

Bacteriology and Antimicrobial Susceptibility of Otitis Media at Dessie Regional Health Research Laboratory, Ethiopia

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Abstract

Background: Otitis media is a major health problem of children in low income countries.

Objectives: This study was done to determine the bacterial isolates and antimicrobial susceptibility of otitis media from children and adults.

Methods: A retrospective record was analyzed on culture results of discharging ears at Dessie Regional Health Research Laboratory in the period 2003-2010. Antimicrobial susceptibility tests were performed using disc diffusion technique as per the standard Kirby-Bauer method.

Results: Out of 897 discharging ear samples, 469 were from children and 428 from adults. Of these, 823 (91.7%) had aerobic bacteria isolated. The most frequent isolates were *Proteus* spp. 223 (26.5%), *S. aureus* 203 (24.6%), *Pseudomonas* spp. 148 (18%) and *E. coli* 146 (17.7%). *Proteus* spp. were the most common isolates in children compared to adults ($p=0.001$). Norfloxacin, ciprofloxacin and gentamicin revealed high level of sensitivity whereas high resistance rates were observed for amoxicillin, tetracycline and erythromycin ($p=0.001$). Antibiograms of isolates showed that 598 (72.6%) of isolates were resistant to two and more antimicrobials.

Conclusion: Otitis media linked with high levels of multiple antibiotic resistant bacteria is a major health concern in all age groups of the study population. There is a need for culture and susceptibility test facilities for appropriate antimicrobial therapy of otitis media and antimicrobial resistant infections. [*Ethiop. J. Health Dev.* 2011;25(2):161-167]

Introduction

Otitis media is an inflammation of the middle ear and mastoid process which could be acute purulent otitis media, otitis media with effusion and chronic suppurative otitis media (1). Worldwide, 65-330 million people suffer from otitis media; of these, about 60% experience significant hearing impairment (2). Otitis media is more common in children, as their eustachian tube is shorter, more horizontal with a more flaccid cartilage which can impair its opening (3). Otitis media is a major health problem of children in developing countries with poor socioeconomic status (2). According to World Health Organization (WHO) survey, countries are clustered into those having louse otitis media when a prevalence rate of otitis media among children is between 1-2% and high when it is 3-6% and Ethiopia belongs to the latter category (4).

In chronic otitis media, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Escherichia coli* found in the skin of the external ear enter into the middle ear through a chronic perforation (5). The frequency of such bacterial isolates could vary in different geographical areas (6, 7). Increased antimicrobial resistant bacteria in chronic otitis media can lead to the development of complications of chronic otitis media like meningitis and brain abscess (8). Moreover, chronic otitis media may lead to cholesteatomas, a tumor in the middle ear as a complication (4, 9, 10). Studies have indicated that

otomycosis, which results from prolonged bacterial treatment of chronic otitis media or primarily by fungal species can complicate the management of chronic otitis media (11, 12).

The etiologies of chronic otitis media differ in geographical area (5, 6). Moreover, antimicrobial resistance profile of bacteria varies among population because of difference in geography, local antimicrobial prescribing practices and prevalence of resistant bacterial strains (10). Thus, up to date information on microbial resistance needs to be available at national and local levels to guide the rational use of the existing antimicrobials.

In Ethiopia, although studies on bacteriology and microbial susceptibility of otitis media have been conducted in children (13-15), no recent data have been reported from adults particularly in the study area. This study was undertaken to determine bacterial pathogens and their antimicrobial resistance profile from children and adult discharging ears obtained in Dessie Regional Health Research Laboratory.

Methods

Study design: A retrospective review of culture results of discharging ears performed in the period 2003 to 2010 at Dessie Regional Health Research Laboratory was made in January, 2011. The age and sex of patients, the organism isolated and the antimicrobial susceptibility

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patterns were retrieved from the registration records using a standard data collection form.

Study area: The study was conducted at Dessie Regional Health Research Laboratory which serves outpatients for culture and sensitivity testes. At present, Dessie Regional Health Research Laboratory renders culture and susceptibility tests to Dessie hospital, nearby health centers, private hospitals and clinics. There was no laboratory facility for isolation of anaerobic bacteria from chronic otitis media and fungi from otomycosis in ear discharges.

Culture and identification: As the standard operation procedures showed, pus swabs from the discharging ears were collected using swab techniques by cotton-wool at the microbiology laboratory. Ear discharge samples were plated on MacConkey agar, blood agar and mannitol salt agar (Oxoid, UK) and then incubated aerobically at 37°C for 24 hours. Bacterial species were identified as per the standard microbiological methods (16).

Antimicrobial susceptibility testing: Antimicrobial susceptibility tests were done on Mueller-Hinton agar (Oxoid, England) using disk diffusion method as described by Kirby Bauer (17). The antimicrobial agents tested were: tetracycline (30 µg), cephalotin (30 µg), erythromycin (15 µg), chloramphenicol (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), cotrimoxazole (25 µg), ceftriaxone (30µg) and amoxicillin (10µg) (Oxoid, England). Resistance data were interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2006) (formerly known as National Committee for Clinical Laboratory Standards / NCCLS) (18). Reference strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used for quality control for antimicrobial susceptibility tests (18).

Statistical analysis: Statistical analysis was done using SPSS version 15 software. Chi-square test was employed to compare the proportion of bacterial isolates with patients' age and comparison of antimicrobial resistances. P-value of < 0.05 was considered to indicate statistically significant difference.

Ethical considerations: Institutional ethical approval was secured from the Research Ethics Committee of Bahir Dar University. Permission from Dessie Regional Health Research Laboratory was also obtained.

Results

Between 2003 and 2010, 897 ear discharge cultures were examined. Four hundred sixty nine (52.2%) ear discharges were from children and 428 (47.8%) from adults. There was almost equal distribution of otitis media among the sexes [females: 464 (51.7%) and males 433 (48.3%)]. The median age of patients with discharging ear was 18 years (range: 2 months to 80 years). Among children (0-18years), peak prevalence of otitis media (28.1%) was documented in children with the age group 0-4 years. From 897 discharging ear cultures, 735 (82%) were bacterial cultures positive. From culture positive ear discharges, 650 (88%) had single isolates and 85 (12%) had mixed ones. Gram negative bacteria accounted for 611 (74.2%) of isolates of the ear discharges. The most commonly identified organisms were: *Proteus* spp., *S. aureus*, *Pseudomonas* spp., and *E. coli*. *Proteus* spp. were the most common isolates in children than in adults (p=0.001) (Table 1).

Proteus spp., *Pseudomona* spp. and *E. coli* showed high levels of susceptibility (87-100%) to norfloxacin, ciprofloxacin and gentamicin. However, high resistance rates were documented for erythromycin (90.8-97%), amoxycillin (80.8-100%) and tetracycline (61.1-89%). *S. aureus* exhibited resistance rates ranging from 40.7-86% to amoxycillin, tetracycline and cotrimoxazole. However, most of the isolates were highly sensitive (90-93.4%) to ciprofloxacin, norfloxacin and gentamicin (Table 2).

The overall antimicrobial susceptibility demonstrated that norfloxacin, ciprofloxacin and gentamicin revealed high level of sensitivity (86.6-94.6%). However, 72-86% resistance rate was documented for erythromycin, tetracycline and amoxycillin (p=0.001) (Table 3).

As displayed in Table 4, antibiogram results showed 78 (9.5%) of the isolates were susceptible to ten antimicrobials tested whereas 745 (90.5%) were resistant to one and more antimicrobials tested. Multiple antimicrobial resistance to two and more antimicrobials was 598 (72.6%) at a time.

Table 1: Age and sex distribution of bacterial isolates of otitis media at Dessie Regional Health Research Laboratory (2003-2010)

Age (yrs)	Sex	Bacterial species										
		Proteus sp	S. aureus	Pseudomonas	E. coli	Enterobacter spp.	Citrobacter spp.	Klebsiella spp.	Providencia spp.	Streptococci spp.	CNS	NG
0-4	F (n=63)	19	15	9	9	5	-	1	-	-	-	13
	M (n=69)	22	9	7	13	2	2	2	-	1	1	14
5-9	F (n=45)	14	12	8	5	-	-	-	1	-	1	8
	M (n=52)	17	12	7	11	2	2	1	-	-	-	4
10-14	F (n=71)	15	16	18	10	1	3	3	2	-	-	12
	M (n=54)	16	11	14	7	2	3	-	-	-	-	7
15-18	F (n=69)	17	16	11	11	3	2	2	-	-	1	14
	M (n=46)	16	8	7	6	1	1	1	-	1	1	7
Paediatric (n=469)		136	99	81	73	16	13	10	3	2	4	79
19-44	F (n=177)	34	42	26	28	14	7	5	-	-	1	32
	M (n=173)	37	43	31	33	3	9	6	2	1	1	31
>44	F (n=39)	4	10	4	8	3	1	1	-	-	-	11
	M (n=39)	10	9	6	4	1	1	1	-	-	-	9
Adults (n=428)		85	104	67	73	21	18	13	2	1	2	83
Total (n=897)		221 (26.8)	203 (24.6)	148 (18)	146 (917.7)	37 (4.5)	31 (3.7)	23 (2.8)	5 (0.6)	3 (0.36)	6 (0.7)	162 (18)
P-value		0.001	0.26	0.51	0.55	0.26	0.24	0.39	NA	NA	NA	0.33

CNS: Coagulase negative staphylococci, NA: not applicable
 NG: no growth of organism

Table 2: Antimicrobial resistance pattern of bacterial isolates from otitis media at Dessie Regional Health Research Laboratory, (2003-2010)

Antimicrobial Agents	Proteus Spp. (221)		S. aureus (203)		Pseudomona Spp. (148)		E. coli (146)		Enterobacter Spp. (37)		Citerobacter Spp. (31)		Klebsiella Spp. (23)		CNS (6)		Providencia spp. (5)		Streptococci Spp. (3)	
	# T	R %	# T	R %	# T	R %	# T	R %	# T	R %	# T	R %	# T	R %	# T	R %	# T	R %	# T	R %
Tetracycline	174	89	192	65	127	84	125	61.6	37	67.5	31	71	15	53	3	33	5	40	3	100
Gentamicin	207	11	223	10	156	7.7	122	34	37	7.8	2	6.4	3	13	4	25	5	20	3	-
Chloramphenicol	179	58.6	180	32	132	69.7	122	34	35	34.3	31	38.7	19	52.6	5	60	5	40	ND	ND
Cotrimoxazol	183	64	184	40.7	120	70	123	51	28	42.8	31	54.8	19	52.6	6	33	5	40	ND	ND
Erythromycin	106	93.4	176	34	120	90.8	77	87	33	97	20	100	10	90	3	-	3	66	3	33
Cephalothin	111	66	82	35.4	53	73.5	54	59	12	66	11	54	14	78.5	2	-	1	100	ND	ND
Ciprofloxacin	93	9.6	107	4.6	113	3.5	55	7.2	36	2.7	22	9	8	-	2	-	4	-	ND	ND
Amoxycillin	94	80.8	75	86	49	91.8	43	86	18	100	11	100	12	91.6	4	75	3	100	2	50
Norfloxacin	15	-	25	8	7	-	7	-	7	-	7	-	7	-	3	-	ND	ND	ND	ND
Ceftriaxone	65	47.7	94	20	67	53.7	42	40.7	13	46	16	62.5	7	28.5	ND	ND	3	66	ND	ND

T: number of isolates tested against each antimicrobial agent
R %: percent of isolates resistance to antimicrobial agent, ND: not done

Table 3: Overall sensitivity and resistance profile of antimicrobial agents tested for bacterial isolates of otitis media, (2003-2010)

Antimicrobial	Number of Antimicrobials Tested	Susceptibility patterns		
		Sensitive, N (%)	Intermediate, N (%)	Resistance, N (%)
Tetracycline	716	172 (24)	18 (2.5)	526 (73.5)
Gentamicin	846	733 (86.6)	25 (3)	88 (10.4)
Chloramphenicol	636	324 (51)	12 (2)	300 (47.1)
Cotrimoxazol	699	313 (44.8)	4 (0.6)	382 (54.6)
Erythromycin	551	145 (26.3)	7 (1.3)	399 (72.4)
Cephalothin	310	120 (38.7)	5 (1.7)	185 (59.6)
Ciprofloxacin	440	412 (93.6)	0	28 (6.4)
Amoxycillin	311	44 (14)	0	267 (86)
Norfloxacin	56	53 (94.6)	0	3 (5.4)
Ceftriaxone	307	180 (58.6)	4 (1.4)	123 (40)
P-value		0.001		

Table 4: **Antibiogram of bacterial pathogens isolated from otitis media at Dessie Regional Health Research Laboratory (2003-2010)**

Bacterial species	Multiple antimicrobial resistance, N (%)							
	R0	R1	R2	R3	R4	R5	R6	R7
<i>Proteus spp.</i> (n=221)	10 (4.5)	24 (10.8)	47 (21.2)	50 (22.6)	55 (24.8)	25 (11.3)	13 (5.8)	0
<i>S. aureus</i> (n=203)	39 (19)	59 (29)	50 (24.6)	33 (16)	13 (6.4)	9 (4.4)	0	0
<i>Pseudomonas spp.</i> (n=148)	6 (4)	14 (9.4)	28 (19)	36 (24)	36 (24)	15 (10)	9 (6)	4 (2.7)
<i>E. coli</i> (n=146)	21 (14.4)	32 (22)	35 (24)	26 (17.8)	20 (13.7)	8 (5.4)	4 (2.7)	0
<i>Enterobacter spp.</i> (n=37)	2 (5.4)	4 (10.8)	9 (24.3)	9 (24.3)	9 (24.3)	1 (2.7)	2 (5.4)	1 (2.7)
<i>Citrobacter spp.</i> (n=31)	0	6 (19.4)	2 (6.4)	9 (29)	7 (22.6)	4 (13)	3 (9.6)	0
<i>Klebsiella spp.</i> (n=23)	0	7 (30.4)	5 (21.7)	4 (17.4)	5 (21.7)	1 (4.3)	1 (4.3)	0
CNS (n=6)	0	3 (50)	2 (33)	1 (17)	0	0	0	0
<i>Providencia spp.</i> (n=5)	0	1 (20)	2 (40)	0	1 (20)	0	1 (20)	0
<i>Streptococci spp.</i> (n=3)	0	0	2 (66.6)	1 (33.4)	0	0	0	0
Total (n=823)	78 (9.5)	150 (18)	182 (22)	169 (20.5)	146 (17.7)	63 (7.6)	33 (40)	5 (0.6)
P-value	0.001							

R0: susceptible to all antimicrobials tested; R1, R2, R3, R4, R5, R6, R7: Resistance to one, two, three, four, five, six and seven antimicrobials, respectively

Discussion

In the study area, ear discharge cultures were one of the most frequently requested clinical specimen for culture and antimicrobial susceptibility testes. This indicated that otitis media is a common health problem of all age groups. Among children, peak-age prevalence was observed in the age group under five years. This is in agreement with previous studies conducted in Ethiopia (15, 19) and Nigeria (20). This is due to the fact that younger children are more prone to otitis media related to the immaturity of their immune status, the shorter and horizontal nature of Eustachian tubes, frequent exposure to upper respiratory tract infections and malnutrition (13).

In the present study, gram-negative bacteria were the dominant isolates (74.2%) of the discharging ears compared to gram-positive bacteria. Moreover, Tesfaye from Addis Ababa (19) and Wariso from Nigeria (20) have reported 60.5% and 75% of gram-negative bacteria, respectively. *Proteus* spp., *S. aureus*, *Pseudomonas* spp. and *E. coli* accounted for 87.2% of isolates. *Proteus* spp., *S. aureus* and *Pseudomonas* spp. were the most dominant isolates. This trend conforms to findings of other studies done in Ethiopia (13, 15, 21, 22). In contrast, Tesfaye from Addis Ababa (19) reported *S. aureus*, *Proteus* spp., and *Pseudomonas* spp. as predominant causes for chronic otitis media. Very low isolation rate of *Pseudomonas* spp. were reported from Gondar (22) in Ethiopia. However, several studies elsewhere in the world have reported that the most common isolated organisms were *Pseudomonas* spp. followed by *S. aureus* (23-25).

Possible explanation to this difference in isolation rate might be related to the effect of climate. Bacterial colonization of otitis media increases as temperatures rise which in turn increases the isolation rate of bacteria (26). Moreover, Mohoney reported that *S. aureus* was the common isolate in patients' complaint in the first week after the onset of ear discharge while *Proteus* spp. and *Pseudomonas* spp. were common isolates in patients presenting lately 2 months after onset of ear discharge (27). *Proteus* spp. were the commonest isolates in pediatrics compared to adults ($p=0.001$). This is in agreement with others studies in Ethiopia (21, 22). However, in India, Saini reported that *S. aureus* was the commonest isolate in pediatric patients (28). The reason may be children with discharging ears may not be noticed for early diagnosis (27).

Previous studies have indicated that otitis media is one of the major causes for frequent antibiotic use in children and being key contributor to antibiotic resistance (29, 30). Thus, this study presents antibiogram trends of the most predominant bacterial isolates in otitis media. *Proteus* spp. showed high resistant rate (90-93%) to tetracycline and erythromycin. Moreover, 96-100% resistance level of tetracycline and erythromycin were reported by Wariso from Nigeria (20). Moderate resistance rate to cotrimoxazol and chloramphenicol (60-

64%) were also documented. This is consistent with report of Abera (21) and Gebre-Selassie (31). However, in this study *Proteus* spp. demonstrated 100% sensitivity to norfloxacin and low level of resistance to gentamicin and ciprofloxacin.

In the present study, *S. aureus* revealed a high level of resistance to amoxicillin which is in agreement with a report from Pakistan (25). *S. aureus* showed low rate of resistance to ceftriaxone which is in parallel with a finding from Addis Ababa (19) but 50% resistance rate was reported from Nigeria (20). Most of *S. aureus* exhibited low levels of resistance (4.6-10%) to ciprofloxacin, gentamicin and norfloxacin which is also reported by (19, 22). However, 23% gentamicin resistance was reported from Felege Hiwote Hospital (21) and Gondar University hospital (22).

Pseudomonas spp. were the most antibiotic resistant isolates in otitis media. Most of the isolates were resistant to amoxicillin, erythromycin, tetracycline, cotrimoxazole and chloramphenicol. This is comparable with results from Ethiopia (15, 22), Nigeria (20) and Iraq (24). However, *Pseudomonas* spp. were highly sensitive to norfloxacin, ciprofloxacin, and gentamicin, similar to reports of findings in Gondar in Ethiopia (22), Brazil (23) and Pakistan (25). However, Ihsan from Iraq reported 25% and 50% resistance rate for ciprofloxacin and gentamicin, respectively (24).

Regarding antibiograms, a statistically significant difference was noted among bacterial isolates ($p=0.001$). *Citrobacter* spp. and *Pseudomonas* spp. were the most antibiotic resistant isolates. Twenty-five (80.6%) of *Citrobacter* spp. and 128 (86.5%) of *Pseudomonas* spp. were multiple resistant to two and more antimicrobials. Overall, susceptibility patterns of bacterial isolates exhibited significant sensitivity to gentamicin, ciprofloxacin and norfloxacin indicating that gentamicin to be the first-line antibiotic in the empirical treatment of otitis media in children since ciprofloxacin and norfloxacin are contraindicated (4). Because of the retrospective nature of the study we could not trace patients' detail clinical data thus the study was limited to describing types of otitis media.

In conclusion, the present study indicated that otitis media is common health problem in children and adults imply a major healthcare burden in the area. Enterobacteriaceae, *S. aureus* and *Pseudomonas* spp. were the dominant isolates in otitis media. Most of the isolates showed high levels of antimicrobial resistance to commonly prescribed antimicrobials. However, gentamicin and fluoroquinolones, have activity against most of the bacterial isolates *In vitro*. Therefore, culture and susceptibility testes have paramount importance for better management of otitis media and drug-resistant infections.

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