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Bacteriological Study of Otitis Media and Its Antibiotic Susceptibility Pattern

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ABSTRACT

Background: Otitis Media is a prevailing and notorious infection in developing countries causing serious local damage and threating complication. Mainly in developing countries like Nepal, Otitis Media results because of illiteracy, poverty and poor hygiene. The aim of this study was to determine the profile of Otitis Media, its causative agents and their antibiotic susceptibility pattern.

Methods: The study included 263 pus samples from 240 patients attending ENT department of Bharatpur hospital from May 2015 to January 2016. Samples were processed in microbiology department for bacteria using standard operating protocol. Antibiotic susceptibility testing was performed for all bacterial isolates by Kirby Bauer disc diffusion method and the results were interpreted according to clinical and laboratory standard institute (CLSI) guideline.

Results: Out of 240 patients, 121 were female and 119 were male. Highest incidence of Otitis Media was observed in 1-10 year age group. Out of 263 samples taken from 240 patients, 216 showed bacterial growth. Gram negative bacteria predominated and the most common bacteria isolated were *Staphylococcus aureus* 36.11% followed by *Pseudomonas aeruginosa* 33.33% and Coagulase Negative Staphylococci 8.08%. All bacterial isolates were sensitive to gentamycin. *Staphylococcus aureus* was sensitive to Amikacin and gentamycin. All gram negative bacterial isolates were sensitive to Imipenem and gentamycin. 100% of *Pseudomonas aeruginosa* was sensitive to Imipenem.

Conclusions: *Staphylococcus aureus* was the most predominant organism isolated from the pus swab followed by *Pseudomonas aeruginosa* and all the isolated organisms were sensitive to Gentamycin.

Keywords: Antibiotic; bacteria; otitis media; *staphylococcus aureus.*

INTRODUCTION

Otitis Media (OM) is an inflammation of the middle ear cleft, with or without intact tympanic membrane. The aetiology and pathogenesis of otitis media are multifactorial and include genetic, infections, allergy, environmental, social & racial factors and eustachian tube dysfunction.[1] Otitis media, both acute and chronic is highly prevalent worldwide.[2]

In Nepalese context, approximately 16% of the population above the age of 5 years suffer from otitis media. More than 55% of these cases occur in school going children, most of them belonging to the lower socio-economic class.[3] Development and spread of resistant bacteria due to the over and indiscriminate use of antibiotics was a global public health threat.[4]

Reduction of the incidence of OM is greatly prioritized with the early diagnosis of OM, its etiological agents and antibiotic susceptibility pattern. Hence, the aim of this study is to identify causative organisms and their sensitivity towards antibiotics

METHODS

A hospital based cross-sectional descriptive analytical study was carried out in the department of microbiology in Bharatpur Hospital, Chitwan from May 2015 to January 2016. Two hundred and forty clinically diagnosed OM patients, having ear discharge with unilateral or bilateral disease attending out-patient department of ENT were included in the study. HIV patients sample were excluded. In order to avoid selection bias, repeated samples from the same person were not included. In case of bilateral infection, samples were taken from both ears. So, in total 263 middle ear samples were collected from the

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total 240 OM patients of all age groups and genders.

Ethical approval was taken from Nepal Health Research Council (NHRC) on 09 September 2015 and the study was approved by authorities of Bharatpur Hospital. The participants were involved in this study after their volition and written informed consent.

An otolaryngologist diagnosed and evaluated OM by otoscopic examination. Each patient requested for culture was directly interviewed for his or her clinical history during sample collection. The questionnaire was filled documenting name, age, sex and clinical information including chief complain, side of ear affected, and types of Otitis Media.

The samples were labelled with date, time, patient's full name, age, sex and registration number. Before sample collection, the external ears were cleansed with sterile cotton swabs moistened with sterile normal saline.[5] A sterile aural speculum of appropriate size was introduced into external auditory canal to avoid contamination and a specially designed sterile swab stick of appropriate size was carefully introduced into the ear and rotated to collect the discharge; the swab stick was then carefully removed to avoid contact of the stick with the external meatus and aseptically replaced into the clean, sterile, leak-proof screw capped tube containing 1 ml peptone water.

Two pus samples were collected for laboratory investigation; one for direct smear stains microscopy and the other for culture. After collection, the samples were immediately transported to the microbiology laboratory for processing. The ear swabs were inoculated onto Chocolate Agar (CA), Sheep Blood Agar (SBA), Mannitol Salt Agar (MSA) and Mac Conkey Agar (MA) plates by the semi-quantitative culture technique using a standard calibrated loop by forming first primary inoculums followed by streaking. The SBA, MSA and MA plates were incubated at 37 ºC for 24 hours in incubator aerobically, whereas CA plates were kept in a candle jar, which can generate about 5 % CO_2 at 37°C for 48 hours.The identification of the isolates was done based on a standard microbiological technique microscopic examination, colony morphology and biochemical tests such as indole test, MR-VP test, citrate utilization test, TSIA test, etc.[6] Bacterial susceptibility test to antimicrobial agents was done *in vitro* by modified Kirby-Bauer disc diffusion method using fresh 0.5 McFarland standard culture suspension of isolates in Muller Hinton II agar medium following CLSI recommendations.[7] Control strains of ATCC i.e. *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used for the quality control. Results obtained were analysed by SPSS version 16.

RESULTS

Total 263 ear swabs were studied from 240 cases of which 216 (82.13%) ear swabs showed growth and 47 (17.87%) showed no growth. Unilateral infection was seen in 220 (91.7%) patients and bilateral in 20 (8.3%). Of the cases studied, 121 (50.4%) were female and 119 (49.6%) male. Similarly, higher incidence of Otitis Media was seen in 1-10 years of age group and it decreases as the age increases. Among the 240 patients, no growth was seen in 24 patients. Of the 216 patients showing microbial growth, 109 were female and 107 male. Age-wise high bacterial growth was observed in 1-10 years (48) and 11-20 years of age group being 48. Table 1 shows gender wise distribution of culture positive and culture negative pus samples.

Table 1. Gender wise distribution of culture positive and culture negative pus samples.

Gender	Growth positive		No growth		Total	P-value
	No.	%	No.	%		
Male	107	89.9%	12	10.1%	119	P>0.05
Female	109	90.1%	12	9.9%	121	
Total	216	90%	24	10%	240	

The growth was found to be higher in female patients 109 (90.1%) than in male patients 107 (89.9%). Pattern of ear swab culture between age and gender was statistically significant (P<0.05). The highest number of patients belonged to age group 1-10 years (55) followed by age group 11-20 years (54). Out of 216 culture positive samples, highest number of samples (23.6%) was from the age group 1-10 years, followed by the age group 11-20 years (22.2%). The patients in the age groups above 41-50 and above 50 were relatively found to be less affected.

Out of 216 bacterial isolates, 99 were Gram positive bacteria, of which *S. aureus* 78 (78.8%) was the most predominant species isolated, followed by Coagulase negative Staphylococci (CoNS) 19 (19.2%) and *Streptococcus* spp. 2 (2.0%) as represented in Figure 1. Out of 216 bacterial isolates, 117 were Gram negative bacteria of which *Pseudomonas aeruginosa* 72 (61.5%) was the most predominant species followed by *Klebsiella pneumoniae* 13 (11.1%), *P. mirabilis* 9 (7.7%), *Acinetobacter* complex. 7 (6.0%), *E. coli* 5 (4.3),

Klebsiella oxytoca 3 (2.6%), *Enterobacter* spp. 2 (1.7%) *Aeromonas hydrophillia* 1 (0.9%), *Proteus vulgaris* 1 (0.9%) and *Citrobacter* spp. 1 (0.9%). Other organisms (*Morganella morganii* and *Burkholderia* spp.) 3% of the gram negative isolates.

Out of 240 cases studied, 15 (69.9%) were of chronic type of otitis media and 65 (30.1%) of acute type. So, the most prevalent type of otitis media was chronic type in this study. *Staphylococcus aureus* were predominant in chronic suppurative otitis media 58 (74.4%) whereas *Pseudomonas aeruginosa* was predominant in acute otitis media 24 (33.3%).

Imipenem was found to be the most effective drug for bacterial isolates which were (95.8%) sensitive, followed by Amikacin (92%), Gentamycin (90.8%), Chloramphenical (74%) and Piperacillin/Tazobactam (72.2%). Amikacin was observed as the most effective drug for both gram positive and gram negative bacterial isolates, which had 92.7% and 91.4% sensitivity for gram ppositive and negative respectively.

The most effective antibiotic against *S.aureus* isolated from the ear pus sample was Amikacin (100%), followed by Gentamycin (86.5%), Chloramphenical (83.4%), Clindamycin (80%), Cefotaxime (76.2%), Ceftriaxone (62.5%) and Cotrimoxazole (55.2%). The least effective antibiotic was found to be Ampicillin (11.3%) and Penicillin-G (5%)(Table 2).

Table 2. Antibiotic susceptibility pattern of *S.aureus*.

Antibiotics used	Susceptible No (%)	Intermediate No (%)	Resistant No (%)	Total
Amikacin (AK30mcg)	44(100%)	_	_	44
Ampicillin (AMP10mcg)	6(11.3%)	10(18.8%)	37(69.9%)	53
Cefotaxime (CTX30mcg)	16(76.2%)	5(23.8%)	_	21
Ceftazidime (CAZ30mcg)	3(16.7%)	5(27.8%)	10(55.5%)	18
Ceftriaxone (CTR30mcg)	30(62.5%)	12(25%)	6(12.5%)	48
Chloramphenicol (30mcg)	60(83.4%)	7(9.7%)	5(6.9%)	72
Ciprofloxacin (CIP5mcg)	30(39.5%)	29(38.2%)	17(22.3%)	76
Clindamycin (CD2mcg)	60(80%)	4(5.3%)	11(14.7%)	75
Trimethoprim/Sulphamethoxazole (COT1.25/23.75mcg)	37(55.2%)	12(17.9%)	18(26.9%)	67
Erythromycin (E15ncg)	22(37.9%)	9(15.5%)	27(46.6%)	58
Gatifloxacin (GAT5mcg)	7(26.9%)	3(11.6%)	16(61.5%)	26
Gentamicin (GEN10mcg)	64(86.5%)	4(5.4%)	6(8.1%)	74
Penicillin-G (P10units)	1(5%)	_	19(95%)	20
Piperacillin/Tazobactam (PIT100/10mcg)	28(54.9%)	18(35.3%)	5(9.8%)	51
Tetracycline (TE30mcg)	14(73.7%)	1(5.3%)	4(21%)	19

Table 3. Antibiotic susceptibility pattern of *P. aeruginosa*.

Antibiotics used	Susceptible No (%)	Intermediate No (%)	Resistant No (%)	Total
Amikacin (AK30mcg)	33(89.2%)	--	4(10.8%)	37
Amoxycillin/Clavulinic Acid (AMC20/10mcg)	9(21.9%)	1(2.4%)	31(75.7%)	41
Cefotaxime (CTX30mcg)	28(65.1%)	10(23.3%)	5(11.6%)	43
Ceftazidime (CAZ30mcg)	24(75%)	4(12.5%)	4(12.5%)	32
Ceftriaxone (CTR30mcg)	32(57.1%)	15(26.8%)	9(16.1%)	56
Chloramphenicol (C30mcg)	26(55.3%)	8(17%)	13(27.7%)	47
Ciprofloxacin (CIP5mcg)	63(87.5%)	1(1.4%)	8(11.1%)	72

Trimethoprim/Sulphamethoxazole (COT1.25/23.75mcg)	13(30.2%)	3(7%)	27(62.8%)	43
Gatifloxacin (GAT5mcg)	23(82.1%)	2(7.2%)	3(10.7%)	28
Gentamicin (GEN10mcg)	64(91.4%)	_	6(8.6%)	70
Imipenem (IPM10mcg)	66(100%)	_	--	66
Meropenem (MRP10mcg)	26(92.9%)	_	2(7.1%)	28
Norfloxacin (NX10mcg)	17(89.4%)	1(5.3%)	1(5.3%)	19
Piperacillin/Tazobactam (PIT100/10mcg)	52(74.3%)	15(21.4%)	3(4.3%)	70
Polymyxin-B (PB300units)	49(98%)	_	1(2%)	50

The most effective drugs against *P. aeruginosa* isolated from the ear samples was Imipenem (100%), followed by Polymyxin-B (98%), Meropenem (92.9%), Gentamycin (91.4%), Amikacin (89.2%), Ciprofloxacin (87.5%), Gatifloxacin (82.1%), Ceftazidime (75.0%), and Piperacillin/Tazobactam (74.3%). The least effective drugs were Amoxycillin/Clavulinic Acid (21.9%) and Cotrimoxazole (30.2%)(Table 3).

DISCUSSION

Out of 240 cases, 119 (49.6%) were male patients and 121 (50.4%) were female patients where female predominance was higher than male The prevalence of otitis media was not significantly affected by gender. The dissertation conducted by Hassan et al also found the similar result of female predominance (52.7%) over male (47.3%).[5] The higher incidence of female was also found in similar studies carried out by Kristo and Buljan[8] and Loy et al.[9] But in study done by Koppad,[10] Lodhi et al.,[11] Yousuf et al.,[12] Kumar and Seth[13] and Gul et al.,[14] male was predominant.

In this study, age group 1-10 years had the high prevalence of OM. A total of 55 (22.9%) patients of the total OM cases were found in this age group. Previous study done by Ahmad and Kudi,[15] Shrestha et al.,[3] Jha et al.[16] and dissertation conducted by Koppad[10] also found similar result. It was in contrast to the study done by Raakhee et al., where the prevalence was in age group of 16-25 years.[17] Due to short eustachian tube, infected material from the nose, adenoids and sinuses more readily pass along the Eustachian tube to the tympanic cavity particularly during coughing, sneezing, vomiting, and forced feeding commonly practiced in our environment with the child's nose blocked, while being held head down and half prone.[18]

In the present study, 98 (45.7%) gram positive and 118 (54.6%) gram negative organisms were isolated. Similar type of studies performed by Abera and Kibert,[19] Kumar and Seth,[13] Gul et al.,[14] and Iqbal et al.,[20] also showed predominance of gram negative bacteria. But it differs with the study done by Vaidya et al.[21] and Loy et al.,[9] where gram positive bacteria predominated. This variation might be due to differences in the etiological distribution of OM in accordance to climatic conditions and cultural practices.[22]

Altogether 13 different bacterial isolates were found in this study. Among the isolates, *Staphylococcus aureus* (36.11%) was found to be the most predominant organism followed by *Pseudomonas aeruginosa* (33.33%), Coagulase Negative Staphylococci (8.80%), *Klebsiella pneumonia* (6.02%), *Proteus mirabilis* (4.17%), *Acinetobacter complex* (3.24%), *E.coli* (2.31%), *Klebsiella oxytoca* (1.39%), *Enterobacter* spp. (0.93%), *Streptococcus* spp. (0.93%), *Aeromonas hydrophila* (0.46%), *Citrobacter* spp. (0.46%), *Proteus vulgaris* (0.46%) and others (1.39%). The result is also in harmony with the study done by Shrestha et al.,[3] Kristo and Buljan,[8] Ayson et al.[23] and Nia et al.[24] The frequency of *Staphylococcus aureus* in middle ear infections can be attributed to their ubiquitous nature and high carriage of resistant strains in the external auditory canal and upper respiratory tract.[25] The second most common organism isolated was *Pseudomonas aeruginosa* (33.33%). Kristo and Buljan[9] also found the similar result where Vaidya et al.[21] found it as third common organism. The warm and wet environment of the ear canal and middle ear cavity may be conductive to the growth of *P. aeruginosa* or its spread from one patient to another via medical instruments in outpatient clinics.[26]

Out of 240 cases studied, 151 (69.9%) were of chronic type of otitis media and 65 (30.1%) of acute type. So, the most prevalent type of otitis media was chronic type in this study. *Staphylococcus aureus* were predominant in chronic suppurative otitis media 58 (74.4%) where as *Pseudomonas aeruginosa* were predominant in acute otitis media 24 (33.3%). Similar result was obtained from the study carried out by Wasihun and Zemene where chronic type was 71% and acute type 29%.[27] Possible explanation to this difference in isolation rate might be

related to the effect of climate. Bacterial colonization of otitis media increases as temperature rises which in turn increases the isolation rate of bacteria.[22]

Among the gram positive bacteria *S.aureus* was 100% sensitive to Amikacin. Gentamycin was 86.5% effective, chloramphenical 83.4% sensitive and clindamycin 80%. Moreover cefotaxime was found to be effective to 76.2% of the *S.aureus* and ceftriaxone 62.5%. In contrast, penicillin was effective to 5%.In the study carried out by Kristo and Buljan, *Staphylococcus aureus* showed a marked sensitivity to gentamycin (88.5%), erythromycin (92.3.0%), azithromycin (92.3%), sulphomethoxzol/ trimethoprim (92%) and clindamycin (91.7%).[8] In the study done by Ahmad and Kudi, 89% *S.aureus* were sensitive to gentamycin, 65% to erythromycin and 61% to chloramphenical.[15] All of the bacterial isolates were resistant to Penicillin. The study conducted by Ayson et al also found that Penicillin was the least effective drug.[23]

The most effective drugs against *Pseudomonas aeruginosa* isolated from the pus samples were Imipenem (100%), followed by by Polymyxin-B (98%), Meropenem (92.9%), Gentamycin (91.4%), Amikacin (89.2%), Ciprofloxacin (87.5%), Gatifloxacin (82.1%), Ceftazidime (75.0%) and Piperacillin/Tazobactam (74.3%). The least effective drugs were Amoxycillin/Clavulinic Acid (21.9%) and Cotrimoxazole (30.2%). Kristo and Buljan reported that *Pseudomonas* also showed a marked sensitivity to third-generation cephalosporins from 78.6% to 100%, imipenem (91.7%) and piperacillin (75%), and moderate sensitivity to gentamycin (52.6%).[10] In study done by Raakhee et al., *Pseudomonas* showed high sensitivity to Ciprofloxacin (92.3%), Gentamicin (84.61%), Imipenem (84.61%), Piperacillin (88.46%).[17] Gul et al., found *P.aeruginosa* to be most sensitive to ciprofloxacin followed by Amikacin, ceftazidime and gentamycin.[14] In contrast, Iqbal et al found Imipenem to be 100% sensitive and gentamycin only 50%.[20]

Inadequate antimicrobial treatment defined as ineffective treatment of infection is an important factor in the emergence of antibiotic resistant bacteria. It is therefore important to monitor the changing trends in bacterial infections and their antimicrobial susceptibility patterns.

CONCLUSIONS

Gram negative isolates were higher (54.17%) than gram positive isolates. *S. aureus* was the most predominant organism followed by *P. aeruginosa, Coagulase negative Staphylococci* and *Klebsiella* spp.. The most effective antibiotic for Gram positive isolates was Amikacin followed by Gentamycin , Chloramphenical, Clindamycin, and Ceftriaxone. Similarly, against Gram negative bacteria, Imipenem, Gentamycin, Meropenem, and Amikacin were most effective. Thus, this study concluded that ear infection remains an ongoing problem. The main culprit for ear infection is the trivial organisms like *P. aeruginosa, E. coli, S. aureus, Klebsiella* spp., etc. Continuous and periodic evaluation of microbiological pattern and antibiotic sensitivity of isolates is necessary to decrease the potential risk of complications by early institution of appropriate treatment.

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