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Research Article

PATTERN OF BACTERIAL AND FUNGAL INFECTION IN EAR INFECTION IN A TERTIARY CARE HOSPITAL

Govind Patel[1], Khyati Jain[2], Sadat Qureshi[3] and Kalpana Sadawarte[4]

[1,2,4]Department of Microbiology,
[3]Department of ENT, People's College of Medical Sciences and Research Center, Bhopal, MP

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A B S T R A C T

Background: Ear discharge is a frequent complaint in ENT clinics. Worldwide, 65-330 million people suffer from ear infection, with 60% having hearing loss. *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Aspergillus niger* are commonly isolated from these species. This study will provide data regarding the aerobic bacterial and fungal profile of ear infection to assist physicians in empirical therapy and setting therapeutic protocols

Materials and Methods: A total of 360 patients were enrolled in the study and the samples were obtained from each patient using sterile cotton swabs and processed for isolation of bacterial and fungal organisms. antibiotic susceptibility testing was done by using Kirby-Bauer disc diffusion method.

Results: 275 specimens (76.38%) had pure single bacterial or fungal growth, 85 (23.61%) no growth. Of 275 isolates, 224 (81.45%) were bacteria and 51 (18.54%) were fungi. The most common microorganisms isolated were *Pseudomonas aeruginosa* (26.54%) followed by *Staphylococcus aureus* (24.72%), *Proteus mirabilis* (12%) and *Candida species* (9.45%).Antibiotic used for the susceptibility testing of Gram positive bacteria; Penicillin, Clindamycin and Gentamicin had the highest susceptibility rate. Regarding Gram negative bacteria the highest susceptibility rate was for Ceftazidime, Ciprofloxacin and Piperacillin/tazobactam.

Conclusion: The types of bacteria causing ear infections varies in different geographical areas and the antimicrobial resistance profile also varies accordingly, due to the local antimicrobial prescribing practices and the prevalence of resistant bacterial strains in that area. So, antimicrobial susceptibility testing should be done for all isolates to help in the choice of drugs for treatment.

INTRODUCTION

Background

Ear discharge is a frequent complaint in ENT clinics. Patients of different ages are candidates for this condition especially children.[1]About 65 to 330 million people suffer from ear infection worldwide and 60 % of them had significant hearing loss.[2] These infections, which can be viral, bacterial, or fungal in origin, are widespread in both children and adults. Patients of all ages, particularly children, are susceptible to this illness. Ear discharge patients were found to have anaerobic bacteria, aerobic bacteria, combined, and fungal microbes.[3, 4]there are two types of ear infections: otitis externa and otitis media. Infection of middle & tympanum is Otitis medium connected by otorrhoea, which can persist for maximum 3 months Permanent dehiscence is caused by bacteria.[5]

An infection of the middle ear space is defined as acute otitis media. There are three types of otitis media: acute otitis media (AOM), chronic suppurative otitis media (CSOM), and otitis media with effusion (OME). Otitis externa affect primarily the external auditory canal. Hot and humid weather is a good condition to develop acute diffuse otitis externa known as (swimmers ear).[6]

Various bacterial species have been found to be associated with ear infections. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are commonly isolated from acute Otitis Externa. *Pseudomonas* and anaerobes are commonly associated with chronic Otitis Externa. The isolates such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Hemophilus influenzae*, and *Moraxella catarrhalis* from an acute infection.[7,8,9] Whereas *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Escherichia coli* are common in chronic otitis media.[10,11] The most common fungi in CSOM are *Aspergillus niger* and *Candida species*.[12] Therefore, up-to-date information about the common Causative organism and their antimicrobial susceptibility pattern is essential for the rational use of the drugs for the treatment. Against this background, this study was done to know the etiological agents causing ear discharge in our

Corresponding author:* **Govind Patel
Department of Microbiology,

environment, with emphasis on the antimicrobial susceptibility patterns of the bacterial isolates, to enable efficacious treatment.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology, Peoples College of Medical Sciences & Research Centre. A total of 360 patients attending the ENT (IPD/OPD) department were included in the study. The duration of this study was 1 year from February 2022 to January 2023.

Samples processing: -Samples were collected in suspected cases of ear infection after written informed consent. Two ear samples/swabs was collected and then all the collected specimens were transported immediately to the laboratory for further processing

First swabwas used for detection of fungal hyphae & budding yeast cells by wet mount (KOH) microscopy &used to prepare smear for Gram staining. Second swab was used for culture onto Blood agar and MacConkey agar and Sabouraud's dextrose agar for detection the growth of bacterial and fungal isolates from each patient.

For the fungal identification One set of inoculated slants was incubated a 22 *C and the other at 37 *C and examined every other day for fungal growth up to 4-6 weeks before discarding as negative. Bacterial isolates obtained were identified by their colony morphology, gram staining and biochemical tests done by standard laboratory procedures.

Antimicrobial susceptibility testing

Antimicrobial sensitivity testing was performed on Mueller Hinton Agar (MHA) by Kirby-Bauer Disc Diffusion Method as per CLSI Guideline 2022.[13]

Screening for MRSA in Staphylococcus aureus- All the strains of *Staphylococcus aureus* isolated were also tested for methicillin resistance by Cefoxitin disc method as recommended by the CLSI.[13]

RESULTS

A total of 360 patients were included in the study, Maximum number of patients (24.36%) belonged to the age group of 11-20 years and minimum number of patients (0.4%) belonged to the age group of 81-90 years.275 specimens (76.38%) had pure single bacterial or fungal growth, 85 (23.61%) no growth. Of the 275 isolates, 224 (81.45%) were bacteria and 51 (18.54%) were fungi.

Out of 79 gram positive bacteria isolates, *Staphylococcus aureas* predominates i.e., 68 isolates (86.10%), followed by 7 *CONS* (8.90%) and *Streptococcus pyogenes* (1.30%). Out of 68 Staphylococcus aureus isolates, 27 showed MRSA (39.70%) and 41 isolate were MSSA (60.29%) (Table1)

Table 1 Bacterial Isolates (n=79)

Gram positive bacteria	Number	%
Staphylococcus aureas	68	86.10%
CONS	7	8.90%
No Growth	3	3.80%
Streptococcus pyogenes	1	1.30%
Total	79	100%

Out of 145 Gram negative bacterial isolates the most commonly isolated organism was *Pseudomonas aeruginosa* i.e., 73 isolates (50.3%). The other organisms isolated were 33 *Proteus mirabilis* (22.8%), *Escherichia coli* 23 (15.9%), 9 *Klebsiella pneumoniae* (6.2%), 4 *Acinetobacter baumannii* (2.8%) and 2 *entrobacter spp.* (1.4%).(Figure 1)

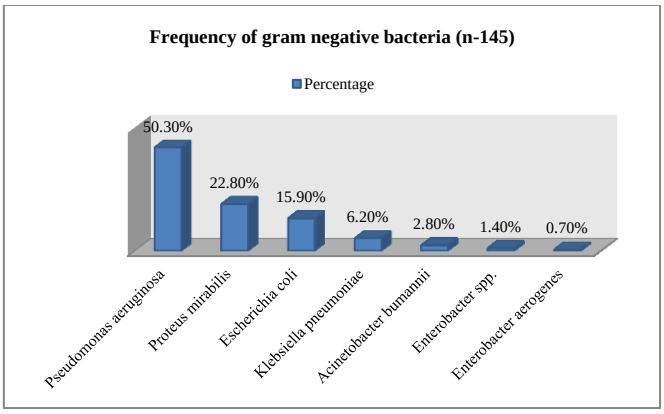

Figure 1 Frequency of gram negative bacteria (n-145)

Out of 51 fungal isolates, Candida spp. predominates i.e., 26 isolates (50.98%), followed by 21 *Aspergillus niger* (41.17%), 3 *Aspergillus fumigatus* (5.88%) and 1 *Aspergillus flavus* (0.7%).

Out Of 79 gram positiveisolates, 50.63% and 59.49% were resistant to Cefoxitin, and Ciprofloxacin respectively. However, low level of resistance was found to Penicillin, Clindamycin, erythromycin and Gentamicin by Gram positive bacteria (Table 2).Regarding Gram negative bacteria the highest sensitivity was for Ceftazidime (84.1%), Co-trimoxazole (75%), and Ciprofloxacin (73.1%).of 145 gram negative bacilli isolates, 40.7%and 32.4% was resistant to Gentamicin and Amikacin, respectively.

Table 2 Antibiotic susceptibility for gram positive bacteria and Gram negative bacteria

Gram positive bacteria. (n =79)		Gram negative bacteria(n =145)	
Name of antibiotics	**Sensitive**	**Name of antibiotics**	**Sensitive**
Penicillin	82.27%	Gentamicin	56.60%
Clindamycin	81.01%	Ciprofloxacin	73.10%
Erythromycin	74.68%	Amikacin	67.60%
Ciprofloxacin	59.49%	Piperacillin/tazobactam	69.70%
Cefoxitin	50.63%	Ceftazidime	84.10%
Gentamicin	75.94%	Cefepime	68.27%
		Co-trimoxazole	75%

All Candida species were found to be sensitive to Fluconazole (80.76%), Itraconazole (69.23%) and Nystatin (76.92%) by disk diffusion method (figure 2).

Figure 2 Antifungal Susceptibility Testing of Yeast

Name of antibiotics	No.	Sensitive
Fluconazole	21	80.76%
Itraconazole	18	69.23%
Amphotericin B	19	73.07%
Ketoconazole	16	61.53%
Nystatin	20	76.92%
Clotrimazole	14	53.84%

DISCUSSION

Patients frequently complain of ear discharge in the ENT clinic, which can be caused by a number of infectious diseases include diffuse otitis externa, otomycosis, and acute and CSOM.[14]the age groups of the patients ranged from 2 years to 90 years. Maximum number of patients (24.36%) belonged to the age group of 11-20 years; similar to a study by Arjyal et.

al. who reported an incidence of 30% cases in the age group of 11-20 years.[4]

Also, study by Agrawal *et al* reporting an incidence of 62.4 % patients, a study in (Burdwan) India reporting 31.9% and in Malaysia 69.3% patients, among age group less than 20 years showed similar results.[15-17]

In our study 275 patients, 56% (155) of them were female and the other 44% (120) were male. Whereas in the study of Bhuvaneshwar *et al*.in Chennai, India, 40 patients among were female consisting of 55% and male 45%.[18] Derese Hailu *et.al*., a study in Ethiopia also found a frequency of 55.7% of male among 368 patients.[19]In the present study the frequency of positive results was high (total =76.38%), other studies, Hailu *et al.* reported single growth with 80.4% frequency.[19] Our study showed 23.61% of specimens with absence of any type of growth, which is compatible with study conducted by Bhuvaneshwar *et al*.[18]

In the present study, out of the total 275 cases, Gram negative bacteria were isolated in 52.72% cases, Gram positive bacteria in 28.72% cases and fungi in 18.54% cases. The total 275 cases, *Pseudomonas aeruginosa* was the most common bacterium isolated (26.54%) followed by *Staphylococcus aureus* (24.72%) and *Proteus mirabilis* (12%). Out of the 521 fungal isolates obtained, *Candida albicans* was the commonest isolate (50.98%) followed by *Aspergillus niger* (41.17%) and *Aspergillus fumigatus* (5.88%). *Pseudomonas aeruginosa* (50.3%) was the commonest gram negative bacteria isolated. A similar varied incidence has been reported ranging from 44% in Bhavnagar. [20]

Staphylococcus aureus was the most common Gram positive bacteria (86.1%) with 30.70% (9.81% of all positive specimens) being methicillin resistant *Staphylococcus aureus* (MRSA). In comparison with studies conducted in India, the first study showed the frequency of MRSA was 7% and the second was 18%.[21]Of the 15 fungal isolates, 26 (50.98%) were *Candida species (Candida albicans).Aspergillus niger* was isolated in 21 cases (41.17%), the maximum number 3 (5.88%) of strains of *Aspergillus* being *Aspergillus fumigatus*. In accordance with our study, Balan *et al*. (2017), studied 28 cases and obtained a maximum growth of Candida in 42.86% cases and a maximum growth of *Aspergillus* in 57.14% cases,[22] while in a Hisar study on 100 patients of ear infection, Harvinder Kumar *et al* (2011) found that fungi accounted for 15% of the isolates and that the fungal organisms which were commonly isolated were *Aspergillus spp* (40%), followed by *Candida sp* (60%).[21]

Regarding antimicrobial susceptibility of Gram positive bacteria, the highest sensitivity was for Penicillin, Clindamycin and Gentamicin with 82.27%, 81.01%, and 75.94% frequency rate respectively. The low sensitivity rate was found for Cefoxitin and Ciprofloxacin, 50.63% and 59.49% respectively. Kaur P *et al* reported that most *Staphylococcus aureus* was resistant to Ciprofloxacin.[23] for Gram negative bacteria; the highest sensitivity rate was for Ceftazidime Co-trimoxazole and Ciprofloxacin with frequency rate 84.1%, 75% and 73.1% respectively.

This trend is similar to reports of other researchers Mohammad Al maayteh. 2019 andKaur P 2018[23, 24]Regarding fungal causative agent our study showed 18.54% of all samples were positive for fungal species, study conducted in Himmatnagar, Gujarat reported 19% frequency rate of fungi,[20] This pattern is consistent with other researchers' findings [25,21,24]

CONCLUSION

The types of bacteria causing ear infections varies in different geographical areas and the antimicrobial resistance profile also varies accordingly, due to the local antimicrobial prescribing practices and the prevalence of resistant bacterial strains in that area. So, antimicrobial susceptibility testing should be done for all isolates to help in the choice of drugs for treatment.

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