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Research Article

Bacterial Isolates and Antibiogram Profile in Clinically Suspected Otitis Media Patient

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Abstract

Background: Otitis media (OM) is a prevalent inflammatory condition of the middle ear that can result in significant morbidity and preventable hearing loss, particularly in the developing world. The management of otitis media is complicated by the emergence of resistance to beta-lactam and other antibiotics among common pathogens. The aim of the study was to identify bacteria causing otitis media and perform their antibiotic susceptibility profile.

Methodology: This study was a laboratory based cross sectional study carried out among all the inpatients and outpatients who visited the hospital during study period. Pus swab samples were collected, processed and identified as per the standard guideline of American Society of Microbiology (ASM). The antibiotic sensitivity test was performed by Kirby bauer disk diffusion method on Mueller Hinton Agar. Among the isolates, phenotypic tests were performed for the detection of Extended-spectrum beta-lactamases (ESBL) and methicillin resistance.

Results: Eight (7.3%) and 88 (80.7%) of the 109 processed specimens had fungal and bacterial growth, respectively. Staphylococcus aureus 34 (38.5%) and Pseudomonas aeruginosa 30 (34.1%) were the most prevalent isolates. It was found that 18(20.5%) of the isolates were multidrug resistant (MDR), and 5(5.7%) of those isolates were gram-negative bacilli that produced ESBL. Eight (23.5%) of the 34 Staphylococcus aureus isolates tested positive for methicillin resistance.

Conclusion: The most prevalent isolate in this study was Staphylococcus aureus, followed by Pseudomonas aeruginosa. Our findings demonstrate that while piperacillin-tazobactam remains the most effective antibiotic for gram-negative bacteria, vancomycin and cefepime provide strong coverage against most gram-positive bacteria. However, a concerning trend was observed with a higher level of MDR among the bacterial isolates. This underscores the urgent need for targeted interventions in antibiotic prescribing practices. To combat the rise of MDR and reduce the risk of ineffective treatment, it is strongly recommended that ear swab cultures and sensitivity tests be conducted prior to antibiotic prescription.

Keywords: Otitis Media; MDR; MRSA; ESBL; Antibiotics

Introduction

Otitis media (OM) is a group of complex infective and inflammatory conditions affecting the middle ear. OM is a leading cause of healthcare visits worldwide and its complications are important causes of preventable hearing loss, particularly in the developing world. There are various subtypes of OM. These include acute otitis media (AOM), otitis media with effusion (OME) and chronic suppurative otitis media (CSOM). AOM is characterized by the rapid onset of symptoms such as otalgia and fever, while otitis media with effusion (OME) involves a chronic inflammation with fluid accumulation in the middle ear but without acute infection signs [1]. Middle ear effusion can present as serous, mucoid, or purulent fluid, often resulting from AOM or OME [2]. CSOM is long-standing suppurative middle ear inflammation, usually with persistently perforated tympanic membrane [3].

OM is particularly prevalent in developing countries, where incidence is higher due to factors such as malnutrition, overcrowding, poor hygiene, inadequate healthcare, and recurrent upper respiratory tract infections [4]. According to WHO, the global burden of OM involves 65–330 million individuals with draining ears, and 60% of these cases (39–200 million) suffer from significant hearing impairment. Chronic suppurative otitis media (CSOM) accounts for 28,000 deaths and over 2 million disability-adjusted life years [5]. More than 80% of children will develop AOM at least once before the age of 3, and 40% will experience six or more recurrences by age 7 [6].

Streptococcus pneumoniae, Haemophilus influenzae, and *Staphylococcus aureus* are the most common bacteria associated with OM, and its management is complicated by the emergence of antibiotic resistance; resistance to β-lactam and other antibiotics [7]. While *Streptococcus pneumoniae* and non-typeable *Haemophilus influenzae* are commonly implicated, the prevalence of resistance is not well documented [8].

The emergence of multidrug-resistant (MDR) strains of *Streptococcus pneumoniae* necessitates a re-evaluation of initial antibacterial therapy for OM and the management of patients who do not respond to treatment. This study addresses key objectives: isolating and identifying bacteria causing OM, determining their antibiotic susceptibility profiles, and identifying methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESBL) producers. This research is particularly significant in developing countries like Nepal, where healthcare resources are limited, and the economic burden of antibiotic-resistant infections is high. Understanding the bacterial etiology and resistance patterns in OM is crucial for developing effective treatment protocols and preventive measures. This can reduce morbidity, mortality, hospital stays, and economic impact, particularly in resource-limited settings.

Materials and Methods

A laboratory based cross-sectional study was conducted over the period of six months (March 2019 to August 2019) at the Department of Laboratory Medicine, Manmohan Memorial Institute of Health Sciences, and Manmohan Memorial Teaching Hospital, in Kathmandu, Nepal.

Sample collection, processing and bacterial isolation

Two ear swab samples were collected aseptically from each individual clinically suspected otitis media. One sample was taken for gram's staining technique and the other for culture and sensitivity test. The samples were inoculated on nutrient agar, Mac-Conkey agar, blood agar and chocolate agar and incubated aerobically at 37°C for 24 hours. But, Chocolate agar plate was incubated in a CO_2 incubator. Then, the isolated bacteria were identified by colonial appearance, gram reaction, cultural characteristics and biochemical tests like Catalase, Coagulase, Oxidase, Citrate utilization test, Urea hydrolysis test and Sulphide Indole Motility test .

Screening and detection of MDR

Antimicrobial susceptibility testing of the isolates was performed by Kirby Bauer Disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines [9] in Mueller Hinton Agar. The antibiotics tested were ciprofloxacin, azithromycin, gentamycin, ceftazidime, chloramphenicol, cotriomoxazole, imipenem and ampicillin. The isolates resistant to at least one antimicrobial agent of three classes of antimicrobial groups were regarded as MDR [10]. The initial screening test for the production of ESBL was performed using aztreonam (30ug) and cefotaxime (30ug) discs (HI Media India). The suspected ESBL producers were then swabbed on to Muller Hinton Agar (MHA) plate, and phenotypic detection of ESBL producers was performed by double disk synergy test and Combination disk method [9]. All gram positive cocci were tested with 30µg Cefoxitin on Muller Hinton Agar for methicillin resistance screening. For each strain, a bacterial suspension adjusted to 0.5 McFarland turbidity standard was used. The zone of inhibition was determined after 24hr incubation at 35ºC. Zone size was interpreted according to CLSI guidelines.

Ethical considerations

Ethical approval was taken from Institutional Research Committee (388/2076-02-10) of Manmohan Memorial Institute of Health Sciences, Soalteemode, Kathmandu. Informed consent was taken from patients before sample collection.

Data analysis

Each sample was encoded with identification number. Finding was manually recorded and entered in Micro-soft Excel 2010. Analysis was done by SPSS Version 20 and interpreted according to frequency distribution and percentage.

Results

Eighty eight (80.7%) of the 109 ear swab samples had bacterial growth, eight (7.3%) had fungal growth, and thirteen (12%) had no growth. Monobacterial growth was the only type of growth seen. The age group of 11–20 years old showed the highest number of bacterial growth, followed by the age group of 1–10 years old, suggesting that youngsters are more susceptible to infection than adults and older people.

Staphylococcus aureus 34 (38.6%) was the most frequently isolated organism, followed by *Pseudomonas aeruginosa* 30 (34.1%). *Proteus mirabilis* 8 (9.1%), *Streptococcus pneumoniae* 7 (8%), *Klebsiella pneumoniae* 5 (5.7%), and CoNS (Coagulase Negative Staphylococcus) 4 (4.5%) are among the other organisms that were recovered [Figure 1].

The majority of gram-positive bacteria were found to be extremely sensitive to vancomycin and cefepime, but resistant to amoxicillin, gentamycin, and cotriomoxazole. The isolates of gram-negative bacteria were shown to be susceptible to piperacillin-tazobactam and extremely resistant to amoxicillin and cotriomoxazole [Table 1].

Out of total 88 bacterial isolates, 18 (20.5%) were found multidrug resistance (MDR), and 70(79.5%) were non-MDR [Table 2]. Among 34 *Staphylococcus,* 8(24%) were resistance to Methicillin (MRSA) and 26(76%) were sensitive to Methicillin (MSSA). In addition, out of 30 *Pseudomonas aeruginosa* isolates, 13.3% were ESBL producer and in *Proteus mirabilis,* it was 12.5% [Table 3].

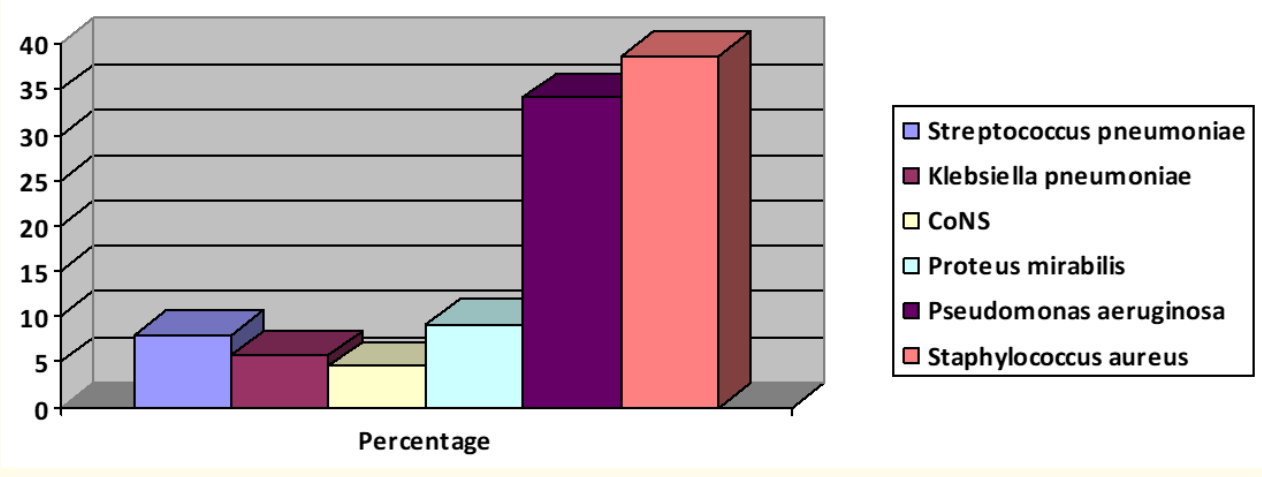

Figure 1: Distribution of bacterial isolates from ear swab samples.

Bacteria isolated		No. (%) CFX	Antibiotic Resistant Pattern of Common Isolates (%)																	
			DXC	TET	LFC	COT	VAN	AMO	LIN	CLI	ERY	CIP	CPM	AZI	GEN	CFM	OFL	CTZ	AMV	PPT	
Gram Positive	*Staphylococcus aureus*	34 (38.6)	23.5	26.5	29.4	29.4	38.2	5.9	67.6	14.7	20.6	35.3	26.5	11.8	26.5	38.2	—	—	—	—	—
	Streptococcus pneumoniae	7 (8)	0	0	14.3	0	28.6	0	100.0	14.3	0	14.3	28.6	0	0	28.6	—	—	—	—	—
	CoNS	4 (4.5)	0	25.0	0	0	25.0	0	75.0	0	0	50.0	25.0	0	0	25.0	—	—	—	—	—
Gram Negative	*Pseudomonas aeruginosa*	30 (34.1)	60.0	76.7	56.7	—	76.7	0	83.3	—	—	—	20.0	40.0	—	33.3	53.3	36.7	33.3	26.7	13.3
	Proteus mirabilis	8 (9.1)	12.5	12.5	25.0	—	25.0	0	50.0	—	—	—	12.5	12.5	—	25.0	12.5	25.0	12.5	0	0
	Klebsiella pneumoniae	5 (5.7)	0	0	0	—	20.0	0	60.0	—	—	—	40.0	0	—	20.0	0	20.0	20.0	0	0
Abbreviations: CFX- cefoxitin, DXC- doxycycline, TET- tetracycline, LFC- levofloxacin, COT- cotriomoxazole, VAN- vancomycin, AMO- amoxicillin, LIN- linezolid, CLI- clindamycin, ERY- erythromycin, CIP- ciprofloxacin, CPM- cefepime, AZI- azithromycin, GEN- gentamycin, CFM- cefixime, OFL- ofloxacin, CTZ- ceftazidime, AMV- amoxyclav, PPT- piperacillin + tazobactam																				

Table 1: Different bacteria isolated and their antibiotic susceptibility pattern.

Bacterial isolates	Total No.	Distribution of Multidrug Resistant Isolates	
		MDR N (%)	Non-MDR N (%)
Staphylococcus aureus	34	8(23.5)	26(76.5)
Pseudomonas aeruginosa	30	10(33.3)	20(66.7)
Proteus mirabilis	8	0(0.0)	8(100.0)
Streptococcus pneumoniae	7	0(0.0)	7(100.0)
Klebsiella pneumoniae	5	0(0.0)	5(100.0)
CoNS	4	0(0.0)	4(100.0)
Total	88	18(20.5)	70(79.5)

Table 2: Distribution of Multidrug Resistant Bacterial Isolates.

Bacterial isolates (n)	Distribution of ESBL Producer Gram Negative Bacilli		Distribution of Methicillin Resistance Gram Positive Cocci	
	ESBL Producer, n (%)	ESBLNon- Producer, n (%)	Methicillin Resistance, n (%)	Methicillin Sensitive, n (%)
Staphylococcus aureus (34)	-	-	08(23.5)	26(76.5)
Pseudomonas aeruginosa (30)	4(13.3)	26(86.7)	-	-
Proteus mirabilis (08)	1(12.5)	7(87.5)	-	-
Streptococcus pneumoniae (07)	-	-	0(0.0)	7(100)
Klebsiella pneumonia (05)	0(0.0)	5(100)	-	-
CoNS (04)	-	-	0(0.0)	4(100)

Table 3: Distribution of ESBL Producer and Methicillin Resistance Isolates.

Discussion

Otitis media (OM) is a leading cause of health care visits and drugs prescription. Its complications and sequelae are important causes of preventable hearing loss, particularly in developing countries. When we took ear swabs from otitis media patients for our investigation, we discovered a very high growth percentage. Eight eight (80.7%) of the 109 ear swab samples had bacterial growth, eight (7.3%) had fungal growth, and thirteen (11.9%) had no growth. Forty-five (51.1%) of the 88 growth-positive samples were obtained from males, and forty-three (48.9%) from females. In India, a study by B.L. Chaudary., *et al*. revealed 80% bacterial growth, which was comparable to the growth rate in our investigation [11].

In our study, otitis media was more common in children and young adults than in older age groups (51.4% in those under 20 and 48.6% in those over 20). In India, a similar study by Poorey VK., *et al*. revealed that children and young adults had a greater prevalence of otitis media [12]. Shrestha B L., *et al*. [13] also noted a similar outcome at Kathmandu University Hospital in Dhulikhel, where 65.2% of the patients were under 20. This observation can be explained by a number of factors, including the fact those children's upper respiratory tract infections are more common than those in adults and that their Eustachian tubes are shorter, narrower, and more horizontal [14]. These results, however, are in contrast to those of another study carried out in Singapore in 2002 by Loy AHC., *et al*. [15], which revealed that the condition was more common in people aged 31 to 40.

The most frequently isolated bacteria that caused OM in our investigation were *Staphylococcus aureus* (38.6%), *Pseudomonas aeruginosa* (34.1%), *Proteus mirabilis* (9.1%), Streptococcus *pneumoniae* (8%), *Klebsiella pneumoniae* (5.7%) and CONS (4.5%). In Yemen (2018), similar results were observed with bacterial growth of *Staphylococcus aureus* (44%), *Pseudomonas aeruginosa* (12.7%) and *Streptococcus pneumoniae* (10%) [16]. However, in the Southern California study by Lieberthal., *et al*. *Haemophilus influenza* and *Moraxella catarrhalis* were the most common organisms that caused otitis media [17]. This could be because of regional differences.

Staphylococcus aureus was shown to be more resistant to amoxicillin (67.6%), followed by gentamycin (38.2%) and cotriomoxazole (38.2%), but more responsive to vancomycin (94.1%), linezolid (85.3%), and cefepime (88.2%). While 83.3% of *Pseudomonas aeruginosa* were found to be resistant to amoxicillin and 76.7% to cotriomoxazole, the bacteria were shown to be more sensitive to piperacillin-tazobactam (86.7%), ciprofloxacin (80%), and amoxyclav (73.3%).

Iqbal K., *et al*. reported similar results in Pakistan in 2011 [18], demonstrating that *P. aeruginosa* was resistant to gentamycin and extremely susceptible to piperacillin-tazobactam. According to a study conducted in Northern Ethiopia by Araya Gebereyesus Wasihun., *et al*. [19], *P. aeruginosa* was extremely resistant to amoxicillin-clavulanic acid (88.9%), while *Staphylococcus aureus* was more resistant to tetracycline (100%) and ampicillin (100%), while being sensitive to ciprofloxacin (79%). The variation in the study population could be the cause of the discrepancy in the results.

As can be seen from the susceptibility pattern *of P. aeruginosa* and *S. aureus*, the susceptibility profile of isolated bacteria offers proof of bacterial resistance to numerous antimicrobial treatments. Antibiotic overuse and illogical use, as well as self-medication, have led to the evolution of resistant strains, creating a concerning situation that makes treating infections difficult. Twenty-five percent of the isolates in our investigation were MDR. Among isolated *E. coli* from different clinical samples, this result is less than that of Baral S. K., *et al*. [20]. Increases in morbidity and death are attributed to MRSA, a significant class of multidrug-resistant organisms [21].

Antibiotic resistance is a major concern because of the prevalence of diseases and the usage of antibiotics, which are readily available "over the counter" in Nepal and frequently do not have an antibiotic susceptibility test [22,23]. Eight (23.5%) of the 34 *Staphylococcus aureus* were determined to be methicillin-resistant (MRSA). 19% of MRSA isolates were found in similar studies carried out in India [24]. 42.5% was found in samples from ICU patients in a research by Mishra., *et al*. [25]. The preferred medication for treating infections brought on by gram-negative bacteria is beta-lactam. However, ESBL, carbapenemase production, and other pertinent processes may be responsible for the growing resistance to them, which poses a problem for the antibiotics that are currently available [26]. Out of the 30 *Pseudomonas aeruginosa* isolates, 4 (13.3%) were discovered to produce ESBL, and 1 (12.5%) of the 8 isolates of *Proteus mirabilis* were identified to produce ESBL. According to a study conducted in Nigeria, 8.2% of ESBL is produced by *Proteus mirabilis* and 28.8% by *Pseudomonas aeruginosa* [27]. These research all show that microbes are becoming more resistant.

Conclusion

This study demonstrated that vancomycin and cefepime were effective against gram-positive bacteria, while piperacillin-tazobactam showed the highest susceptibility among gram-negative bacteria in ear swab samples. Notably, the bacterial isolates exhibited increased levels of multidrug resistance, highlighting a significant public health concern. Given these findings, it is imperative that culture and sensitivity testing of ear swab samples be conducted prior to initiating antimicrobial therapy. This approach will not only ensure the effectiveness of the prescribed antibiotics but also help mitigate the risk of developing further resistance.

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