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Clinical application of a bacteriology study on Otitis media.

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Abstract

Otitis media (OM) is a notorious inflammation of middle ear that mainly affects tympanic membrane and a major health problem in developing countries causing serious local damage and threatening complications.
The focus was mainly on aerobic bacteria, involved in active OM in adults as well as children. An attempt was made, despite resource and man power constraints, to have a glimpse of the current antibiotic sensitivity pattern Early and effective treatment based on the knowledge of causative micro-organisms and their antimicrobial sensitivity ensures prompt clinical recovery and possible complications can thus be avoided.

Keywords: Otitis media, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Ciprofloxacin

1. Introduction

Otitis media is the inflammation of middle ear that may affects tympanic membrane [1, 2]. Based on duration of symptoms like ear discharge or perforation of the tympanic membrane, otitis media may be classified as acute, sub-acute, and chronic suppurative otitis Media (CSOM) [3]. When the symptoms present beyond 12 weeks it is diagnosed as chronic suppurative otitis media. Middle ear infection is primarily seen in young children (80%) compared to adults (20%) [4, 9].

The lower immunity of children as compared to adult, the shorter and more horizontal Eustachian tube in children which permits easier access of microorganisms from the nasopharynx, the fact that bacteria adhere better to epithelial cells of children than adults and soft and thin tympanic membrane in children compared to adults that allows the easy penetration of pathogenic micro-organisms have been suggested as possible reasons for the higher prevalence in children [6, 12].

Otitis media is more common among males. Male: female ratio is 1.4:1. This predominance in males may be due to their more exposed way of life [5, 8].

The disease usually occurs after upper respiratory viral infections followed by invasion of pyogenic organisms. Many studies have shown that common organisms isolated from cases of otitis media are *Pseudomonas spp*,*Staphylococcus aureus*, *Klebsiella pneumonia* and *Proteus spp*. [6, 7]

Anything that interferes with normal functioning of Eustachian tube predisposes the middle ear infection.

It could be:- Recurrent attacks of common cold, upper respiratory tract infections,extanthematous fevers (Measles, Diphtheria, Whooping cough), infections of tonsils and adenoids, nasal allergy, cleft palate, tumours of nasopharynx, smoking of any family

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members and immunodeficiency e.t.c.[10,11]

Factors which can reduce morbidity and mortality of otitis media include: - proper vaccination, breast feeding, better general health nutrition and public awareness [4, 12]

Prevalence of otitis Media is more in developing and underdeveloped countries, this incidence is also common in poorer sections of the developed world with highest percentage among low hygiene populations and malnutrition [12].The wide spread use of antibiotics has precipitated the emergence of multiple resistant strains of bacteria which can produce both primary and postoperative infection [13, 14].

The purpose of this study is to acquire data on pattern of causative agents of otitis media & the antibiotic sensitivity of the isolated organism prevalent in NIMS hospital.

2. Materials and methods

The study was conducted at the NIMS hospital, Department of Microbiology and Department of Otorhinolaryngology. The hospital is a tertiary care institution with a referral status.

2.1. Study type

Cross-sectional study.

2.2. Study duration

The study was conducted over a period of 12 months from March 2015 to March 2016.

2. 3.Sample size

A total of 89 patients, 49 (55%) males and 40(45%) females with signs and symptoms of otitis media attending Department of Otorhinolaryngology in NIMS hospital were included in this study.

2.4. Study population

Ethical committee approval was obtained from the Ethics and Research Committee of the NIMS University and consent form was also taken before starting the study. All Patients who were not on antibiotics within 7days were included in the study.

2.5. Specimen collection and processing

Detail clinical history regarding sex, age, history of ear discharge, antibiotic therapy, family history, smoking history e.tc. Was taken. Two sterile swabs were used to collect ear discharges from each patient. All specimens were transported to the laboratory and analyzed within one hour of collection. One of the swabs was used for direct gram stain.The second swab was inoculated onto blood and MacConkey agar plates. All plates were incubated aerobically at 37°C for 24 to 48 hours.

A single colony was taken from each primary positive culture on blood agar , and on MacConckey agar and it has been identified depending on its morphology (colony shape, Size, colour, border, and texture),and then examined by the microscope after being stained with Gram's stain.

After staining, the biochemical tests have been done on each isolate to complete the final identification .The antimicrobial susceptibility testing was done by the agar discs diffusion method [11].

3. Results

During the study period, ear swabs from patients have been subjected to aerobic culture on Blood and MacConkey agar. Isolates of various micro-organisms were identified on the basis of cultural characteristics, Gram staining and biochemical reactions.

Age and sex Distribution of cases

The isolates were subjected to antimicrobial susceptibility test. From these findings, Out of 89 patients, 18(20%) patients were below 15 years of age, 62(69%) patients were in age range of 15-40years while 9(11%) patients were above 40 years of age, however, all 18 patients below 5 years of age were diagnosed with OM (Table 1 and Figure 1).

Table 1 : Age and sex distribution of cases.

Age (years)	Male	Female	Total
d" 15	10(11%)	8(9%)	18(20%)
15 - 40	35(39%)	27(30%)	62 (69%)
e" 40	4(5%)	5(6%)	9 (11%)

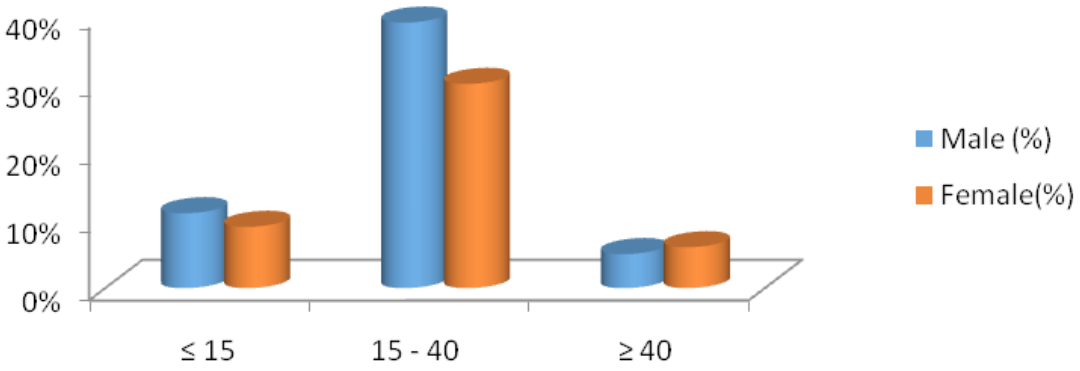


Fig. 1: Age and sex distribution of the cases (%).

Age and sex distribution of positive cases.

The results revealed that 61(68%) samples were positive, whereas 28 (32%) samples have shown negative results out of 89 total culture samples (Table2and Figure2)**.**

Table 2 : Age and sex distribution of positive cases.

Sex	Positive	Negative
Male	34(38%)	15(17%)
Female	27(30%)	13(15%)

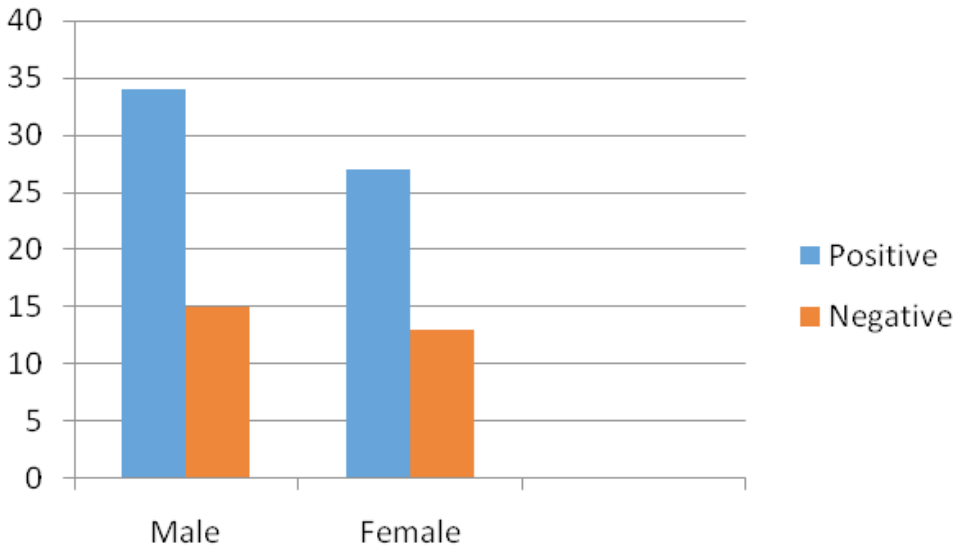


Fig. 2 : Age and sex distribution of positive cases.

Organisms isolated.

The most common organism isolated in this study was *Pseudomonas aeroginosa* 24(39%) followed by *Staphylococcus aureus* 20(33%), *Coagulase negative staphylococcus (CONS)* 13(21%), *Escherichia coli* 3 (5%), *Proteus vulgaris* 1(2%) (Table 3 and figure3).

Table 3 : Organisms isolated.

Name of bacteria	Number of isolates	Percentages (%)
Staphylococcus aureus	20	33
Coagulase negative staphylococcus (CONS)	13	21
Pseudomonas aeroginosa	24	39
Escherichia coli	3	5
Proteus vulgaris	1	2

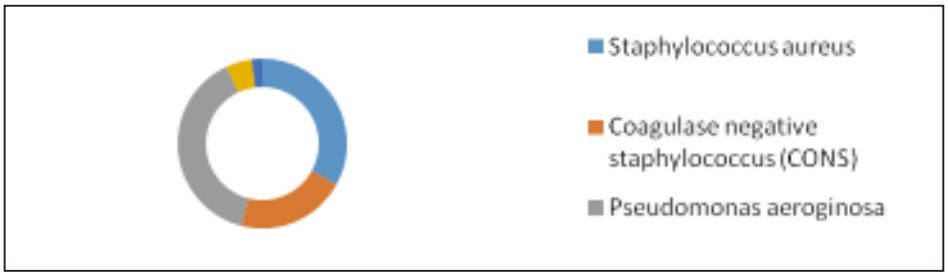


Fig. 3: Frequency of isolated organisms.

Antibiotic susceptibility pattern in *Pseudomonas aeroginosa*(n=24).

Among 24 isolates of *Pseudomonas aeroginosa*, it was sensitive for Ciprofloxacin (90%), Ceftazidime (76%), and Amikacin (68%) Imipenum (58%) and resistant to Gentamicin (62%) Cefetaxime (60%), Cefoperazone (60%), Cefepime (54%) (Table 4 and Figure 4**).**

Table 4 : Antibiotic susceptatibility pattern in Pseudomonas aeroginosa (n=24).

Antibiotics	Sensitive (%)	Resistant (%)
Ciprofloxacin	22 (90)	2(10)
Ceftazidime	18(75)	6 (25)
Amikacin	16(67)	8 (33)
Imipenum	14(58)	10(42%)
Gentamicin	9(37)	15(63)
Cefotaxime	10(42)	14 (58)
Cefoperazone	10(42)	14 (58)
Cefepime	11(46)	13(54)

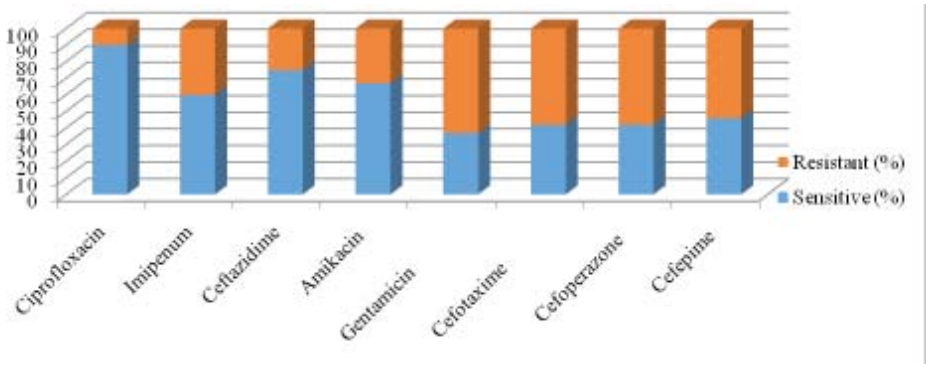


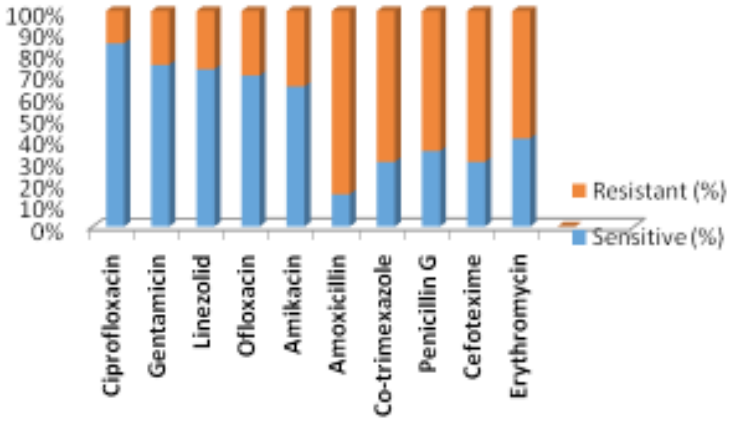
Fig. 4: Frequency of antibiotic susceptatibility pattern in Pseudomonas aeroginosa

Antibiotic susceptibility pattern in *Staphylococcus aureus* (n=20).

Among 20 isolates of *Staphylococcus aureus* (table 5 and figure 5)was sensitive to Ciprofloxacin (85%) Gentamicin (73%), linezolid (73%), Ofloxacin (70%), Amikacin (66%) and resistant to Amoxicillin (85%), Co-trimexazole (70%), Penicillin G (65%), Cefatexim (70%) and Erythromycin (65%).

Table/Fig. 5: Antibiotic susceptibility pattern in Staphylococcus aureus (n=20)

Antibiotics	Sensitive (%)	Resistant (%)
Ciprofloxacin	17 (85)	3(15)
Gentamicin	15(73)	5(27)
Linezolid	15 (73)	5(27)
Ofloxacin	14 (70)	6(30)
Amikacin	13(65)	7(35)
Amoxicillin	3 (15)	17(85)
Co-trimexazole	6 (30)	14(70)
Penicillin G	7 (35)	13 (65)
Cefotexime	6(30)	14 (70)
Erythromycin	6 (35)	13 (65)



Antibiotic susceptibility patterns in *Coagulase negative staphylococcus (n=13).*

Among 13 isolates of *Coagulase negative staphylococcus (CONS) (*table 6 and figure 6) was sensitive to Ciprofloxacin (92%), Gentamicin (77%), Ofloxacin (69%), and Amikacin (62%) linezolid (54%)

and resistant to Amoxicillin (85%), Co-trimexazole (77%), Cefataxime (62%) and Erythromycin (69%).

Antibiotics	Sensitive (%)	Resistant (%)
Ciprofloxacin	12 (92)	1(8)
Gentamicin	10(77)	3(23)
Ofloxacin	9 (69)	4(31)
Amikacin	8(62)	5(38)
Linezolid	7 (54)	6(46)
Amoxicillin	2(15)	11(85)
Co-trimexazole	3(23)	10(77)
Cefotexime	5(38)	8(62)
Erythromycin	4(31)	9(69)

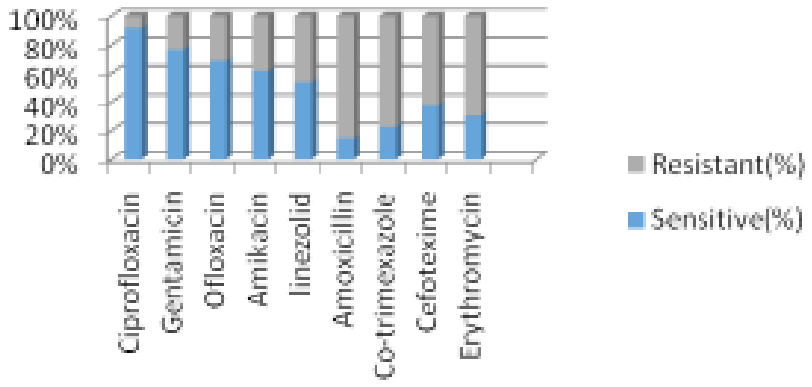


Fig. 6 : Sequence of Antibiotic susceptibility patterns in *Coagulase negatives staphylococcus [CONS].*

Few Gram negative bacteria isolated other than*Pseudomonas aeroginosa*were mostly sensitive to Ciprofloxacin (75%) and Amikacin (50%), Levofloxacin (50%) and resistant to Tetracycline (75%), Cefoperazone (75%) Cefotaxime (84%), Ofloxacin (50%).

Observations from this study, shows that *Staphylococcus spp,Pseudomonas, Proteus* are more prevalent in males than in females while *E.coli* is only isolated in males. According to this study, the disease was found to be more common in lower and middle social- economic strata of the society

4. Discussion

In this study, out of 89 samples (Ear swabs) collected from Department of Otorhinolaryngology , 61 (69%) sample were bacterial culture positive on MacConkey and blood agar for different aerobic bacteria and 28(31%) were culture negative (Table2 and figure2). In this study, 18(20%) patients were below 15 years of age, 62(69%) patients were in age range of 15-40years while 9(11%) patients were above 40 years of age, however, all 18 patients below 5 years of age were diagnosed with OM (Table 2 and Figure 2).

This is comparable to another study in which Otitis Media was most prevalent in young children than in adults, where by 20 (19.4%) were aged 18 years and below and 40 (38.5%) were aged 18 years and above. This is due to shorter, narrow and more horizontal Eustachian tube in children than in adults and also frequent upper respiratory tract infections which are more common in young people [6, 9]. The findings obtained from this study, Otitis Media was found to be common in males 49(55%) than in females 40(45%) (Table2 and Figure2**)**. This is in agreement with other studies like; Lakshmipathi and Bhaskharan

(2000), Arya and Mohapatra (2014), Baruahetal(2015), singh and Bhaskhar (2012) whose results depicted male: female ratio of (1.4:1).Male predominance may be because of their more exposed way of life [9]. According to this study, the disease was found to be more common in lower and middle social- economic strata of the society. This is because of poor nutrition, improper hygiene and lack of healthy education, causing hearing loss, an impact on speech and language development and also affects the school performance and social interactions. It was observed that both Gram positive and Gram negative organisms were responsible for middle ear infection but gram positive bacteria were more than gram negative bacteria (Table 3 and Figure 3). This is not in agreement with another study where gram negative rods 84 (72.4%) out number gram positive rods 32 (27.6%).This is due to geographic factors and variations of organisms in different communities [12]

Otalgia was the commonest mode of onset among the Otitis Media patients during the course of this study, which was high compared to another report from the study done in Iraq where Otolgia was present only in 50 (41.7%) patients. This onset of acute pain was characterized by purulent foul smelling an indication of middle ear infection [16].

The most common isolated organism was *Pseudomonas aeroginosa* followed by *Staphylococcus aureus,Coagulase negative staphylococcus* and other enteric bacteria (Table3 and Figure3). This is not different from many other studies, conducted worldwide which vary from study to study. *Pseudomonas aeroginosa* is the predominant organism in this study because it is opportunistic extracellular pathogen which thrives in the warm damp external auditory meatus Otitis Media patients [17]. This is different from the study carried out in rural area of Malawi where *Proteus mirabilis* was commonest isolated organisms (54%).

Another comparative study was done in urban areas of Kenya and Nigeria [19, 25] which showed that *Proteus mirabilis* was commonest isolate and also in urban areas of Congo and Ethiopia. Another study done by kenna *et al.,* [18] also found that *Pseudomonas* was predominant organism (67%).

In study done by S.Nikakhlagh et al [34] the most common bacterial was *Staphylococcus aureus* (32.4%) followed by *Pseudomonas aeroginosa* (21.69%).This could be attributed to the effect of climate, Ethic, geographic factors, variation of organisms in different communities and localities and different study sites which are either hospital or community based [4,12] .

In this study *E.coli* 3(5%) was isolated as the only coli form-organism (Table3 and Figure3); this is comparable to another study which reported 13 (8.4%) *E.coli* and 6(4%) *Klebsiella [21]*. More frequent isolation of faecal bacteria like *E.coli* and *Klebsiella* species depicts the individuals are at the high risk of infections due to poor hygiene conditions.

All pathogenic strains isolated in the present series were tested against various antibiotics in order to determine the suitable drug of each isolated organisms.

Antibiogram of the isolated Pathogens

The observation from this study, indicates that ciprofloxacin (more than 70%) was found to be the most effective drug against all infectious bacteria isolated, both gram negative and gram positive bacteria, then followed by Amikacin, Cefoperazoneetc (Table 4,5,6 & Figure,4,5,6).

Antibiogram of *Pseudomonas aeroginosa*

According to our study, (Table4 &Figure4**)**, 90% of the isolates of *Pseudomonas aeroginosa* were sensitive to Ciprofloxacin (90%), Ceftazidime (76%), Amikacin (68%) which was similar to the study conducted by C.Manikandan and A.Amsath (2013) sensitivity to Ciprofloxacin, Amikacin and Ceftazidime was (88%),(100%) and (73%) respectively.

In another study done by Arshi *et al.,* (2007), sensitivity to Ceftazidime, Ciprofloxacin, Gentamicin, Piperacillin and Tobromycicin was 50%, 33.33%,45.8%,78.3% and 54.2% respectively compared to the sensitivity of in this study. This difference may be due to difference in anti-microbial policies which vary from hospitals to hospitals.

Antibiogram of *Staphylococcus aureus*

A total of 20 *Staphylococcus aureus* were isolated in this study. *Staphylococcus aureus* was sensitive to Ciprofloxacin (86%) Gentamicin (73%), Linezolid (73%), Ofloxacin (69%), Amikacin (66%), and resistant to Amoxicillin (85%), Penicillin G (65%), Co-Trimexazole (70%), Cefatexime (70%) and Erythromycin (65%) (Table5 & Figure5). This was in accordance to other studies conducted by Sikkim [22] where Erythromycin was (69%) resistant, and (65%) resistant in a study done by Mongore which was found to be sensitivity to Gentamicin 70%. This study was also in agreement with the study done by Srikanth *et al.,* [23] where Erythromycin was (67%) resistant.

Antibiogram of *Coagulase negative staphylococcus (CONS)*

The present study showed a higher sensitivity to Ciprofloxacin (92%), Gentamicin (77%), Ofloxacin (69%), Amikacin (62%), linezolid (54%) and resistant to Amoxicillin (85%), Co-trimexazole (77%), Cefataxime (62%) and Erythromycin (69%) (Table 6 & Figure 6).These results were consistent with those of other studies conducted by U.Mohan et al and Rani et al [24] who also showed a higher resistance to Erythromycin i.e. 75% and 91.2% respectively and more than 80% resistant to Amoxicillin.

Antibiogram of Enterobacteraceae

In this study **three** isolates of *E.coli* and **one** isolates of *Proteus* were isolated from various samples amongst these, most of them, were sensitive to Ciprofloxacin (75%) and Amikacin (50%), Levofloxacin (50%) and resistant to Tetracycline (75%), Cefoperazone (75%) Cefotaxime (86%), Ofloxacin (50%).These results were consistent with those of Sikka et al (2012) who showed 94.4% resistance to Cefotaxime. If this finding is compared with results of various workers done in India, like the study done by prayagana, N.srinivas, moorthy and Sudhakah Ciprofloxacin drug has emerged as the most effective antibiotic useful in for patients in their study which was sensitive against more than 80% of *E.coli*, *Proteus* and other Pathogens.

Other studies like Gulati et al (2014), Mishra et al (2008) have got different results compared to this study [25]. One fact becomes obvious that bacteriology and antibiotic sensitivity pattern of Otitis Media has been changing from time to time. This is clear indication of emergence of antibiotic resistance is becoming more common in this era of antibiotics. Human negligence is a factor responsible for the development of antibiotic resistance. As soon as symptoms subside, many patients stop taking the antibiotics before completion of the therapy and allow partially resistant microbes to flourish, such practice should be condemned strongly and people should be educated to avoid the same.

5. Conclusion

Continuous variation of Bacterial profile due to mise-use of drugs, variations in climate, community and patient populations has been associated with the emergency of the drug resistant strains.Hence routine use of topical antibiotics for any case of OM as the empirical therapy must be reviewed and judicial use of antibiotics should be recommended.

Appropriate antimicrobial drugs should be prescribed after.

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