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BACTERIA ISOLATES AND ANTIBIOTIC SUSCEPTIBILITY OF EAR INFECTIONS AMONG CHILDREN IN GONDAR, ETHIOPIA

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Abstract

Ear infections are of different types with Otitis media being the most common, occurring mostly in children. It has been shown that with the development and widespread use of antimicrobial drugs, the types of the pathogenic organism and their antibiotic sensitivity have changed over time, making continuous and periodic surveillance necessarily in guiding appropriate antimicrobial therapy This study designed to identify the commonest bacterial agents and their antibiotic susceptibility pattern from children with discharging ear at University of Gondar Teaching Hospital. Between January 2011 and May 2012, 120 children with ear discharge were investigated. Ear discharge specimens were collected aseptically and cultured. Antibiotic susceptibility was performed for the isolates. Data entry and analysis was done using SPSS version computer 16 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. Of the 120 patients with ear discharge, 56.7% were males and 43.3% were females (p>0.05) resulting an overall male to female ratio of 1:1.3. A total of 116 bacteria were obtained from 108 culture positive ear discharges. *Staphylococcus aureus* accounted for 30.2% of the total isolate followed by *Pseudomonas aeruginosa(25.9%) and proteus mirabilis* (12.1%). Both gram-positive and gram-negative bacteria isolated from ear discharge showed low level of resistance to most (60%) of anti microbial agents tested. In general ceftriaxone, ciprofloxacin, norfloxacine and gentamicin were the most effective drugs against the tested gram positive and gram negative bacteria. High bacteria isolation rate of 90% was observed among children having ear discharges. The gram negative bacteria were the predominant in prevalence compared to the gram positive bacteria. Ceftriaxone, ciprofloxacin, norfloxacine and gentamicin were the most effective drugs.

Keywords: Discharging ear, Bacteria, Antibiotic susceptibility, Ethiopia.

Introduction

The ear is the organ responsible for hearing and also maintaining balance, its divided into the outer, middle and inner ear with the outer and middle regions being most susceptible to injury and infections[1]. Ear infections are of different types with Otitis media being the most common, occurring mostly in children. The frequency of this infection in children has been attributed to the

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shorter length of the Eustachian tube and more a horizontally inclined orientation in children than in adults[2]. Sources of ear infections include bacteria, fungi and viruses with bacteria being the commonest cause[3]. The route of infection is varied but the principal target is the estachian tube which leads to the nasopharynx, as a result the infection may arise from the nose, throat such as tonsillitis or the outer ear[4].

Infections such as otitis media, can be symptomatic or asymptomatic, acute symptomatic infections are characterized by signs such as moderate to severe pain, irritation, rashes ear discharge (pus) and sometimes fever[4]. A complication of major ear infections such as otitis media varies depending on the duration of microbial colonization, severity of infection and associated microorganisms. Depending on the clinical presentation otitis media can be subdivided into 2 types chronis suppurative otitis media (CSOM) and Acute otitis media (AOM)[5].

The bacteriology of acute Otitis media has been delineated for paediatric disease. *Streptococcus pneumoniae, Heamophilus influenza* and *Moraxella catarrhalis* are the most common organism in non neonates where as a group B streptococci and gram-negative bacilli are important in neonates. Viruses, either alone or with bacteria are found in one quarter of paediatric cases. Small studies of acute otitis media in adults have also found *S. pneumonia and H. influenza* to be the most predominant organism[6].

In chronic supportive otitis media, patients discharge chronic purulent drainage from their ears through perforated tympanic membrane. Aerobic culture of draining fluid from children reveals a high percentage of *Staphylococcus aureus, Pseudomonas aeruginosa* and enteric gram-negative bacilli (*Klebsiella, Escherichia coli, and proteus*). Anaerobes including prevetella, Fusobacterium, porphyromonas and some bacteriodes species are found in 50 percent of cases[6].

Otitis media (OM), both acute and chronic is highly prevalent worldwide[7]. The prevalence of otitis media has been reported to be high in developing countries compared to advanced countries[8]. OM is the leading cause of hearing loss, and the most frequent indication for antimicrobial or surgical therapy in children. The morbidity associated with OM is substantial in the aggregate, for example the costs of therapy estimated over $1 billion each year for medical/surgical treatment and more than 1 million operative procedures being performed annually in the USA[9].

In Ethiopia, as in most developing countries, although it is one of the real health problems, there is very little information on bacterial causes of ear infections in children[10, 11]. It has been shown that with the development and widespread use of antimicrobial drugs, the types of the pathogenic organism and their antibiotic sensitivity have changed over time[12], making continuous and periodic surveillance necessarily in guiding appropriate antimicrobial therapy. To readdress this situation this study was conducted to identify the commonest bacterial agents and their antibiotic susceptibility pattern from children with discharging ear at University of Gondar (UOG) Teaching Hospital. The finding also provides up-to-date information for appropriate management of ear infection in children.

Methods

Study subjects and area

A cross sectional study was conducted between January 2011 and May 2012. Subjects examined were 120 children with discharging ear visiting UOG Teaching Hospital paediatrics clinic during the study period. It is a tertiary level teaching and referral hospital catering 400 beds for inpatients and rendering referral health services for over 4 million inhabitants in the North-west Ethiopia.

Sample Collection, Handling and Transport

Ear discharge specimens were collected aseptically using sterile cotton swab, after the patient's ear is washed by normal saline (0.85% NaCl) by 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 Microbiology laboratory of UOG Teaching Hospital.

Culture and Identification

All ear specimens were inoculated 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. Preliminary identification of all positive cultures was based on their characteristic appearance on their respective media and Gram-staining reaction. Confirmation was done using pattern of biochemical reactions following standard method[13].

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[14].The drugs for disc diffusion testing were in the following concentrations: Ampicillin (AMP) (10 µg), Amoxicillin (AMX) (25 µg), Erythromycin (ERY) (15 µg), Ceftriaxone (CRO) (30 µg), Ciprofloxacin (CIP) (5 µg), Chloramphenicol (C) (30 µg), Gentamicin (CN) (10 µg), Norfloxacin (NOR) (10 µg), Tetracycline (TE 30 µg) and Trimethoprim-Sulphamethoxazole (SXT) (25µg). Diameters of the zone of inhibition around the disc were measured to the nearest millimetre using an electronic digital calliper, and the isolates were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the NCCLs[14]. High, intermediate and low level of resistance is defined when the percentage of resistance is >80%, 60-80% and < 60% respectively.

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 16 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 study was approved by the Ethics committee of the UOG College of Medicine and Health Science. Patients were included in the study only after getting an informed written consent from their parents/guardians. Positive children for bacteria were treated with antibiotics following local drug prescription guidelines.

Results

Study Population

A total of 120 children with discharging ear were included in the study from paediatrics clinic of UOG Teaching Hospital between January 2011 and May 2012. Of the 120 patients, 68 (56.7%) were males and 52 (43.3%) were females (p>0.05) resulting an overall male to female ratio of 1:1.3.

Microbiological data

From 120 ear discharges, positive culture results were obtained for 108 individuals giving a rate of 90%. Statistically non significant amount of bacteria was isolated in age group between 0-5 years and in female (Table1).

Table No. 01: Bacteria isolate in relation to sex and age among children with ear discharge

Variables	N(%)	n (%)	P-value
Age groups(yrs)			
0-5	46(38.3)	44(95.7)	
6-11	39(32.5)	35(89.7)	0.21
12-17	35(29.2)	29(82.9)	
Sex			
Male	68(56.7)	60(88.2)	0.15
Female	52(43.3)	48(92.3)	

N=Total number of ear discharge
n= Total number of positive for bacteria

A total of 116 bacteria were obtained from 108 culture positive ear discharges. The gram positive and gram negative bacteria accounted for 50/116 (43.1%) and 66/116 (56.9%) respectively. *S.aureus* accounted for 30.2% of the total isolate followed by *Pseudomonas aeruginosa(25.9%) and P.mirabilis* (12.1%). Coagulase negative Staphylococci (CONS) was detected in 5/120(4.2%) of the specimens (data not presented here) (Table 2).

Table No. 02: Gram Bacteria Isolated from children with ear discharge

Bacteria Isolates	Frequency	Percent
Gram Positive		
Staphylococcus aureus	35	30.2
Streptococcus pneumonia	10	8.6
Streptococcus pyogenes	5	4.3
Gram Negative		
Pseudomonas aeruginosa	30	25.9
proteus mirabilis	14	12.1
proteus vulgaris	10	8.6
Klebsiella pneumonia	6	5.2
Citrobacter baraaki	4	3.4
Escherichia coli	2	1.7
Total	116	100

Antimicrobial susceptibility data

Gram positive bacteria

The susceptibility pattern of gram positive bacteria (n=50) isolated from discharging ear against 10 antimicrobial agents were presented in Table 3. All isolates showed intermediate level of resistance (60-80%) toampicilline, erythromycine, tetracycline and trimethoprim-Sulphamethoxazole and low level of resistance (<60%) to the rest of tested drugs. Most gram positive isolates 40/50 (80%) showed multiple drug resistance (resistance to two or more drugs) (data not presented here) (Table 3).

Table No. 03: Antimicrobial susceptibility pattern of Gram positive bacteria

Bacteria Isolation		Antimicrobial susceptibility (%)									
		AMP	**AMX**	**CRO**	**CAF**	**CIP**	**CN**	**NOR**	**TE**	**SXT**	**ERY**
S.aureus											
n = 35	**S**	31.4	42.9	74.3	25.7	82.9	85.7	71.4	40	31.4	31.4
	I	0	2.9	5.7	2.9	5.7	2.9	0	0	2.9	0
	R	68.6	54.2	20	71.4	11.4	11.4	28.6	60	65.7	68.6
S.pnemoniae											
n = 10	**S**	40	60	80	80	90	90	80	40	30	50
	I	10	10	10	0	10	0	0	0	0	10
	R	40	30	10	20	0	10	20	60	70	40
S.pyogenes											
n = 5	**S**	40	60	100	100	60	60	60	0	20	60
	I	20	40	0	0	0	0	0	0	0	0
	R	40	0	0	0	40	40	40	100	80	40
Total											
n=50	**S**	34	48	78	76	82	84	72	36	30	38
	I	4	8	6	2	6	2	0	0	2	2
	R	62	44	16	22	12	14	28	64	68	60

S = Sensitive **CRO** = Ceftriaxone **I** = Intermediate **CAF**= Chloramphenicol
R = Resistance **CIP** = Ciprofloxacin **AMP** =Ampicilline **CN** = Gentamicin
AMX =Amoxacilline **NOR** = Norfloxacine **TE** = Tetracycline **SXT** = Trimethoprim-Sulphamethoxazole
ERY =Erythromycin

Gram Negative bacteria

The susceptibility pattern of gram negative bacteria (n=66) isolated from discharging ear against 10 antimicrobial were presented in Table 4. All the isolates showed high resistance to erythromycin (>80%), intermediate level resistance (60-80%) to ampicilline, chloramphenicol, tetracycline and trimethoprim-Sulphamethoxazole and low level of resistance (<60%) to the remaining drugs. Of 66-gram negative isolates, 44 (66.7%) isolates were also identified as multiple drug resistant (data not presented here). In general ceftriaxone, ciprofloxacin, gentamicin and norfloxacine were the most effective drugs against the tested gram positive and gram negative bacteria (Table 3 & 4).

Table No. 04: Antimicrobial Sensitivity pattern of gram negative bacteria isolated from ear discharge.

Bacteria		Antimicrobial Susceptibility in %									
		AMP	**AMX**	**CRO**	**CAF**	**CIP**	**CN**	**NOR**	**TE**	**SXT**	**ERY**
P. Mirabilis											
n = 14	S	62.5	83.3	100	41.7	100	50	83.3	16.7	41.7	18
	I	4.2	0	0	0	0	15	0	4.2	4.2	0
	R	33.3	16.7	0	58.3	0	35	16.7	79.1	54.1	82
P. vulgaris											
n = 10	S	35	50	65	35	90	60	85	10	30	15
	I	5	5	10	0	5	10	0	5	5	0
	R	60	45	25	65	5	30	15	75	65	85
Pseudomonas aerugins											
n = 30	S	0	0	50	0	90	55	80	20	0	0
	I	0	0	0	0	0	10	0	0	0	0
	R	100	100	50	100	10	35	20	80	100	100
K. Pneumonia											
n = 6	S	0	0	33.3	66.7	83.3	66.6	66.7	83.3	33.3	0
	I	0	0	16.7	0	0	16.7	0	0	0	0
	R	100	100	50	33.3	16.7	16.7	33.3	20	66.7	100
Citrobacter baraaki											
n = 4	S	25	25	100	25	0	25	25	0	0	0
	I	0	25	0	0	25	0	0	0	0	0
	R	75	50	0	75	75	75	75	100	100	100
E. Coli											
n = 2	S	0	50	50	100	100	100	100	50	50	0
	I	50	0	0	0	0	0	0	0	0	0
	R	50	50	50	0	0	0	0	50	50	100
Total											
n=66	S	34.8	48.5	74.2	34.8	87.9	60.5	77.3	21.2	28.8	7.6
	I	4.5	3	4.5	0	3	20.4	0	3	3	0
	R	60.7	48.5	21.3	65.2	9.1	19.1	22.7	75.8	68.2	92.4

S = Sensitive **CRO** = Ceftriaxone **I** = Intermediate
CAF= Chloramphenicol **R** = Resistance **CIP** = Ciprofloxacin
AMP=Ampicilline **CN** = Gentamicin **AMX**=Amoxacilline
NOR= Norfloxacine **TE** = Tetracycline **SXT** = Sulphamethoxazole
ERY=Erythromycin

Discussion and Conclusions

The prevalence of ear infections due to bacteria has been reported to be higher in developing countries like Ethiopia compared to developed countries[8]. The rate of bacteria isolation from discharging ear in our study was 90% and slightly higher in female compared to male and age group between 0-5years. Similar result also reported in previous studies done in Ethiopia[10,12] and other developing countries[15].

The observation in this study is that gram-negative bacteria were the predominant isolations (56.9%) when compared to gram-positive bacteria (43.1%) from 120 patients with ear discharge (Table 2). This finding is in agreement with the previous studies in Ethiopia[10,12] and elsewhere in the world[8, 16, 17],but is contradictory to other reports such as a study from University of Uyo teaching hospital Nigeria[18] which reported a gram-positive bacteria rate of 67.1%. *Staphylococcus aureus* was the most common isolates (30.2%) followed by *Pseudomonas aeruginosa (25.9%) and Proteus mirabilis* (12.1%). Generally the kinds of bacteria isolated from ear discharge in our study were inline with previous findings done in Ethiopia[10] and Nigeria[18]. The frequency of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated attributed to the ubiquitous nature of *Pseudomonas aeruginosa* and the availability of *Staphylococcus aureus* as normal flora of mouth, nares and other non sterile sites[18], the virulence nature and rapid colonization properties of these 2 organisms contributes for their high rate of recovery.

However, if we see the distribution of specific bacteria our study reported in contrast with previous studies done in Ethiopia[10] and Nigeria[5] where *Pseudomonas aeruginosa* was the most common isolates followed by *Staphylococcus aureus* and *Proteus mirabilis*. In addition similar study in Ethiopia[12] and Sudan[15,20] indicated that Proteus species was the most common isolates followed by *Staphylococcus aureus*. These indicated that the pattern of bacteria isolated

changed over time. Coagulase negative Staphylococci (CONS) was detected in 5/120(4.2%) of the specimens (data not presented here). These were considered as contaminants in this study since the normal bacterial flora of the external ear canal is predominately CONS and Corynebacterium[21].

This study also provides insights in to the sensitivity profile of bacteria isolated from ear discharge (Table 3&4). Our results have demonstrated that in general both gram-positive and gram-negative bacteria isolated from ear discharge showed low level of resistance to most (60%) of anti microbial agents tested. This is in agreement with previous study done in Ethiopia[11, 22]. However, there are reports from different parts of the world with high resistance to these antimicrobial agents except Ciprofloxacin, norfloxacin, gentamicine and Ceftriaxone[9, 15,23]. This difference in the sensitivity profile might be due to the frequency of usage of these drugs for the treatment of ear infection in different geographical locations. In our study, ceftriaxone, ciprofloxacin, norfloxacin and gentamicine 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[10, 11, 12, 15]. The effectiveness of these drugs against the tested organisms in our study could be the reflection of infrequency prescription of these drugs by the physicians in Ethiopia and expense of the drugs in addition to the difficulty of administration (personal communication). In this study, multiple drug resistance (resistance to two or more drugs) where observed in 40/50 (80%) and 44/66 (66.7%) gram positive and gram negative bacteria, respectively. Comparable result also reported in similar studies[10, 11, 19]. It is well known fact that microbial drug resistance is growing global problem.

An overall high bacteria isolation rate of 90% was observed among children having ear discharges. The gram negative bacteria were the predominant in prevalence compared to the gram positive bacteria. Among all isolates, *S. auerus* is the most predominant. ceftriaxone, ciprofloxacin, norfloxacine and gentamicin were the most effective drugs when compared to other drugs tested against the gram negative and gram positive bacteria. Prescription of antibiotics for ear infection in the Ethiopian setting is empirical; therefore it is recommended that treatment of ear infection should be based on culture and sensitivity. Continuous surveillance is needed for the resistance bacteria to provide the basis of alternative treatment.

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