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### RESEARCH ARTICLE

#### MICROBIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF AURAL SWAB IN TERTIARY CARE HOSPITAL OF NORTHERN INDIA

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#### Manuscript Info

##### Manuscript History

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#### Abstract

**Background:** Ear discharge is often linked with antibiotic prescription, hearing impairment, severe disability and is a public health threat in developing countries.

**Objective:** This study is aimed at determining microbiological etiologic agents and their antimicrobial susceptibility patterns among patients of all age groups in DRPGMC, a tertiary care hospital in Northern India.

**Methods:** Retrospective data recorded on culture and antimicrobial susceptibility profile were retrieved for analysis. Pus swabs from discharging ears collected and processed for aerobic bacterial and fungal cultures. Total of 81 aural pus swabs were received over a period of one year from August 2021 to July 2022 for culture and antibiotic susceptibility testing.

**Results:** Of total 81 aural swab cultures, 68 showed growth of pathogenic microbes, 5 of contaminants and 8 were sterile. Gram positive bacterial isolates were 35% and gram-negative bacteria were 60% while 5% fungal isolates were obtained. Predominant bacterial isolate obtained was *Pseudomonas aeruginosa* (41.25%) followed by *Staphylococcus aureus* (31.25%), *Klebsiella pneumoniae* (6.25%), *Proteus spp.* (3.75%) and CoNS (2.5%). In Fungal isolates two each were *Aspergillus spp.* and non albicans candida. Maximum samples were from age group 1-20 years (22.2%). Highest resistance was observed for Ciprofloxacin, Gentamycin, Ampicillin, Penicillin, cotrimoxazole while Amikacin, Imipenem, Piperacillin-tazobactam, ceftazidime was found effective against the isolated bacteria.

**Conclusion:** Periodical monitoring of microbiological profile is essential for making effective empirical protocol for the cases of ear discharge in a particular geographical area and treatment to be guided by antibiotic susceptibility testing of isolates.

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#### Introduction:-

#### Background:-

Ear infection may be acute or chronic purulent type<sup>1</sup>. Chronic suppurative otitis media (CSOM) or otitis media (OM) is the inflammation of mucoperiosteal layer of middle ear cleft and tympanum with otorrhoea lasting for 2 weeks to more than 3 months<sup>2,3</sup>. In India poor living conditions, overcrowding, poor hygiene and malnutrition are

causes of its wide spread<sup>4</sup>. Otitis media is a major health problem in both developed and developing countries<sup>5</sup> and 60% of patients suffering from it may had significant hearing loss<sup>6</sup>. Ear infection is primarily a disease of infants and young children, it can also affect adults<sup>7</sup>. The disease may begin in childhood or as a complication of untreated or inadequately treated acute suppurative otitis media (ASOM) or may be chronic from onset<sup>5</sup>. The children have higher predisposition to ear infection than adults due to anatomy of eustachian tube which permit easier access to microbes through the nasopharynx and incidence is higher in males than females<sup>5,8</sup>.

The prevalence varies from place to place. The purulent otitis media if untreated leads to perforation, recurrent acute otitis media, persistence of middle ear effusion and often leads to hearing impairment, mastoiditis, meningitis, CSOM, brain abscess and even sepsis<sup>8</sup>. Also, the aetiology, frequency and antimicrobial resistance pattern of ear infection is different in different geographic areas and climatic conditions<sup>1,9</sup>. Many pathogenic bacteria, both aerobic and anaerobic have been found to cause CSOM leading to persistence of infection. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Escherichia coli* are the predominant agents<sup>8,9,10</sup>.

The increased and irrational use of antibiotics has led to development and spread of antimicrobial resistance (AMR)<sup>1,11</sup>. The increased AMR can lead to complications like meningitis, brain abscess<sup>9</sup> and ear infection may lead to tumor in middle ear, post aural swelling and aural sinus complications<sup>9,12</sup>. Due to limited microbiology laboratory setup, clinicians prescribe antibiotics without antimicrobial susceptibility tests which can further lead to emergence of drug resistance.

### **Objective:-**

This study was undertaken to determine the microbial etiological agents of ear infection and their antimicrobial susceptibility pattern among patients of all age groups which may be useful in prescribing the appropriate antibiotics.

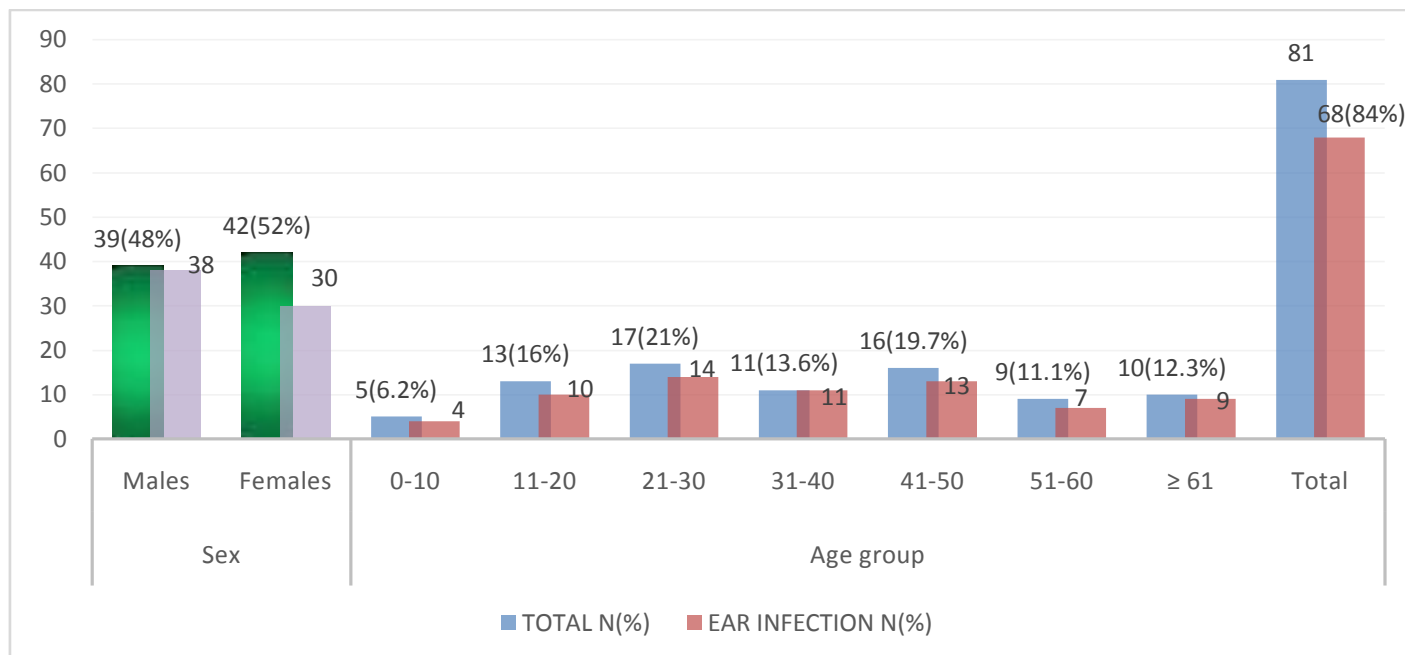
### **Methods:-**

A retrospective cross-sectional study was conducted at DRPGMC based on review of records of aural swab (pus) samples from patients clinically suspected of otitis media over a period of one year from 1<sup>st</sup> August 2021 to 31<sup>st</sup> July 2022. The age, sex profile, culture isolates and their antimicrobial susceptibility pattern were retrieved.

The standard microbiological techniques<sup>13</sup> were followed for collection of samples by using sterile cotton wool swabs. The swabs obtained were inoculated on Nutrient agar, Blood agar and MacConkey's agar and incubated at 37°C for 18-24 h. Bacterial species were identified by biochemical tests and antibiotic sensitivity pattern were determined on Muller-Hinton agar using Kirby-Bauer disc diffusion Method<sup>14</sup>. The antibiotic susceptibility pattern was interpreted based on Clinical and Laboratory Standards Institute (CLSI) guidelines<sup>15</sup>. A standard bacteriological procedure was followed to maintain correct test results. For quality control standard reference American Type Culture collection (ATCC) strains (*S. aureus* ATCC 25923; *E. coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853) were used for culture and susceptibility testing.

### **Results:-**

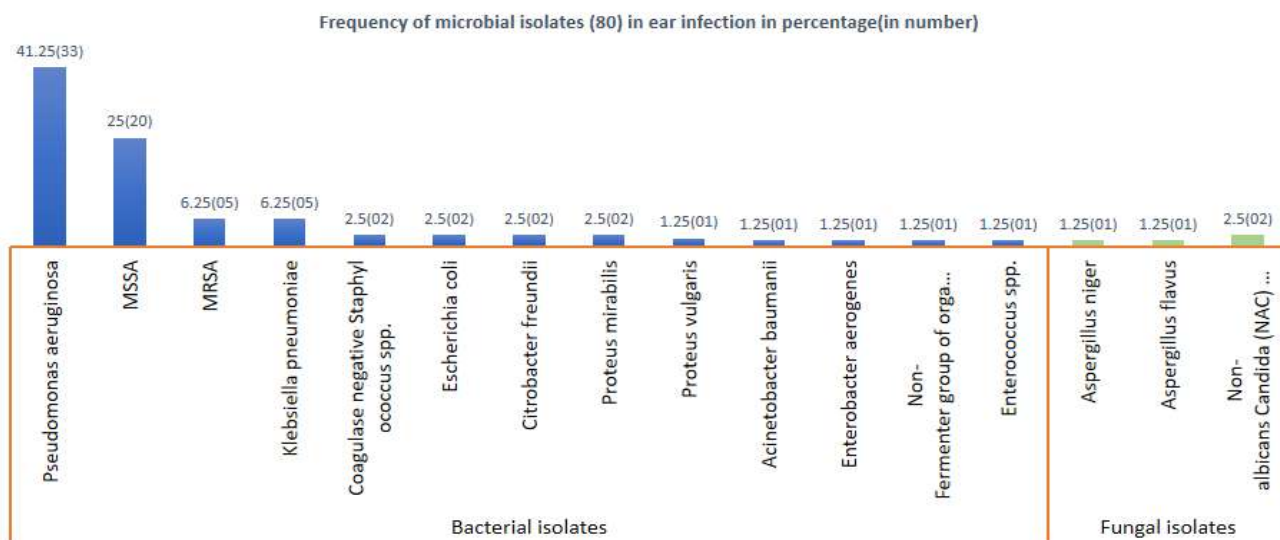
From clinically suspected patients of otitis media, a total of 81 samples were received, tested and analysed. Out of 81 samples 39 (48%) were males and 42 (52%) were females. In 68 (84%) samples microbial growth was obtained, 8 (9.9%) were sterile and 5 (6.1%) growth positive samples were reported as contaminants. The proportion of ear infection in males was 97.4% (38/39) and in females was 71.4% (30/42). In 68 growth positive sample, a total of 80 microbial isolates were obtained with mixed pathogenic microbial growth was obtained in 12 (17.65%) samples and only one isolate from 56(82.35%) samples. Out of 80 isolates, 76 (95%) were bacterial and 4 (5%) fungal isolates were obtained.



**Table 1:-** Frequency of microbial isolates (80) of ear infection.

<b>BACTERIAL ISOLATES (76)</b>	<b>RATE OF INFECTION</b>	
	<b>Frequency</b>	<b>Percentage</b>
<i>Pseudomonas aeruginosa</i>	33	41.25
<i>Staphylococcus aureus</i>	25 (MSSA=20; MRSA=05)	31.25
<i>Klebsiella pneumoniae</i>	05	6.25
Coagulase negative <i>Staphylococcus</i> spp.	02	2.5
<i>Escherichia coli</i>	02	2.5
<i>Citrobacter freundii</i>	02	2.5
<i>Proteus mirabilis</i>	02	2.5
<i>Proteus vulgaris</i>	01	1.25
<i>Acinetobacter baumannii</i>	01	1.25
<i>Enterobacter aerogenes</i>	01	1.25
Non-Fermenter group of organisms	01	1.25
<i>Enterococcus</i> spp.	01	1.25
<b>FUNGAL ISOLATES (4)</b>	<b>RATE OF INFECTION</b>	
	<b>Frequency</b>	<b>Percentage</b>
<i>Aspergillus niger</i>	01	1.25
<i>Aspergillus flavus</i>	01	1.25
Non-albicans <i>Candida</i> (NAC) group	02	2.5
<b>Total</b>	<b>80</b>	<b>84</b>

Gram positive bacterial isolates were 35 % (28), gram negative bacteria were 60% (48) and 5% (4) were fungal isolates.



Gram positive bacterial isolates were 35 % (28), gram negative bacteria were 60% (48) and 5% were fungal isolates.

Of 25 *Staphylococcus aureus*, 05 were Methicillin resistant *Staphylococcus aureus* (MRSA) and 20 were Methicillin sensitive *Staphylococcus aureus* (MSSA). CoNS were isolated from 02 samples and 01 sample showed growth of *Enterococcus spp.*

Among gram negative bacteria, *Pseudomonas aeruginosa* (33) followed by *Klebsiella pneumoniae* (05) then *E. coli*, *Citrobacter freundii* and *Proteus mirabilis* ( 2 each) and 1 isolate each of *Proteus vulgaris*, *Acinetobacter baumannii*, *Enterobacter aerogenes* and Non-fermenter group of organisms were obtained.

Of 4 fungal isolates, 2 were non-albicans candida and 1 each was of *Aspergillus niger* and *Aspergillus flavus*.

**Table 2:-** Samples showing mixed pathogenic microbial growth :

Combination of organisms	Frequency
<i>Pseudomonas aeruginosa</i> and <i>Citrobacter freundii</i>	02
<i>Pseudomonas aeruginosa</i> and <i>Acinetobacter baumannii</i>	01
<i>Pseudomonas aeruginosa</i> and MRSA	01
<i>Pseudomonas aeruginosa</i> and MSSA	04
<i>Pseudomonas aeruginosa</i> and <i>Proteus vulgaris</i>	01
<i>Pseudomonas aeruginosa</i> and <i>Klebsiella pneumoniae</i>	01
<i>Klebsiella pneumoniae</i> and <i>Enterobacter aerogenes</i>	01
MRSA and Non-albicans candida (NAC)	01
<b>Total</b>	<b>12</b>

### Antibiotic Resistance pattern:

**Table 3: Gram negative bacteria :**

Gram negative Bacteria (48)	CEF <sup>a</sup>	GEN <sup>b</sup>	CIP <sup>c</sup>	AMK <sup>d</sup>	PZ <sup>e</sup>	IMP <sup>f</sup>	AMP <sup>g</sup>	CFU <sup>h</sup>	AMC <sup>i</sup>
<i>P.aeruginosa</i> (33)	9%	15%	27%	3%	0%	0%	-	0%	-
<i>K.pneumoniae</i> (5)	-	20%	40%	-	20%	0%	80%	0%	40%
<i>C.frendii</i> (2)	0%	0%	0%	0%	0%	0%	100%	-	-
<i>E.coli</i> (2)	50%	50%	50%	0%	0%	0%	50%	-	-
<i>Proteus spp.</i> (3)	0%	100%	66%	0%	0%	0%	-	-	0%

<i>A. baumannii</i> (1)	0%	0%	0%	0%	0%	0%	-	0%	-
<i>E.aerogenes</i> (1)	0%	0%	0%	0%	0%	0%	-	-	0%
Non-fermenter organisms (1)	0%	0%	0%	0%	0%	0%	-	0%	-

*Pseudomonas aeruginosa* showed highest resistant to Ciprofloxacin(CIP<sup>c</sup>) and Gentamycin(GEN)<sup>b</sup> while sensitive to Ceftazidime(CEF<sup>a</sup>), Amikacin (AMK<sup>d</sup>), Piperacillin-tazobactam (PZ<sup>e</sup>) and Imipenem(IMP<sup>f</sup>). *Klebsiella pneumoniae* is resistant to Ampicillin(AMP<sup>g</sup>), Amoxycillin-clavulanate(AMC<sup>i</sup>) and Ciprofloxacin and is sensitive to Cefuroxime(CFU<sup>h</sup>), Imipenem and Piperacillin-tazobactam. Other gram-negative bacterial isolates showed resistant to Gentamycin, Ciprofloxacin and Ceftazidime but are sensitive to Amikacin, Piperacillin-tazobactam and Imipenem.

**Table 4:-** Gram positive bacteria :

Gram positive Bacteria (27)	PEN <sup>j</sup>	ERY <sup>k</sup>	CLIN <sup>l</sup>	COT <sup>m</sup>	CFX <sup>n</sup>	VAN <sup>o</sup>	LIN <sup>p</sup>
<i>S. aureus</i> (25; 5MRSA & 20 MSSA))	76%	32%	24%	28%	20%	0%	0%
CoNS (2)	100%	50%	0%	0%	0%	0%	0%

MSSA showed highest resistant to Penicillin(PEN<sup>j</sup>), Erythromycin(ERY<sup>k</sup>) and Cotrimoxazole (COT<sup>m</sup>) followed by Clindamycin (CLIN<sup>l</sup>) and is sensitive to Cefoxitin (CFX<sup>n</sup>), Vancomycin (VAN<sup>o</sup>) and Linezolid (LIN<sup>p</sup>). MRSA have highest resistant to Penicillin, Cefoxitin followed by cotrimoxazole, Erythromycin and Clindamycin but is sensitive to Vancomycin and Linezolid. CoNS are resistant to Penicillin and Erythromycin only.

Non-albicans candida are sensitive to Voriconazole and Fluconazole .

## Discussion:-

Ear infection is one of the frequent diseases for which patient take antibiotics<sup>16</sup>. In this study 84% cases of ear discharge were culture positive. In the study by Itzhak Brook and Bethesda on the bacteriology of cholesteatoma, they have reported that *Staphylococcus aureus* was the predominant gram-positive pathogen and *Pseudomonas aeruginosa* the most predominant gram-negative organism followed by *Proteus mirabilis*, *Klebsiella pneumoniae* and *Escherichia coli*. They have also reported mixed growth in 8 patients<sup>17</sup>. Studies done by Poorey et al<sup>18</sup> showed *Pseudomonas* species as the most common isolate. *Pseudomonas* species has been shown to be the commonest organism responsible for CSOM by Bansal et al<sup>19</sup>, Moorthy et al<sup>20</sup> and Varshney et al<sup>21</sup>. Its incidence has been reported to vary from 18% to 67%. Predominance of aerobic bacteria is well known in studies by Bansal et al<sup>19</sup>, Mishra et al<sup>22</sup> and Poorey et al<sup>18</sup>. The proportion of ear infection is higher in males compared to females. The results obtained from this study also concur with the data obtained from other studies<sup>10</sup>.

In this study, the highest proportion of otitis media was found among patients from 1 to 20 years of age (22.2%) which agrees with reports from other countries<sup>8,10,12,23</sup>. High rates of ear infection among young age groups might be due to the short, broad and straight nature of the eustachian tube, lower immune status, frequent exposure to upper respiratory tract infections, lack of hygiene and malnutrition<sup>1,8,10,12</sup>.

In the present study, gram-negative bacteria are the dominant isolates (60%) of ear infection compared to gram-positive bacteria (35%) which is in agreement to earlier studies<sup>1,3,9,10,23</sup>. In this study single bacterial infection of the ear was seen in 82.35% of patients which also has been observed in other studies<sup>8,9</sup>. In this study, *P. aeruginosa* (43.4 %) followed by *S. aureus* (32.9 %) were the leading isolates. This trend is similar to reports of other researchers<sup>23,24,25</sup>. The most common isolation, *P. aeruginosa* in our study could be due to its ability to survive in competition with other organisms and antibiotic resistance. *P. aeruginosa* uses its pili to attach to the necrotic or diseased epithelium of the middle ear and once attached, the organism produces enzymes to elude the normal defense mechanism of body required for fighting infections<sup>8</sup>. A study in India reported 8% and 4% of *K. pneumoniae* and *E. coli* respectively<sup>26</sup> though this study observed 6.61% of *K. pneumoniae* and 2.6% *E. coli*. Poor hygienic conditions may be the reason of isolation of fecal bacteria like *K. pneumoniae* and *E. coli*.

## Conclusion:-

Microbial cultures of aural swab showed growth of both gram positive and negative pathogenic bacteria as well as pathogenic fungus, while gram negative pathogens form the majority isolates. *P. aeruginosa*, *S. aureus*, *K. pneumoniae* and *Proteus* spp. were the dominant isolates.

The treatment of ear discharge/otitis media needs to be guided by antibiotic susceptibility testing of isolates. The microbial profile and the antibiotic susceptibility pattern of microbes keep changing overtime. It is, therefore

important to monitor the microbiological profile periodically along with antimicrobial susceptibility pattern, for making effective empirical treatment protocol.

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