Original article

Microbiology of discharging ears in Ethiopia

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Abstract

Objectives: To isolate and identify the bacterial etiologic agents, including their antibiotic susceptibility pattern isolated from patients with discharging ear infections. **Methods**: Between September 2006 and February 2007, 178 patients with discharging ear visiting ENT clinics of St. Paul and Tikur Anbessa University Hospitals Addis Ababa, Ethiopia were investigated. **Results**: Of the patients investigated, 52.8% were males and 47.2% were females resulting in an overall male to female ratio of 1.1:1. Ear discharge was the commonest clinical finding followed by hearing problem (91.2%), otalgia (ear pain) (74.7%), fever (17.9%) and itching of external ear (5.1%). *S. aureus* accounted for 30.2% of the total isolates followed by *Proteus* ssp. (*P. mirabilis*, *P. vulgaris*) (25.4%), and *P. aeruginosa* (13.4%). Both gram positive and negative bacteria isolated from ear infections showed low resistance rates to most antimicrobial agents tested. Overall ceftriaxone and ciprofloxacin were the most effective drugs when compared to other drugs tested against the gram-positive and gram-negative bacteria. **Conclusion**: Otitis media was the most common clinical finding in patients with ear infection. With discharging ear, the gram-negative bacteria were the predominant isolates. The susceptibility pattern of isolates from the study showed that ceftriaxone, ciprofloxacin and gentamicin were the most effective drugs. It is recommended that treatment of ear infections should be based on culture and sensitivity at the study sites. Therefore, efforts should be directed towards early diagnosis and treatment of acute ear infection and continued re-evaluation of the resistant patterns of organisms to optimize treatments and reduce complications.

Keywords: Otitis externa; Otitis media; Bacterial etiologic agents; Antibiotic susceptibility pattern; Ethiopia

INTRODUCTION

Inflammatory diseases of the external ear and middle ear are one of the commonest conditions encountered by pediatricians [1]. Otitis externa (OE) is an inflammation or infection of the external auditory canal. Otitis media (OM) is an inflammatory disease of the mucosal lining of the middle ear, the leading cause of hearing loss, and the most frequent indication for antimicrobial or surgical therapy in children[2]. *P. aeruginosa* and *S. aureus* are the most common organisms isolated from cases of otitis externa and chronic otitis media [3]. *S. pneumoniae* is the leading bacterial pathogens followed by non-typeable strains of *H. influenzae* and *M. catarrhalis* isolated from acute otitis media[4]. Bacterial infections of the middle ear normally originate from the upper respiratory tract, with the bacteria entering the ear through the auditory (Eustachian) tube, the principal portal of entry to the ear [2]. Inner ear inflammations are less common and need special and urgent attention. In Ethiopia there are few published reports concern-

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ing the problem and etiologies of ear infections [5, 6]. It has been shown that bacterial predominance and antibiotic sensitivity have changed overtime, making continuous and periodic surveillance necessary in guiding appropriate antimicrobial therapy [7]. With the development and widespread use of antibiotics, the types of pathogenic organisms and their resistance to antibiotics have changed. Knowledge of the species and resistance rates of current pathogens is important for determining the appropriate antibiotics for patients with ear infections. To readdress this situation, this study was undertaken to isolate and identify the bacterial etiologic agents, including their antibiotic susceptibility pattern isolated from patients with ear infections visiting ENT clinics of St. Paul and Tikur Anbessa University Hospitals Addis Ababa, Ethiopia and also provide update information for appropriate management ear infections.

MATERIALS AND METHODS

Study population

A cross-sectional prospective study was conducted during the period of September 2006 to February 2007. A total of 1 930 patients with ear problem were seen at the ENT Department of St. Paul Hospital (n = 1 410) and Pediatrics and Child Health Department of Tikur Anbessa University Hospital (n = 520). Of these, 442 (22.9%) were clinically diagnosed to have otitis externa and media. Out of 442 patients, 178 informed and consented patients with ear discharge (adults, n = 86; children, n = 92) were investigated for bacterial infection. Sample was not collected from the remaining 264 individuals with chronic otitis media with dry perforation of tympanic membrane.

Working definitions of ear infections

Otitis externa: inflammation of external auditory canal characterized by severe pain when manipulating pinna or the presence of spontaneous discharge with a normal tympanic membrane.

Acute otitis media: is characterized by red with either retracted or bulged intact tympanic membrane or discharging ear with perforated tympanic membrane less than 3 months of duration since infection.

Chronic otitis media: discharging or dry ear with perforated tympanic membrane with a duration of 3 months and above since infection.

Sample collection, handling and transport

Ear swab/ discharge specimens were collected aseptically, after the patient's ear was washed by normal saline (0.85% NaCl) from each patient with discharging ear by the attending physician. Samples were kept in Amies Transport Media (Oxoid, Hampshire, England, UK) to maintain the viability of microorganisms until the specimen is processed. The specimens were transported within one hour to the Core Laboratory of Faculty of Medicine, Addis Ababa University.

Culture and identification

All ear specimens were inoculated directly on blood, chocolate and MacConkey agar (Oxoid, Ltd). The blood and MacConkey agar plates were incubated in aerobic and chocolate agar in microaerophilic atmosphere using a candle jar at 37°C for 24-48 hrs. All positive cultures were identified by their characteristic appearance on their respective media, gram-staining reaction and confirmed by the pattern of biochemical reactions using the standard method [8].

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed for all isolates according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLs) by disk diffusion method [9]. The drugs for disc diffusion testing were in the following concentrations (Oxoid): Ampicillin (AMP) (10 μg), Amoxicillin-Clavulanic Acid (AMC) (30 μg), Ceftriaxone (CRO) (30 μg), Ciprofloxacin (CIP) (5 μg), Chloramphenicol (C) (30 μg), Erythromycin (E) (15 μg), Gentamicin (CN) (10 μg), Kanamycin (K) (30 μg), Methicillin (MET) (5 μg), Penicillin (P) (10 units), Streptomycin (S) (10 μg), Trimethoprim-Sulphamethoxazole (SXT) (25μg) and Vancomycin (30 μg). Penicillin, Vancomycin, Erythromycin and Methicillin were tested only for Gram-positive bacteria. The isolates were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the NCCLs [9].

High, intermediate and low level of resistance is defined when the percentage of resistance is > 80%, 60-80% and < 60% respectively.

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Reference strains

P. aeruginosa (ATCC-27853), *S. aureus* (ATCC-25923) and *E. coli* (ATCC-25922) were used as a quality control throughout the study for culture and antimicrobial susceptibility testing.

Statistical analysis

Data entry and analysis was done using SPSS version computer 11.5 software. Comparisons were made using Chi-square test with Fisher exact tests. A *P*-value of <0.05 was considered indicative of a statistically significant difference.

Ethical consideration

The research project proposal was approved by the Department of Microbiology, Immunology and Parasitology, ethically cleared by the Faculty Research Publications Committee-II (FRPC-II) and endorsed by the Faculty Academic commission. Official permission from the study sites was obtained.

RESULTS

Study Population

A total of 178 patients were included in the study from St. Paul Hospital ($n=124$) and Tikur Anbessa University Hospital ($n=54$), between September 2006 and February 2007. Of the 178 patients, 94 (52.8%) were males and 84 (47.2%) were females ($P>0.05$) resulting in an overall male to female ratio of 1.1:1.

Patterns of ear infection

The pattern of ear infections observed in 178 patients at St. Paul's and Tikur Anbessa University Hospitals is presented in Table 1. Of the 178 ear infection cases, 96.6% and 3.4% accounted for otitis media and otitis externa respectively ($P<0.05$). Acute otitis media and chronic otitis media accounted for 12.9 and 83.7%%, respectively ($P<0.05$).

Clinical features

Ear discharge was the commonest clinical finding observed in 178 patients investigated for ear infections with ASOM and CSOM (100%), followed by hearing problem (91.2%), otalgia (ear pain) (74.7%), fever (17.9%) and itching of external ear (5.1%). The ear problem was unilateral (right = 65 or left ear = 80) in 145 (81.5%) and bilateral (both ears) 33 (18.5%) patients. The most common type of ear discharge was serous (10.1%), purulent (80.3%), bloody (7.3%) and greenish (2.2%).

Microbiological data

A total of 157 bacterial pathogens were isolated from otitis externa ($n=6$) and otitis media ($n=151$) as shown in Table 2. *S. aureus* accounted for 30.2% of the total isolates followed by *Proteus* ssp. (*P. mirabilis and P. vulgaris*) (25.4%), *P. aeruginosa* (13.4%), *E. coli* (8.3%), *Streptococcus* spp. (*S. pneumoniae* and *S. pyogenes*) (8.2%), *Klebseiella* spp. (*K. pneumoniae* and *K. oxytoca*) (6.3%), *H. influenzae* and *C. barakii* each with an incidence of 2.5% and *S. marcescens* and *E. cloaceae* accounted for 1.3% and 0.6% respectively. Coagulase negative Staphylococci (CNS) was detected in 5/178 (2.8%) specimens (data not shown). The Gram-positive and negative bacteria accounted for 62/157 (39.5%) and 95/157 (60.5%) respectively ($P<0.05$).

Use of antibiotics and outcome

Of the patients who received antibiotic/s, 82/102 (80.3%) had positive culture results, while those who did not receive any antibiotics 75/76 (98.6%) had positive culture results ($P<0.05$).

Antimicrobial susceptibility data

Gram positive bacteria

The susceptibility patterns of gram-positive bacteria ($n=62$) isolated from ear infection against 13 antimicrobial agents are presented in Table 3. All isolates showed intermediate level of resistance (60%-80%) to penicillin and ampicillin and low level of resistance (<60%) to amoxicillin-clavulinic acid, ceftriaxone, ciprofloxacin, chloramphenicol, erythromycin gentamicin, kanamycin, methicillin, streptomycin, trimethoprim-sulphamethoxazole and vancomycin. Most gram-positive isolates, 48/62 (77.4%) showed multiple drug resistance (resistance to two or more drugs) (data not shown).

Gram negative bacteria

The susceptibility patterns of gram-negative bacteria ($n=95$) isolated from ear infections against 9 anti-

microbial agents are presented in Table 4. All isolates showed intermediate level of resistance (60%-80%) to ampicillin and low level of resistance (< 60%) to amoxicillin-clavulinic acid, ceftriaxone, ciprofloxacin, chloramphenicol, gentamicin, kanamycin, streptomycin and trimethoprim-sulphamethoxazole. Of the 95-gram negative isolates, 59 (62.1%) strains were also identified as multiple drug resistant (data not shown). In general ceftriaxone, ciprofloxacin and gentamicin were the most effective drugs against the tested gram-positive and gram-negative bacteria (Tables 3 and 4).

Table 1 Patterns of ear infections observed in 178 patients visiting St. Paul and Tikur Anbessa University Hospitals Addis Ababa, Ethiopia.

Type of ear infection	St. Paul Hospital No. (%)	TAH No. (%)	Total No. (%)
Otitis media			
Acute otitis media	12 (9.7)	11 (20.4)	23 (12.9)
Chronic otitis media	108 (87.1)	41(75.9)	149 (83.7)
Otitis externa			
Acute otitis externa	4 (3.2)	2 (3.7)	6 (3.4)
Total	124 (69.7)	54 (30.3)	178 (100)

Table 2 Bacterial etiologic agents isolated from patients with discharging ear visiting ENT Department of St. Paul and Tikur Anbessa Hospitals, Addis Ababa, Ethiopia.

Etiologic agents	Otitis Media			
	Otitis Externa No. (%)	Acute No. (%)	Chronic No. (%)	Total No. (%)
Staphylococcus aureus	2 (33.3)	3 (13.0)	44 (34.4)	49 (30.2)
Proteus mirabilis	– –	– –	30 (23.4)	30 (19.1)
Pseudomonas aeruginosa	4(66.7)	– –	17 (13.3)	21 (13.4)
Escherichia coli	– –	1 (4.3)	12 (9.4)	13 (8.3)
Proteus vulgaris	– –	– –	10 (7.8)	10 (6.4)
Klebsiella oxytoca	– –	– –	8 (6.3)	8 (5.1)
Streptococcus pneumoniae	– –	7 (30.4)	– –	7 (4.5)
Streptococcus pyogenes	– –	6 (26.1)	– –	6 (3.8)
Haemophilus influenzae	– –	4 (17.4)	– –	4 (2.5)
Citrobacter barakii	– –	– –	4 (3.1)	4 (2.5)
Klebsiella pneumoniae	– –	2 (8.7)	– –	2 (1.3)
Serratia marcescens	– –	– –	2 (1.6)	2 (1.3)
Enterobacter cloaceae	– –	– –	1(0.8)	1(0.6)
Total	6 (100)	23 (100)	128 (100)	157 (100)

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Table 3 Susceptibility patterns of Gram – positive bacteria isolated from ear infections.

Organisms		Antimicrobial agents (%)												
		AMP	AMC	CRO	CIP	C	E	CN	K	MET	P	S	SXT	VA
Staphylococcus aureus (n=49)	S*	28.6	61.2	91.8	93.9	67.3	57.1	83.7	69.4	61.2	20.4	71.4	63.3	69.4
	I*	6.1	24.5	2.0	2.0	6.1	4.1	–	6.1	36.7	–	8.2	–	–
	R*	65.3	14.3	6.1	4.1	26.5	38.8	16.3	24.5	–	79.6	20.4	36.7	30.6
Streptococcus pneumoniae (n=7)	S	57.1	85.7	100	100	100	71.4	71.4	85.7	42.9	42.9	42.9	100	57.1
	I	14.3	–	–	–	–	28.6	14.3	14.3	–	–	14.3	–	42.9
	R	28.6	14.3	–	–	–	–	14.3	–	57.1	57.1	42.9	–	–
Streptococcus pyogenes (n=6)	S	16.7	33.3	83.3	83.3	66.7	67.7	100	100	83.3	16.7	83.3	66.7	83.3
	I	–	16.7	–	–	–	–	–	–	–	–	16.3	–	16.7
	R	83.3	50.0	16.7	16.7	33.3	33.3	–	–	16.7	83.3	–	33.3	–
Total (n=62)	S	30.6	61.3	91.9	93.6	71.0	59.7	83.9	74.2	61.3	22.6	69.4	67.7	69.4
	I	6.5	21.0	1.6	1.6	4.8	6.4	1.6	6.5	1.6	–	9.7	–	30.6
	R	62.9	17.7	6.5	4.8	24.2	33.9	14.5	19.4	37.1	77.4	20.9	32.3	–

* S = Sensitive * I = Intermediate * R = Resistant

AMP: Ampicillin; AMC: AmoxicillinClavulanicacid; CRO: Ceftriaxone; CIP: Ciprofloxacin; C: Chloramphenicol; E: Erythromycin; CN: Gentamicin; K: Kanamycin; MET: Methicillin; P: Penicillin; S: Streptomycin; SXT: Trimethoprim – sulphamethoxazole; VA: Vancomycin

DISCUSSION

The clinical findings of patients with ear problem (predominantly CSOM), prevalence and peak-age prevalence (mostly in children younger than 15 years of age) in our study are similar to findings of previous studies done in Ethiopia [5, 6] and other developing countries [10-14]. In developed countries, however, ASOM is 20-30 times more commonly seen in hospitals than CSOM [15].

The observation in this study is that gram-negative bacteria were the predominant isolates (60..5%) when compared to gram-positive bacteria (39.5%) from 178 patients with discharging ear (Table 2). This is in agreement with previous studies done in Ethiopia [5, 6] and elsewhere the world [14, 16-21]. In the present study, *P. aeruginosa* was the most common isolate (66.7%) followed by *S. aureus* (33.3%) from the cases otitis externa. Similar findings have been observed elsewhere [3, 18, 22]. Our findings showed that the microbiologic testing results of otitis other media were predominantly *S. aureus* (30.2%) followed by *Proteus* spp. (*P. mirabilis* and *P. vulgaris*) (25.4%) and *P. aeruginosa* (13.4%) (Table 2). Generally this pattern of isolated organisms agrees with previous findings done elsewhere [14, 16, 18-20, 21, 23]. The bacteriologic findings in this study in both acute and chronic otitis media were different (Table 2). *S. pneumoniae* (30.4%), *S. pyogenes* (26.1%) and *H. influenzae* (17.4%) were predominately isolated from cases with acute otitis media. This is comparable with most studies done elsewhere [18, 24-26]. In the present study, *S. aureus* (34.4%), *Proteus* spp. (*P. mirabilis* and *P. vulgaris*) (31.3%) and *P. aeruginosa* (13.3%) were most common isolates from cases of chronic otitis media. The pattern of isolates is similar to other studies done elsewhere [12, 13, 18-21, 24, 27], even though there are slight differences in the frequency of isolation. However, this is in contrast with previous study done in Ethiopia where *Proteus* spp. (37.7%) were the commonest isolates followed by *S. aureus* (32.1%), *P. aeruginosa* (9.3%) and gram negative enterics (9.5%) in chronic otitis media [5]. In a related study conducted in Indonesia anaerobic bacteria were the most commonly isolated organisms (71%) in cases of chronic otitis media [2]. The overall culture results in this study in both ASOM and CSOM, *S. aureus* accounted for 30.2% of the isolates, followed by

Table 4 Susceptibility patterns of Gram – negative bacteria isolated from ear infections.

Organisms		Antimicrobial agents (%)								
		AMP	AMC	CRO	CIP	C	CN	K	S	SXT
Proteus mirabilis (n=30)	S*	70.0	80.0	100	100	53.3	96.7	100	83.3	73.3
	I*	6.7	–	–	–	13.3	–	–	3.3	3.3
	R*	23.3	20.0	–	–	33.3	3.3	–	13.3	23.3
Pseudomonas aeruginosa (n=21)	S	14.3	19.0	52.4	81.0	28.6	57.1	42.9	19.0	28.6
	I	–	14.3	28.6	4.8	–	19.0	–	–	4.8
	R	85.7	66.7	19.0	14.3	71.4	23.8	57.1	81.0	66.7
Escherichia coli (n=13)	S	30.8	46.2	100	84.6	46.2	76.9	76.9	28.5	53.8
	I	7.7	–	–	–	23.1	15.4	15.4	23.1	7.7
	R	61.5	53.8	–	15.4	30.8	7.7	7.7	38.5	38.5
Proteus vulgaris (n=10)	S	20.0	40.0	100	100	20.0	90	80	70	50
	I	–	20.0	–	–	–	–	–	20	–
	R	80.0	40.0	–	–	80.0	10	20	10	50
Klebsiella oxytoca (n=8)	S	12.5	75.0	100	100	75.0	75.0	50	37.5	75
	I	–	12.5	–	–	–	12.5	37.5	37.5	–
	R	87.5	12.5	–	–	25.0	12.5	12.5	12.5	25
Haemophilus influenzae (n=4)	S	25.0	75.0	100	100	75.0	100	100	75	75
	I	–	–	–	–	–	–	–	–	–
	R	75.0	25.0	–	–	25.0	–	–	25	25
Citrobacter baraaki (n=4)	S	25.0	25.0	75.0	100	75.0	100	75	25	50
	I	25.0	25.0	25.0	–	–	–	25	–	25
	R	50.0	50.0	–	–	25.0	–	–	75	25
Klebsiella pmeumoniae (n=2)	S	–	50.0	100	100	–	–	100	100	–
	I	–	–	–	–	–	–	–	–	–
	R	100	50.0	–	–	100	100	–	–	100
Serratia marcescens (n=2)	S	–	–	100	100	100	100	50	–	100
	I	–	50.0	–	–	–	–	50	100	–
	R	100	50.0	–	–	–	–	–	–	–
Enterobacter cloacae (n=1)	S	–	–	100	100	100	100	100	–	100
	I	–	–	–	–	–	–	–	100	–
	R	100	100	–	–	–	–	–	–	–
Total (n=95)	S	34.7	51.6	88.4	93.7	46.3	81.0	75.8	53.7	56.8
	I	4.2	8.4	7.4	1.0	8.4	7.4	7.4	12.6	4.2
	R	61.1	40.0	4.2	5.3	45.3	11.6	16.8	33.7	39.0

* S = Sensitive * I = Intermediate * R = Resistant

AMP: Ampicillin; AMC: Amoxicillin-Clavulanic acid; CRO: Ceftriaxone; CIP: Ciprofloxacin; C: Chloramphenicol; CN: Gentamicin; K: Kanamycin; S: Streptomycin; SXT: Trimethoprim sulphamethoxazole

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Proteus spp. (25.4%) and *P. aeruginosa* (13.4%). This is in agreement with a study done in Taiwan [7] and Poland [28], which showed that *S. aureus* had become more common than *P. aeruginosa* in discharging ear, in contrast to most studies which found *P. aeruginosa* followed by *S. aureus* as predominant agents of acute and chronic otitis media [3,12,18]. Coagulase negative Staphylococci (CONS) was detected in 5/178 (2.8%) specimens (data not shown). These were considered as contaminants in this study since the normal bacterial flora of the external ear canal is predominately CONS and *Corynebacterium* [29]. Even though there was statistically significant difference in culture positivity in patients who received antibiotic/s (80.3%) and did not receive any antibiotics (98.6%), the percentage of culture positivity in patients who have taken antibiotics is high which shows the importance of culture and sensitivity in the management of ear infections.

The study also provides insights into the susceptibility profile of bacteria isolated from ear infections (Tables 3 and 4). Our results have demonstrated that in general both gram positive and negative bacteria isolated from ear infections showed low level of resistance to most antimicrobial agents tested. This is in agreement with previous study done in Ethiopia [5]. However, there are reports from different parts of the world with high resistance to these antimicrobial agents except for gentamicin [12-14, 16, 20, 23]. This difference in the susceptibility profile might be due to frequency of usage of these agents for the treatment of ear infections in different geographic locations. In the present study, ceftriaxone and ciprofloxacin were the most effective drugs when compared to other drugs tested against the gram-positive and gram-negative bacteria. This is comparable with other studies done elsewhere [12, 14, 21-23, 28]. The in vivo efficacy of gentamicin, ceftriaxone and ciprofloxacin against tested organisms in our study is the reflection of infrequent prescription of these drugs by ENT specialist in Ethiopia (Personal communication) mainly due to severe side effects in case of gentamicin and expense of ceftriaxone and ciprofloxacin, in addition to difficulty of administration. In this study, multiple resistances (resistant to two or more drugs) were observed in 77.2% and 62.1% gram positive and gram-negative bacteria, respectively.

It is a well-known fact that microbial drug resistance is a growing global problem. In gram-negative bacteria, the most resistant pathogens are *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. and *P. aeruginosa*, with increasing trends observed for all major anti-gram negative agents (beta-lactams, fluorquinolones and aminoglycosides) [30]. Serious infections caused by gram-positive bacteria are increasingly difficult to treat because of pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and penicillin-resistant *Streptococcus pneumoniae*. The more recent emergence of vancomycin intermediate and -resistant MRSA (VISA and VRSA) has further compromised treatment options [31]. The detection of multidrug resistant isolates may further limit therapeutic options. In the present investigation, it was not possible logistically to include anaerobic bacteria, fungi and other pathogens due to budget constraints and the laboratory setup where the research was conducted.

In conclusion, in the present study the most common symptom of ear infections is ear discharge (otorrhea). Otitis media accounted for 96.6% of the patients included in the study. The gram-negative bacteria were the predominant isolates from patients with discharging ear. Ceftriaxone, ciprofloxacin and gentamicin were the most effective drugs when compared to other drugs tested against the gram-positive and gram-negative bacteria. Prescription of antibiotics for ear infections in the Ethiopian setting is empirical, there fore it is recommended that treatment of ear infections should be based on culture and sensitivity. Continuous surveillance is needed for resistant bacteria to provide the basis of alternative treatment.

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