



PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIAL ISOLATES CAUSING EAR INFECTION AMONG THE PATIENTS ATTENDING TERTIARY CARE HOSPITAL, PUDUCHERRY.

Dr. G. Vaishnavadevi

Assistant Professor , Department of Microbiology, Sri Lakshmi narayana institute of medical sciences, Puducherry.

ABSTRACT Ear infection can cause pain, discharge, hearing impairment ,deafness and its related complications . Ear infection affects both children and adults particularly in developing countries. Since the emergence of antibiotic resistant organisms increasing the risk of treatment failure. The prevalence and susceptibility of isolates to antibiotics is important for patient treatment. The aim of the study to assess the prevalence of isolated bacterial pathogen and their antimicrobial susceptibility profile among patient with ear infection attended SLIMS , puducherry between Febuary 2021 – January 2022 .

Materials And Methods: Retrospective data was collected for analysis from ear samples subjected for bacterial culture and antimicrobial susceptibility pattern . Specimens such as ear swabs and aspirated fluid was collected and processed for microbiological diagnosis as per standard operating procedure (SOP) .Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standard Institute (CLSI) guidelines. Statistical Package for the social sciences (SPSS) was used to analyzed the data .

Results: Gram positive and gram negative organisms were isolated from 109 ear samples . Gram positive cocci accounted for 75 (68.8 %) and Gram negative bacilli for 34 (31.2 %) . *Staphylococcus aureus* (33%) and *pseudomonas spp* (21.1%) were the most commonly isolated organisms. In drug susceptibility testing, gentamicin had coverage against 68(90.7%) of 75 gram positive cocci and 30(88.2 %) of 34 gram negative bacilli which were tested. Antimicrobial resistance were observed for penicillin , Erythromycin ,clindamycin whereas ciprofloxacin, gentamicin, chloramphenicol and cotrimoxazole were found to be susceptible against the isolated bacteria.

Conclusion: The predominant isolate was *Staphylococcus aureus* and *Pseudomonas spp*. The antibiotic with the greatest bacterial coverage in drug susceptibility pattern was Gentamicin. Gentamicin showed good coverage against gram positive cocci and Gram negative bacilli isolated from ear infections. Ciprofloxacin showed the lowest resistance rates to all bacterial isolates.

KEYWORDS : Ear infection, Antimicrobial susceptibility pattern, Gram positive cocci, Gram negative bacilli.

INTRODUCTION:

Globally, 360 million people are suffering from hearing loss . About 60% of hearing loss is due to ear infection of which 40% can be preventable. Ear infection affect the external ear (Otitis Externa), middle ear (Otitis Media) and inner ear (Otitis Interna) .¹ Ear infection is a major problem for all age groups due to the factors : i)shorter and horizontally placed Eustachian tube ii) flaccid cartilage and iii) low immunity, especially in children . The etiology and prevalence of ear infection varies with geographical region and climatic conditions. Even skin normal flora such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Escherichia coli* that can easily enter through perforated ear causing otitis media .² Ear infection include various etiologies which can be bacteria, viruses or fungi. More than 90% cases of otitis externa caused by *Pseudomonas aeruginosa* (22–62%) and *Staphylococcus aureus* (11–34%).³

Other bacterial pathogens such as *Streptococcus pneumoniae* , *Streptococcus pyogenes* , *Haemophilus influenzae* and *Proteus* species are also responsible for the infection. *Pseudomonas aeruginosa* and *Staphylococcus aureus* produce biofilms and evoke innate inflammatory responses lead to the chronicity of Otitis media and progress to chronic suppurative otitis media despite of treatment. ⁴ Other risk factors include Upper respiratory tract infection, previous infection of Acute Otitis Media(AOM) , low social economic status, smoking, poor nutrition status .Chronic Otitis Media can cause hearing impairment and delay in speech. Chronic Otitis Media (COM) can cause intracranial and extracranial complications such facial nerve paralysis, meningitis and mastoiditis.⁵ However antibiotics are not administered based on culture and antibiotic susceptibility testing (AST) and furthermore injudicious antibiotic use increase the emergence of antimicrobial resistance. This causes change in the microbiology and antimicrobial susceptibility pattern of microorganism causing ear infections.

Therefore continuous and ongoing monitoring and surveillance is needed. ⁶ COM accounted for 90% of infection and 18.8% of hearing loss are due to the consequence of ear infection. It is a major global infectious cause of hearing impairment and deafness in developing countries mainly seen in the South East Asian region , Western Pacific and African regions where COM accounts for 34.5%, 23.7% and 16.4% of hearing loss respectively.⁷ Hence, current knowledge about prevalence and antimicrobial susceptibility pattern of the pathogenic bacteria needed to be available at hospital level to guide the rational use of antibiotics for the treatment of ear infection. Thereby prevent emergence of drug resistant bacterial strains.

MATERIALS AND METHODS:

A Retrospective study conducted from February 2021 – January 2022 at SLIMS, Puducherry. All patients who were attended ENT department with symptoms such as pain, ear discharge ,hearing impairment and clinically diagnosed patients suspected with ear infection were included in this study . We obtained demographic characteristics of patients age, gender, organisms isolated and its antibiogram from the microbiology laboratory record books . Ear swabs, Aspirated fluid were collected under sterile aseptic technique . All swab and aspirated fluid samples were inoculated onto blood agar base, MacConkey agar and chocolate agar. The inoculated blood agar and chocolate agar media were incubated at 37 degree Celsius for 18 - 24 hours within candle jar (5-10% CO₂) except MacConkey agar which incubated in aerobic conditions . Pure bacterial isolates were identified by colony morphology, gram stain, pigment production and species identification was done by biochemical test such as catalase, coagulase test for gram positive organisms .Indole production test, urease test, citrate utilization test, Triple sugar Iron test, motility test, Oxidase test, Phenylalanine deaminase test (PPA) for gram negative organisms.⁸

Antibiotic susceptibility test (AST) was performed by a modified Kirby-Bauer disc diffusion method on Mueller –Hinton Agar for identification of bacterial isolates as recommended by CLSI guidelines 2021. The bacterial suspension was prepared and equivalent to the McFarland standard (0.5CFU) followed by inoculated on Mueller –Hinton Agar plates and paper impregnated antibiotic disks was placed , then incubate for 18- 24 hours at 37 degree celsius . The following antibiotic were used for testing such as:- Gentamicin (10µg), Erythromycin (15µg), ciprofloxacin(50µg), Trimethoprim-sulphamethoxazole (1.25/23.75 µg), Penicillin (10 µg),Clindamycin (2µg), cefoxitin (30µg), Linezolid (30 µg), Chloramphenicol(30µg), Ceftazidime(10µg) , Cefotaxime(30 µg), Cefuroxime (30 µg), Cefepime (30 µg), meropenem (10µg) and Imipenem (10 µg) . Sample collection was done by standard operating procedure and laboratory analysis were followed strictly. ⁹ The known control strains were used such as *S.aureus* (ATCC 25923) and *P.aeruginosa* (ATCC 27853) for quality control .Data were collected , entered and analyzed using SPS version 20 software and results were obtained.

Ethical Consideration:

Ethical clearance was obtained from Institute Ethics Committee.

RESULTS:

A total of 204 patient samples were obtained from clinically

suspected ear infection cases . Among gender distribution , 136(66.6%) were males and 68(33.3%) females. Overall 109 (53.4%) samples yield microbial isolates. A total of 109 patients 72(52.9%) were males and 37(54.4%) females (Table:1).

Table:1 Demographic Characteristics Of Patients With Ear Infection

Demographic characteristics		
Gender	Ear infection N(%)	Total N(%)
Male	72(52.9%)	136(66.6%)
Female	37(54.4%)	68(33.3%)

Microbial isolates predominantly seen in age group between 21-30(31.2%) years of age followed by 11-20(24.8%) years of age (Table: 2).

Table:2 Demographic Characteristic Of Patient With Ear Infection

Age in years	Frequency(%)
≤10	6(5.5%)
11-20	27(24.8%)
21-30	34(31.2%)
31-40	18(16.5%)
41-50	9(8.2%)
51-60	12(11%)
≥ 61	3(2.7%)

Among culture positive bacterial isolates gram positive cocci accounted for 75(68.8 %) and gram negative bacilli for 34(31.2 %). The more common isolated organisms were *S.aureus* 36 (33%) ,*Pseudomonas* spp 23(21.1 %) , Methicillin resistant staphylococcus aureus 22(20.1%),Methicillin resistant coagulase negative staphylococcus 17(15.6%),*Proteus* spp 11(10.1%) (table:3).

Table 3: Bacterial Strains Isolated From Ear Infections.

Organisms	Total number of isolates (n= 109)	Percentage of Isolates(%)
<i>Staphylococcus aureus</i>	36	33%
<i>Methicillin resistant staphylococcus aureus</i>	22	20.1%
<i>Methicillin resistant Coagulase negative staphylococcus</i>	17	15.6%
<i>Pseudomonas spp</i>	23	21.1%
<i>Proteus spp</i>	11	10.1%

The drug susceptibility pattern of the gram positive bacteria (n=75) showed that 48% of isolates were sensitive to cefoxitin . Among the 36 isolates of *Staphylococcus aureus* , it was 100% sensitive to gentamicin, chloramphenicol, Linezolid, Cotrimoxazole and Penicillin 32(88.9%), Ciprofloxacin 31(86.1%), Clindamycin 17(47.2%), Erythromycin 15(41.7%) .

Methicillin resistant staphylococcus aureus was sensitive to gentamicin, chloramphenicol, Linezolid, Cotrimoxazole 22 (100%) each followed by Ciprofloxacin 18(81.8%), Penicillin 8(36.4%), Clindamycin 6(27.3%), Erythromycin 3 (13.6%).

Methicillin resistant Coagulase negative staphylococcus was sensitive to chloramphenicol, Linezolid 17(100%) each and Cotrimoxazole 11(64.7%), Gentamicin 10(58.9%), Ciprofloxacin 8(47%), Clindamycin 4(23.5) , Penicillin and Erythromycin 2(11.8) each (Table: 4).

Table :4 Susceptibility Pattern Of Gram Positive Bacterial Isolates (n= 75)

S.No	Bacterial Isolates	Pattern	Antibiotics								
			P	CX	E	CD	G	CIP	C	CO	LZ
1	<i>Staphylococcus aureus</i>	S	88.9%	100%	41.7%	47.2%	100%	86.1%	100%	100%	100%
		R	11.1%	-0	58.3%	52.8%	0	13.9%	0	0	0
2	<i>Methicillin resistant staphylococcus aureus</i>	S	36.4%	0	13.6%	27.3%	100%	81.8%	100%	100%	100%
		R	63.7%	100%	86.4%	72.7%	0	18.1%	0	0	0
3	<i>Methicillin resistant Coagulase negative staphylococcus</i>	S	11.8%	0	11.8%	23.5%	58.9%	47%	100%	64.7%	100%
		R	88.2%	100%	88.2%	76.5%	41.2%	52.9%	0	35.3%	0

P: Penicillin; CX: Cefoxitin; E: Erythromycin; CD: Clindamycin; G: Gentamicin ; CIP: Ciprofloxacin; C: Chloramphenicol; CO: Cotrimoxazole ; LZ: Linezolid.

Table :5 Susceptibility Pattern Of Gram Negative Bacterial Isolates (n=34)

S. No	Bacterial Isolates	Pattern	Antibiotics								
			CAZ	G	CIP	PIT	Ce	CXM	CPM	MEM	IMP
1	<i>Pseudomonas</i>	S	100%	86.9%	91.3%	100%	ND	ND	100%	100%	100%
		R	0	13%	8.7%	0			0	0	0
2	<i>Proteus</i>	S	ND	90.9%	81.8%	100%	81.8%	63.6%	ND	100%	100%
		R		9%	18.1%	0	18.2%	36.4%		0	0

CAZ: Ceftazidime; G:Gentamicin; CIP: Ciprofloxacin; PIT: Piperacillin + Tazobactam; Ce: Cefotaxime; CXM: Cefuroxime; CPM: Cefepime; MEM: Meropenem; IMP: Imepenem.

The drug susceptibility pattern of Gram negative bacilli were 100% sensitive for ceftazidime , Piperacillin+Tazobactam, Cefepime, meropenem and Imepenem. Among 23 isolates of *Pseudomonas* spp , it was sensitive to Ciprofloxacin 21(91.3%) and Gentamicin 20 (86.9%). *Proteus* spp was sensitive to Gentamicin 10(90.9%), Ciprofloxacin and Cefotaxime 9(81.8%) each ,Cefuroxime 7(63.6%) (Table:5).

DISCUSSION:

In the present study ,109 (53.4%) out of 204 ear samples were culture positive. Several studies conducted in Ethiopia by Wasihun et al., Seid et al. , Muluye et al., Abera and Kibret et al. showed 98.2%, 89.4%, 89.5%, 91.7% of bacterial isolates.^{2,7,10,11} Culture positivity rate differs in the study based on the types of study design and study participants. In the present study, ear infection was predominantly

higher in males compared to females . Similar finding seen in Nigeria by Egbe et al. ¹²A study by Hassan et al showed that females were more commonly affected than males.¹³

In this study, ear infection seen in age group between 10-30 years . This study result is very similar with other similar studies conducted by Ahmad and Iseh et al. ^{14,15} Majority of ear infection seen among younger age groups could be due to the short, horizontal position of the Eustachian tube, poor hygiene, lower immunity, recurrent upper respiratory tract infections and malnutrition.^{2,7,10,14}

The more common isolated organisms in this present study were *S.aureus* 36 (33%) ,*Pseudomonas* spp 23(21.1 %) , Methicillin resistant staphylococcus aureus 22(20.1%),Methicillin resistant coagulase negative staphylococcus 17(15.6%), *Proteus* spp

11(10.1%). A study conducted in tertiary care hospital in Bangladesh reported both gram negative bacilli (55%) and gram positive bacilli (45%) as etiologies of ear infection along with *S. aureus* (37%) and *Pseudomonas* species (31.5%).¹⁶

A study by Kumar et al, among 79 bacterial isolates, *P. aeruginosa* was the predominant pathogen 43 (54.43%) followed by *S. aureus* in 12 (15.19%), coagulase-negative staphylococci 9 (11.39%), *K. pneumoniae* 7 (8.86%), *E. coli* 4 (5.06%), *P. vulgaris* 2 (2.53%), *P. mirabilis* 1 (1.27%), and *S. pneumoniae* 1 (1.27%).¹⁷ A similar findings by Nikakhlagh et al showed that *S. aureus* (32.4%) is common isolate followed by *P. aeruginosa* (21.7%).¹⁸ In this study, the antimicrobial susceptibility patterns of isolates were different. Gentamicin, chloramphenicol and co-trimoxazole were effective antibiotics against more than 90% for some of the isolates. Ciprofloxacin found to be more than 80% sensitive for some of the isolates. Study by Ihsan et al showed that Ciprofloxacin, Gentamicin were (70 to 80%) sensitive to different species of Gram negative and Gram positive bacteria in CSOM.¹⁹ Another study by Maji P K et al, 86% and 46.6% *Pseudomonas* isolates and 87.5% and 64.3% *Staphylococcus aureus* was sensitive to Gentamicin and ciprofloxacin respectively.²⁰

CONCLUSION:

In our study, we reported the bacterial profile and their anti-microbial susceptibility pattern of ear infection in the tertiary care hospital. The predominant isolates of gram positive and gram negative bacteria were *S. aureus* and *pseudomonas* respectively. Bacterial isolates showed the highest susceptibility rates to Cefazidime, gentamicin, ciprofloxacin, cotrimoxazole. The antibiotics may be used to treat ear infection and thus prevent the development of antibiotic resistant bacteria and complication. The spectrum of bacterial profile and anti-microbial susceptibility pattern can guide the physicians to select the effective drug for the treatment of ear infection based on drug susceptibility test.

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