivity tests for GPB and

Oxidase production, Triple Sugar Iron utilization, Citrate utilization, Urease production, Motility, and Indole production tests for GNB. Antibiotic susceptibility testing was carried out using Kirby-Bauer Disc-diffusion technique[34] on Muller Hinton agar (OXOID, UK) following the CLSI guidelines (2012–2017). The bacterial suspension was standardized using 0.5 McFarland standard and inoculated on Mueller–Hinton agar. The antibiotic discs were dispensed after drying the plate for 3–5 min and incubated at 37°C for 24 hrs. Penicillin G (10IU), Cefoxitin (30µg), Ampicillin (10µg), Amoxicillin (10µg), Erythromycin (15µg), Clindamycin (2µg), Cotrimoxazole (25µg), Oxacillin (1µg), Co-amoxiclav (20/10µg), Cefuroxime (30µg), Ceftriaxone (30µg), Gentamicin (10µg), Tobramycin (10µg), Ciprofloxacin (5µg), Norfloxacin (10µg), Meropenem (10µg), Tetracycline (30µg), Nalidixic acid (30µg), Vancomycin (30µg), Amikacin (30µg), and Chloramphenicol (30µg) were used to test the susceptibility of bacterial isolates. The control strains *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853) were used for quality control.[35]

Data Analysis and Interpretation

Data were entered and analyzed using SPSS version 20 software, and results were presented using tables. The Chi-square test was applied to compare the proportions of ear-discharge isolates with patient characteristics. A p-value < 0.05 was considered as a statistically significant association.

Results

Over the six years, 369 ear-discharge samples were collected and then tested in the UoGCSH microbiology laboratory. The male to female ratio was 1.71:1, with 233 (63.1%) were male patients. The mean age of the study subjects was 17.46 years (±15.26 SD), with 105 (28.45%) were below the age of 5 years. Most of the ear discharge samples, 282 (76.4%), were from outpatient wards. Most of the samples, 122 (33.1%), were tested in 2013, followed by in 2014 (25.2%) and 2015 (18.2%). Overall, 283 (76.7% (95% CI = 72.4–81.3)) of the samples were culture positive, with 14 (4.95% (95% CI: 2.8–7.7)) were with mixed bacteria (data not shown). The culture positivity rate was slightly higher among patients with age groups of 25–29, females, and inpatients than other respective groups. Higher culture positivity rate was also observed in 2016 (88.2%), followed by 2013 (86.1%), and 2014 (73.1%) (Table 1).

There were about 269 ear-discharge samples with single bacterial isolates. Gram-negative bacteria 159 (59.1%) were isolated predominantly comprised to GPB 110 (40.9%). The predominant bacterial isolate from ear discharge samples was *S. aureus* 75 (27.88%), followed by *P. mirabilis* 28 (10.4%), *P. vulgaris* 23 (8.55%), *E. coli* 20 (7.43%), and *P. aeruginosa* 19 (7.06%) (Table 2).

Between 2013 and 2018, the UoGCSH microbiology laboratory used ten antibiotic classes and 18 individual antibiotics to identify the ASP of bacteria isolated from ear discharge samples. Tables 3 and 4 show the ASP of GPB and GNB, respectively. The overall resistance rate in GPB was 46.3%, with a range of 5.7% to 65.6%. More than half of Gram-positive isolates were resistant to Tetracycline (65.6%), Co-trimoxazole (54.1%), Erythromycin (52.6%), and Penicillin groups (50%). However, most of them were sensitive to Vancomycin (94.3%), Gentamicin (83.7%), Clindamycin (77.8%), Quinolones (77.1%), Chloramphenicol (74.4%), and Cephalosporins (71.1%) drugs (Table 3).

The overall resistance rate in GNB was 49.1%, with a range of 12.5% to 90.9%. There was a high level of resistance rate in the Tetracycline group (86.36%), Penicillin group (79%), Clindamycin (70%), Chloramphenicol (64.6%), and Co-trimoxazole (50.8%) among Gram-negative isolates. However, these isolates were relatively sensitive to quinolones (76.5%), Aminoglycosides (73.9%), Cephalosporins (69.7%), and Meropenem (66.7%) drugs (Table 4).

From the total 269 bacterial isolates tested against common antibiotics, 123 (45.72%) were MDR. Gram-negative isolates 81 (50.9%) showed a higher MDR rate than Gram-positive isolates 42 (38.2%). The range of MDR rate among isolates is between 0–72.7%, with 69.57%, 57.9%, 55%, 42.6%, and 41.3% of *P. vulgaris, P. aeruginosa, E. coli, P. mirabilis*, and *S. aureus* isolates were MDR, respectively. More than 26%, 23%, 21%, and 19% of the isolates were resistant to at least one antibiotic in three, one, two, and five and more antimicrobial classes, respectively (Table 5).

Discussion

Worldwide, over 430 million people (~ 5% of the world's population) living with disabling hearing loss, which affects the quality of life of individuals. The prevalence of hearing loss is higher in developing countries, with

Table 1 The Distribution of Ear Infection in Relation to Age, Sex, Ward, and Diagnosis Year of Patients at the UoGCSH from Jan 2013 to Dec 2018

Variables		Frequency	Culture Result		Chi-Square
			Positive	Negative	
Age group	< 5	105 (28.45)	77 (73.3)	28 (26.7)	0.538
	5–14	74 (20.05)	57 (77.0)	17 (23.0)	
	15–24	80 (21.68)	64 (80.0)	16 (20.0)	
	25–34	67 (18.16)	56 (83.6)	11 (16.4)	
	35–44	18 (4.87)	12 (66.7)	6 (33.3)	
	45–54	10 (2.71)	7 (66.7)	3 (33.3)	
	> 54	15 (4.07)	10 (66.7)	5 (33.3)	
Sex	Male	233 (63.1)	177 (76.0)	56 (24.0)	0.703
	Female	136 (36.9)	106 (80.0)	30 (20.0)	
Ward	OPD	282 (76.4)	215 (76.2)	67 (23.8)	0.773
	IPD	87 (23.6)	68 (78.2)	19 (21.8)	
Diagnosis year	2013	122 (33.1)	105 (86.1)	17 (13.9)	0.007
	2014	93 (25.2)	68 (73.1)	25 (26.9)	
	2015	67 (18.2)	45 (67.2)	22 (32.8)	
	2016	34 (9.2)	30 (88.2)	4 (11.8)	
	2017	24 (6.5)	16 (66.7)	8 (33.3)	
	2018	29 (7.85)	19 (65.5)	10 (34.5)	
Total		369 (100)	283 (76.7)	86 (23.3)	

Table 2 Frequencies of Bacteria Isolated from Patients with Ear Infection at the UoGCSH from Jan 2013 to Dec 2018

Bacterial Isolates		Frequency	Percentage
Gram-positive bacteria	*Staphylococcus aureus*	75	27.88
	Streptococcus pyogenes	12	4.46
	Coagulase -ve *staphylococci*	8	2.97
	Streptococcus pneumoniae	5	1.86
	Viridans streptococci	5	1.86
	Other *streptococcus* species	5	1.86
	Total	**110**	**40.9**
Gram-negative bacteria	*Proteus mirabilis*	28	10.4
	Proteus vulgaris	23	8.55
	Escherichia coli	20	7.43
	Pseudomonas aeruginosa	19	7.06
	Klebsiella pneumoniae	11	4.09
	Citrobacter species	15	5.58
	Other LF G-ve rods	11	4.09
	Other NLF G-ve rods	11	4.09
	Other *Proteus* species	5	1.86
	Other *Pseudomonas* species	5	1.86
	Salmonella species	5	1.86
	Other *Klebsiella* species	4	1.49
	Haemophilus influenzae	2	0.74
	Total	**159**	**59.1**
Grand total		**269**	**100**

Abbreviations: LF, lactose fermenter; NLF, non-lactose fermenter; G-ve, Gram-negative.

Table 3 Antimicrobial Susceptibility Pattern of GPB Isolated from Patients with Ear Infection at the UoGCSH from Jan 2013 to Dec 2018

Drug (Tested)	*S. aureus*		*S. pyrogens*		*S. pneumoniae*		*V. streptococci*		Other *Streptococcus* spps		CoNS		Total (%)	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
PEN (53)	11	23	4	2	4	0	1	1	2	1	3	1	25 (47.2)	28 (52.8)
AMP (31)	6	11	4	2	1	1	2	1	1	1	0	1	14 (45.2)	17 (54.8)
AMX (22)	7	7	2	1	1	1	–	–	2	0	1	0	13 (59.1)	9 (40.9)
OXA (20)	9	9	–	–	0	1	0	1	–	–	–	–	9(45)	11 (55)
AUG (8)	4	2	1	0	–	–	–	–	1	0	–	–	6 (75)	2 (25)
CRO (48)	22	9	6	0	1	0	3	1	1	2	2	1	35 (72.9)	13 (27.1)
CRX (19)	10	6	–	–	–	–	–	–	0	1	2	0	12 (63.2)	7 (36.8)
CTX (16)	6	3	2	0	2	0	–	–	1	0	1	1	12 (75)	4 (25)
CIP (52)	29	8	6	0	–	–	2	1	1	1	3	1	41 (78.8)	11 (21.2)
NOR (31)	14	5	5	2	1	0	1	0	0	1	2	0	23 (74.9)	8 (25.8)
GEN (43)	21	4	6	0	2	1	3	2	–	–	4	0	36 (83.7)	7 (16.3)
TET (64)	16	29	0	6	3	1	2	2	1	1	0	3	22 (34.4)	42 (65.6)
ERY (78)	25	30	7	3	1	1	0	4	0	2	4	1	37 (47.4)	41 (52.6)
VAN (70)	43	4	10	0	4	0	2	0	2	0	5	0	66 (94.3)	4 (5.7)
SXT (37)	11	15	1	2	1	2	2	0	–	–	2	1	17 (45.9)	20 (54.1)
CAF (39)	18	9	3	0	5	0	1	0	1	1	1	0	29 (74.4)	10 (25.6)
CLI (36)	22	6	3	0	–	–	–	–	–	–	3	2	28 (77.8)	8 (22.2)
Overall DSP (%)	**274 (60.4)**	**180 (39.6)**	**60 (76.9)**	**18 (23.1)**	**26 (76.5)**	**8 (23.5)**	**19 (59.4)**	**13 (40.6)**	**13 (54.2)**	**11 (45.8)**	**33 (73.3)**	**12 (26.7)**	**425 (63.7)**	**242 (46.3)**

Abbreviations: PEN, penicillin; AMP, ampicillin; AMX, amoxicillin; OXA, oxacillin; AUG, augmentin; CRO, ceftriaxone; CTX, cefoxitin; CRX, cefuroxime; CIP, ciprofloxacin; NOR, norfloxacin; GEN, gentamicin; TET, tetracycline; ERY, erythromycin; VAN, vancomycin; SXT, co-trimoxazole; CAF, chloramphenicol; CLI, clindamycin; CoNS, coagulase-negative *staphylococci*; DSP, drug-susceptibility pattern.

middle ear disease (one of the common causes of hearing impairment) has the highest incidence.[1,36,37] As a result, reporting bacterial etiologies of ear disease and their AST result is vital to prevent the multi-dimensional effect of the ear infection and guide the empirical treatment in the low-resource areas.

In the study area, ear discharge from patients suspected with ear infections is one of the routinely ordered specimens for microbiological analysis. This retrospective study showed that 76.7% (95% CI = 72.4–81.3) of the ear discharge samples were culture positive, with 4.95% of them had mixed isolates. This result is consistent with results reported in Ethiopia, including 80.4% in Bahir-Dar (2013–2015, 38) and 75.6% in Hawassa (a cross-sectional study).[28] However, it was lower than reported from other parts of Ethiopia, such as Gondar (2009–2012) (89.5%),[29] Dessie (2001–2011) (83.6%),[39] and Mikelle (98.2%).[27] Our result was slightly higher than reported from Bangladesh (70.8%).[40] The differences in culture positivity rate might be affected by the types of study design and study participants used in the study.

In this retrospective analysis, GNB accounted for 59.1% of bacterial ear infections, and *S. aureus* (27.88%), *Proteus* spp. (20.82%), *Streptococcus* spp. (10.4%), and *Pseudomonas* spp. (8.92%) were predominant isolates. A previous retrospective study (2009–2012) from the same study area also reported GNB (56.4%) as the leading cause of ear infections, and *Proteus* species (27.5%), followed by *S. aureus* (26.5%) were the predominant isolate.[29] Similar to our finding, previously reported data from other parts of Ethiopia also reported these isolates as the main etiologies of bacterial ear infections.[27,31,39] A review article in Sub-Saharan Africa also reported similar bacterial isolates as the cause of OM.[18] Furthermore, a recently published article from a tertiary care hospital in Bangladesh reported both GNB (55%) and GPB (45%) as etiologies of bacterial ear infection, with *S. aureus* (37%) and *Pseudomonas* species (31.5%) were the leading isolates.[40] Different literatures also mentioned *S. aureus, P. aeruginosa, Proteus* species as the most common isolates from bacterial ear infection.[41–46] Since the natural habitats of most of these bacteria can be skin, environment and soil, ear infection, particularly OE from these isolates[42,46] is usually common.

Table 4 Antimicrobial Susceptibility Pattern of GNB Isolated from Patients with Ear Infection at the UoGCSH from Jan 2013 to Dec 2018

Drug (Tested)	*P. mirabilis*		*P. vulgaris*		*E. coli*		*P. aeruginosa*		*Citrobacter* spps		*K. pneumoniae*		Other NLF G-ve Rods		Other LF G-ve Rods	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
AMP (119)	5	17	2	14	2	13	1	14	0	12	0	8	1	6	4	4
AMX (44)	4	5	1	6	0	2	0	5	0	3	3	0	3	2	3	1
AUG (32)	3	3	2	1	0	3	0	3	0	6	0	2	0	2	0	2
CRO (95)	13	6	9	2	11	2	11	1	6	3	3	1	4	2	6	2
CTX (25)	3	5	4	0	1	2	0	1	2	1	-	-	0	1	-	-
CRX (22)	4	1	2	2	2	0	0	3	-	-	1	1	-	-	2	0
CIP (102)	13	4	12	0	12	4	11	1	11	1	6	2	5	1	6	0
NOR (52)	8	0	8	3	5	2	5	0	2	1	4	0	4	0	3	2
NAL (33)	0	3	4	8	1	4	1	2	2	0	1	0	1	1	0	2
GEN (120)	17	8	14	4	12	3	13	2	8	4	5	1	2	4	5	1
TOB (8)	3	1	-	-	-	-	-	-	1	0	1	0	-	-	-	-
AMK (6)	0	1	-	-	1	0	1	0	-	-	1	0	-	-	-	-
TET (77)	2	11	1	10	3	10	1	11	2	6	1	1	0	7	1	3
DOX (11)	-	-	0	1	0	2	-	-	0	1	-	-	1	3	0	1
CAF (79)	8	2	2	14	1	7	2	8	3	4	3	2	3	3	3	1
SXT (61)	6	5	5	6	2	6	1	5	4	1	3	3	2	3	1	1
CLI (10)	0	1	3	2	0	2	-	-	-	-	-	-	-	-	0	2
MER (6)	0	2	-	-	-	-	-	-	1	0	1	0	-	-	-	-
Overall DSP (%)	**89 (54.3)**	**75 (45.7)**	**69 (48.6)**	**73 (51.4)**	**53 (46.1)**	**62 (53.9)**	**47 (45.6)**	**56 (54.4)**	**42 (49.4)**	**43 (50.6)**	**33 (61.1)**	**21 (38.9)**	**26 (42.6)**	**35 (57.4)**	**34 (60.7)**	**22 (39.3)**

Drug (Tested)	Other *Klebsiella* spps		Other *Proteus* spps		Other *Pseudomonas* spps		*Salmonella* spps		*H. influenzae*		Total N (%)	
	S	R	S	R	S	R	S	R	S	R	S	R
AMP (119)	0	4	1	4	0	1	3	2	0	1	19(16	100 (84)
AMX (44)	0	1	0	2	0	3	-	-	-	-	14 (31.8)	30 (68.2)
AUG (32)	2	1	-	-	0	1	1	0	-	-	8(25)	24 (75)
CRO (95)	2	0	3	1	1	1	2	1	2	0	73 (76.8)	22 (23.2)
CTX (25)	1	0	1	0	0	1	1	0	1	0	14(56)	11(44)
CRX (22)	-	-	0	2	0	1	1	0	-	-	12 (54.5)	10 (45.5)
CIP (102)	2	0	4	1	2	0	2	1	1	0	87 (85.3)	15 (14.7)
NOR (52)	1	0	2	1	-	-	1	0	-	-	43 (82.7)	9 (17.3)
NAL (33)	-	-	-	-	2	0	1	0	-	-	13 (39.4)	20 (60.6)
GEN (120)	2	2	4	1	3	0	3	2	-	-	88 (73.3)	32 (26.7)
TOB (8)	-	-	-	-	2	0	-	-	-	-	7 (87.5)	1 (12.5)
AMK (6)	-	-	-	-	1	0	-	-	-	-	4 (66.7)	2 (33.3)
TET (77)	0	1	0	2	0	3	0	1	-	-	11 (14.3)	66 (85.7)
DOX (11)	-	-	-	-	0	2	-	-	-	-	1 (9.1)	10 (90.9)
CAF (79)	0	3	0	1	0	3	1	3	2	0	28 (35.4)	51 (64.6)
SXT (61)	1	1	1	0	1	0	1	0	2	0	30 (49.2)	31 (50.8)
CLI (10)	-	-	-	-	-	-	-	-	-	-	3(30)	7 (70)
MER (6)	-	-	-	-	2	0	-	-	-	-	4 (66.7)	2 (33.3)
Overall DSP (%)	**11 (45.8)**	**13 (54.2)**	**16 (51.6)**	**15 (48.4)**	**14 (46.7)**	**16 (53.3)**	**17 (63)**	**10(37)**	**8 (88.9)**	**1 (11.1)**	**459 (50.9)**	**443 (49.1)**

Abbreviations: AMP, ampicillin; AMX, amoxicillin; AUG, augmentin; CRO, ceftriaxone; CTX, cefoxitin; CRX, cefuroxime; CIP, ciprofloxacin; NOR, norfloxacin; NAL, nalidixic-acid; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; TET, tetracycline; DOX, doxycycline; CAF, chloramphenicol; SXT, co-trimoxazole; CLI, clindamycin; MER, meropenem; NLF, non-lactose fermenter; LF, lactose fermenter; DSP, drug-susceptibility pattern.

Over the six-year (2013–2018) period, the UoGCSH bacteriology laboratory used more than ten antibiotic classes to test the susceptibility of ear-discharge isolates. We observed a higher overall resistance rate in Tetracycline (77.63%), Penicillins (67.2%), Co-trimoxazole (52.04%), Chloramphenicol (51.7%), and Erythromycin (52.6%). However, there was a lower degree of resistance rate in Fluoroquinolones (23.33%),

Table 5 Multidrug Resistance Patterns of Bacteria Isolated from Patients with Ear Infection at the UoGCSH from Jan 2013 to Dec 2018

Bacterial Isolates		Antibiotic Resistance (%)							MDR (%)
		R0	R1	R2	R3	R4	R5	R6	
GPB	*S. aureus* (n =75)	9 (12)	17 (16)	18 (24)	16 (21.3)	8 (10.67)	5 (6.67)	2 (2.67)	31 (41.3)
	CoNS (n = 8)	1 (12.5)	4 (50)	1 (12.5)	2 (25)	–	–	–	2 (25)
	S. pyogenes (n = 12)	3 (25)	4 (33.3)	2 (16.67)	3 (25)	–	–	–	3 (25)
	S. pneumoniae (n = 5)	–	4 (80)	1 (20)	–	–	–	–	0 (0.0)
	V. streptococci (n = 5)	–	1 (20)	1 (20)	2 (40)	1 (20)	–	–	3 (60)
	Other *strep* spps (n = 5)	1 (20)	–	1 (20)	2 (40)	1 (20)	–	–	3 (60)
	Total (n = 110)	**14 (12.7)**	**30 (27.3)**	**24 (21.8)**	**25 (22.7)**	**10 (9.1)**	**5 (4.5)**	**2 (1.8)**	**42 (38.2)**
GNB	*P. mirabilis* (n = 28)	1 (3.57)	6 (21.43)	9 (32.14)	7 (25)	4 (14.3)	1 (3.57)	–	12 (42.86)
	P. vulgaris (n = 23)	1 (4.35)	3 (13)	3 (13)	8 (34.78)	4 (17.4)	4 (17.4)	–	16 (69.57)
	Other *Proteus* spps (n = 5)	–	1 (20)	2 (40)	–	1 (20)	1 (20)	–	2 (40)
	P. aeruginosa (n = 19)	–	2 (10.53)	6 (31.58)	9 (47.37)	2 (10.53)	–	–	11 (57.9)
	Other *Pseudomonas* spps (n = 5)	–	1 (20)	1 (20)	2 (40)	1 (20)	–	–	3 (60)
	E. coli (n =20)	2 (10)	3 (15)	4 (20)	5 (25)	3 (15)	2 (10)	1 (5)	11 (55)
	K. pneumoniae (n = 11)	2 (18.2)	4 (36.36)	2 (18.2)	2 (18.2)	–	1 (9.1)	–	3 (27.3)
	Other *Klebsiella* spps (n = 4)	–	–	2 (50)	1 (25)	1 (25)	–	–	2 (50)
	Citrobacter spps (n = 15)	–	5 (33.3)	3 (20)	4 (26.67)	1 (6.67)	1 (6.67)	1 (6.67)	7 (46.7)
	Other LF G–ve rods (n = 11)	2 (18.2)	4 (36.36)	1 (9.1)	2 (18.2)	1 (9.1)	1 (9.1)	–	4 (36.4)
	Other NLF G–ve rods (n = 11)	–	1 (9.1)	2 (18.2)	6 (54.55)	1 (9.1)	1 (9.1)	–	8 (72.7)
	Salmonella spps (n = 5)	1 (20)	2 (40)	–	–	2 (40)	–	–	2 (40)
	H. influenzae (n = 2)	1 (50)	1 (50)	–	–	–	–	–	0 (0.0)
	Total (n = 159)	**10 (6.3)**	**33 (20.8)**	**35 (22)**	**46 (28.9)**	**21 (13.2)**	**12 (7.5)**	**2 (1.3)**	**81 (50.9)**
Grand total (269)		**24 (8.9)**	**63 (23.4)**	**59 (21.9)**	**71 (26.4)**	**31 (11.5)**	**17 (6.3)**	**4 (1.5)**	**123 (45.72)**

Notes: R0, R1, R2, R3, R4, R5, and R6 stands for resistance of the isolates for none, one, two, three, four, five, and six antibiotic classes tested in this study, respectively. MDR = ≥R3.

Abbreviations: CoNS, coagulase negative Staphylococci; LF, lactose fermenter; NLF, non-lactose fermenter; G-ve, Gram-negative; MDR, multidrug resistant.

Aminoglycosides (23.73%), and Cephalosporins (29.78%). Studies from different parts of Ethiopia[28,38,39,47,48] also reported that most of the ear-discharge isolates were resistant to drugs in the Penicillin, Tetracycline, and Macrolide class. However, they reported that drugs in the Fluoroquinolones, Aminoglycosides, and Cephalosporins were better in treating ear-discharge bacterial isolates. The acquisition of genetic elements carrying resistance genes, mutations within the drug binding site, chromosomal mutation, production of inactivating enzymes, or efflux pumps contribute to the resistance of antibiotics, including Tetracycline, Penicillins, Co-trimoxazole, and Chloramphenicol.[49–51]

The most effective antibiotics for GPB were Vancomycin (94.3%), Gentamicin (83.7%), Clindamycin (77.8%), Fluoroquinolones (77%), Chloramphenicol (74.4%), and Cephalosporins (71%), and the most effective antibiotics for GNB were antibiotics in the Fluoroquinolones class (Ciprofloxacin (85.3%) and Norfloxacin (82.7%)), Ceftriaxone (76.8%), Aminoglycosides (73.8%), and Meropenem (66.7%). In this study, *S. aureus* was more sensitive to Vancomycin (91.5%), Gentamicin (84%), Clindamycin (78.6%), and Fluoroquinolones (76.8%) than other antibiotics used in this study. More than 62% of *P. mirabilis* and *P. vulgaris* were sensitive to Fluoroquinolones, Gentamicin, and Cephalosporins. Moreover, the antibiotic sensitivity result of *P. aeruginosa* showed that Gentamicin (86.7%), Fluoroquinolones (85%), and Cephalosporins (68.7%) were more sensitive than other antibiotics used in this study. These results were supported by reports from different parts of Ethiopia.

In this study, frequently isolated bacteria, including *S. aureus, P. mirabilis, P. vulgaris*, and *P. aeruginosa* showed a high level of resistance to Penicillins (58.4%, 80.8%, 67.6%, and 95.65%, respectively) and Tetracyclines (64.4%, 91.7%, 84.6%, and 91.7%, respectively). These results suggest that the UoGCSH should avoid using antibiotics in the Penicillins and Tetracyclines class unless they are supported with

evidence that they are sensitive to the ear-discharge bacterial isolates. Ear-discharge isolates resistant to beta-lactam antibiotics, including Penicillins and Tetracyclines was also reported in other parts of Ethiopia.[27,38,39]

In this study, the overall MDR rate among ear-discharge bacterial isolates was 45.72% (123/269) (95% CI = 40.5–51.4%). This was consistent with the study conducted in Bahir Dar, where MDR prevalence was reported as 43%.[52] In this study, 50.9% and 38.2% of Gram-negative and Gram-positive isolates were MDR, respectively. Worldwide, there is an increasing population of MDR bacteria, which is becoming a serious concern for healthcare professionals and the population at large. Due to different economic and social-related factors, the burden of AMR is greater in the developing countries, where lack of antimicrobial stewardship program, limited diagnostic facilities, inadequate patient educations, or non-human use of antimicrobials are prevalent.[53–55] The higher MDR rate in GNB might be due to extended-spectrum β-lactamase (ESβL) and carbapenemase enzyme in isolates. This claim was supported by a previous report, where 54.2% and 12.5% of the Gram-negative isolates from the UoGCSH were ESβL and carbapenemase producers.[56] According to a recent meta-analysis report, an estimated 48.9% of Gram-negative (Enterobacteriaceae) clinical isolates in Ethiopia are ESβL producers, most of them are classified as MDR because they usually carry genes encoding resistance to antibiotics other than beta-lactams.[57]

Limitation

Because of the limited patient details recorded on the laboratory logbook, it was impossible to include data related to clinical and other diagnosis information. Since, the types of ear infection (OM, OE, OI, or others) were not indicated in the laboratory logbook, we could not compare the association between bacterial isolates with the types of ear infection.

Conclusion and Recommendations

This retrospective study revealed that about two-thirds of the ear discharge samples were bacterial culture positive, which implies that bacterial ear infection is one of the health problems in the study area. *S. aureus, P. mirabilis, P. vulgaris, E. coli*, and *P. aeruginosa* were the predominant bacteria isolated from patients suspected of ear infections in the study area. Fluoroquinolones, Aminoglycosides, and Cephalosporins were effective against most of the ear-discharge isolates. However, more than half of the isolates had a high resistance level against Tetracycline, Penicillins, Co-trimoxazole, Chloramphenicol, and Erythromycin. This study also indicated that more than 45% of ear-discharge isolates were MDR. Therefore, periodic and continuous follow-up of antibiotic usage at the UoGCSH is necessary. For successful patient management and prevention of the emergence of MDR bacteria, treating bacterial ear infections based on culture and AST results in the study area is advisable.

Abbreviations

AOM, Acute Otitis Media; AMP, Antimicrobial Susceptibility Pattern; AST, Antimicrobial Susceptibility Testing; ASP, Antimicrobial Susceptibility pattern; CLSI, Clinical and Laboratory Standard Institute; GNB, Gram-negative bacteria; GPB, Gram-positive bacteria; MDR, Multidrug resistant; OI, Otitis Interna; OM, Otitis Media; COM, Chronic Otitis Media; OME, Otitis Media with Effusion; UoG, University of Gondar; UoGCSH, University of Gondar Comprehensive Specialized Hospital.

Data Sharing Statement

All data generated or analyzed during this study were included in this article. Data that support the findings of this study are also available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

Before the commencement of the study, we obtained ethical clearance (Ref. No/SBMLS/2123/2019; 22 Feb 2019) from the UoG, School of Biomedical and Laboratory Sciences ethical review committee, and an official letter of co-operations was provided to UoGCSH. Before data collection, we explained the study objective to the heads of the hospital director and bacteriology laboratory personnel. Since we used secondary data for this study, we didn't require the patient's informed consent. We conducted the study following the Declaration of Helsinki.[58] To ensure confidentiality of information from participant's record, we didn't record any personal identifiers on the data collection sheet, and secured data from participant records were not available to anyone except for the investigators.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no competing interests in this work.

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