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Original Article

Antimicrobial Susceptibility Pattern of Bacterial Pathogens in Our Setup: Ear, Nose and Throat Perspective

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ABSTRACT

With a high prevalence of infections involving the ear, nose, and throat, and the occurrence of drug resistance the antimicrobial susceptibility pattern of bacterial pathogens is of immense importance. **Objective:** To determine the antimicrobial sensitivity profile of pathogenic bacteria isolated from representative infected areas of patients with ear, nose, and throat infections. **Methods:** This cross-sectional observational study was conducted at Ear, Nose and Throat (ENT) Outpatient Department (OPD), Capital Hospital Islamabad, over two years. The sample included 639 pathogenic bacterial culture specimens, grown from the representative infected ear, nose, or throat of patients who attended ENT outpatients of the hospital. The bacterial cultures were subjected to the standard disc agar diffusion method to know the antimicrobial susceptibility profile. Data collected included the patient's age, gender, area/ site of infection, bacterial pathogen isolated, and sensitivity to antibiotics. **Results:** The sample included 49.30% males and 50.70% females and mean age of 30.13±19.24 years. Gram-positive organisms were predominant [n=441, (69.01%)] with *Staphylococcus aureus* being the commonest isolate (67.92%) followed by *Pseudomonas spp.* (26.13%). Gram-positive isolates were sensitive to Cefoperazone+Sulbactam, Ceftazidime, Cefoperazone, Amikacin, Pipercillin+Tazobactam, Vancomycin, Gentamycin, Linezolid, Amoxicillin + Clavulanate, and Ceftriaxone with resistance to Cefixime, while the Gram-negative isolates were sensitive to Vancomycin and Meropenum, Ciprofloxacin, Levofloxacin, Ceftriaxone and Ceftazidime and highly resistant to Cefixime, Cefuroxime, Amoxicillin+Clavulanate and Co-trimoxazole. **Conclusions:** Since ENT infections are predominated by Staphylococcus aureus and Pseudomonas spp. When unavoidable the empirical therapy should cover these pathogens, however culture and sensitivity studies are justified keeping in view the growing resistance to antimicrobials.

INTRODUCTION

Ear, Nose and Throat (ENT) infections are the most prevalent ENT disorders [1, 2], and result in significant morbidity and death. Among the ENT disorders, Otitis media of the aural issues, rhinitis among nasal issues and pharyngitis and tonsillitis among throat issues are most prevalent [3], with a pooled incidence of sore throat alone of 82.2 events /100 child years [4]. Some may be difficult to control due to development of antibiotic resistance and biofilm formation, requiring advanced antibiotics [5]. In ENT, there is evidence of formation of biofilms even in cholesteatoma, otitis media with effusion (OME), tonsillitis, rhino-sinusitis and adenoid tissue in chronic rhino-sinusitis cases and devices like tranchy tubes and gromets [6]. Hence, infections must be treated promptly with optimum dose and duration of antibiotics according to sensitivity profile, since decline in antibiotic sensitivity of organisms is being observed [7] with other interventions where required, otherwise, they escort health problems including complications, development of drug resistance and spread of resistant organisms in the community. Hence, inappropriate prescription of antibiotics with wide spectrum in infection of ENT domain need to be

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discouraged [8]. Chew et al., in their study noted predominance of different pathogenic bacteria in ENT infections differing between tropical and non-tropical regions and proposed that tropical areas should not adopt the antibiotic guidelines meant for non-tropical in toto [9]. Also, literature search revealed numerous other studies on bacterial flora and antimicrobial sensitivity of isolates worldwide including Pakistan with variation in bacterial flora in ENT infections in different areas including different areas of Pakistan [10-13]. The difference may be due to so many factors including socioeconomic, access of masses to healthcare, food preferences and usage of different antimicrobials [14].

Since different bacterial pathogens predominate in different parts of country, empirical prescription of antibiotics can contribute to drug resistance [15], therefore current study was conceived to find out the locally prevalent bacterial flora and antimicrobial sensitivity pattern in a clinical setup to improve future drug prescription and hamper development of drug resistance. This study is of importance, since it will help clinicians in better prescription of antibiotics to their patients and hamper development of drug resistance.

METHODS

This cross-sectional observational study using convenient sampling, was conducted at Department of Otorhinolaryngology, Capital Hospital, and Islamabad, Pakistan over a period of two years. Study was conducted after obtaining ethical approval from Institutional Research Board of Capital Hospital vide letter No. 2024-03-007. The sample of the study included 639 pathogenic bacterial culture specimens. A sample size of n=664 was calculated using the formula: n = [DEFF*Np(1-p)]/ [(d2/Z21-α/2*(N-1)+p*(1-p)], with DEFF=1, confidence limit of 5%, and population 1000000, and n=25 cultures with fungal growths excluded, leaving behind a sample of N= 639 which was utilized for the study, which were grown by routine microbiological culture methods form representative infected ear (535, 83.89%), nose (97, 15.18%) and throat (7, 0.93%) of patients, who visited Otorhinolaryngology outpatients of Capital Hospital, Islamabad with respective ENT infections and including both genders and all age groups. These were subjected to antimicrobial sensitivity testing using standard disc agar diffusion method and the inoculum to be tested were compared with 0.5 McFarland turbidity standard, to know the antimicrobial susceptibility profile of bacterial pathogens. The antimicrobial discs used included Ceftriaxone (CRO) n=576, Ceftazidime (CAZ) n=196, Cefuroxime (CXM) n=265, Cefoperazone (CFP) n=4), Cefixime (CFM) n=516, Levofloxacin (LVX) n=587, Ciprofloxacin (CIP) n=605, Vancomycin (VA) n=395, Amikacin (AMK) n=154, Gentamycin (GEN) n=454, piperacillin+ tazobactam (TZP) n=208, Cefoperazone + Sulbactam (SCF) n=32,Co-trimoxazole n=11, Meropenem (MEM) n=1, Linezolid (LZD) n=402, Amoxicillin + clavulanic acid (AMC) n=631 and Erthromycin (ERY) n=7. Following placement of antibiotic discs, plates were incubated for 16 to 18 hours at 37oC and zone size was interpreted against each antimicrobial disc for each organism and measured in millimeter (mm). Sensitivity was recorded as resistant, intermediate and sensitive using zone interpretation chart [16]. Data collected and recorded included patient's age, gender, site of infection, bacterial isolate and sensitivity to antimicrobials. Data were collected, and analyzed using Microsoft Excel Worksheet and expressed in frequency, percentage and cumulative percentage and cross tabulated. Data were then compared with the local and international literature and deductions made were discussed.

RESULTS

Of a total number of 639 samples (swabs) with pathogenic bacterial cultures isolated for the study obtained from infected patients' representative infected areas of ear, nose and throat. These included samples from 49.30% males and 50.70% females (Figure 1) with mean age of 30.13 + SD 19.24 years with majority i.e., 73.08% pathogenic cultures isolated in less than 40 years age groups (Table 1).

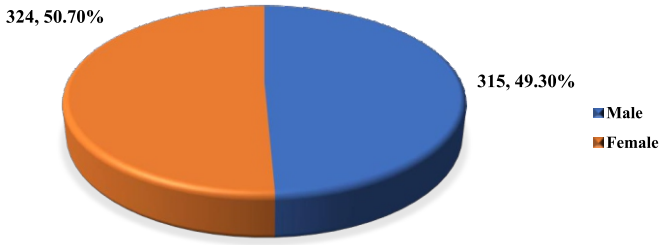

Figure 1: Gender Distribution of Study Population (n=639)

Table 1: Age-wise Prevalence: Age Groups * Bacterial Group. Cross Tabulation (n= 639)

Age Group	Pathogens (n=639)		
	Gram-Positive	Gram-Negative	Total
	n (%)	n (%)	n (%)
1-10	80 (70.18)	34 (29.82)	114 (17.84)
11-20	79 (73.15)	29 (26.85)	108 (16.90)
21-30	80 (61.07)	51 (38.93)	131 (20.50)
31-40	76 (66.67)	38 (33.33)	114 (17.84)
41-50	53 (70.67)	22 (29.33)	75 (11.74)
51-60	40 (81.63)	9 (18.37)	49 (7.67)
> 60	33 (68.75)	15 (31.25)	48 (7.51)
Total	441 (69.01)	198 (30.99)	639 (100)

Despite being an extensive study, only 5 genera of pathogens were isolated with Gram Positive organisms being more prevalent (n=441, 69.01%) compared to Gram Negatives (n=198, 30.99%) with *Staphylococcus Aureus*

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being the commonest pathogen isolated (n=434, 67.92%) followed by *Pseudomonas* (n=167, 26.13%) (Table 2).

Table 2: Frequency Distribution of Bacterial Isolates (n=639)

Group	Bacteria Isolated	Frequency (%)
Gram Positive (n=441, 69.02%)	*S. aureus*	434 (67.92)
	Streptococcus pneumoniae	7 (1.10)
Gram Negative (n= 198, 30.98%)	*Pseudomonas spp.*	167 (26.13)
	Proteus spp.	26 (4.07)
	E. coli	5 (0.78)
Total		639 (100)

In this study (table 3), the overall susceptibility of Gram Positive bacteria was high with, 100% sensitivity to SCF, CAZ, CFP, and AMI followed by TZP (99.27%), VA (98.47%), GEN (97.58%), LZD (95.23%), AMC (93.58%) and CRO (91.83%). Reduced sensitivity was noted with ERY (85.71%), CXM (83.62%), LVX (83.59%) and CIP (81.71%). Gram positive organisms were found to be highly resistant to CFM.

Table 3: Antimicrobial Susceptibility Pattern of Gram Positive and Gram Negative Bacteria: Antimicrobial Drug * Microbial Group. Cross Tabulation (n = 639)

Antimicrobial Drugs		Microbial Group					
		Gram Positive			Gram Negative		
Drug Disc Used	Frequency	(n=441)			(n=198)		
		R	I	S	R	I	S
Cefoperazone + Sulbactam	32	0 (0.00%)	0 (0.00%)	8 (100%)	0 (0.00%)	0 (0.00%)	24 (100%)
Ceftriaxone	576	25 (6.19%)	8 (1.98%)	371 (91.83%)	18 (10.47%)	3 (1.74%)	151 (87.79%)
Ceftazidime	196	0 (0.00%)	0 (0.00%)	371 (91.83%)	4 (100%)	0 (0.00%)	0 (0.00%)
Cefuroxime	265	16 (13.79%)	3 (2.59%)	97 (83.62%)	89 (59.73%)	16 (10.74%)	44 (29.53%)
Cefoperazone	4	0 (0.00%)	0 (0.00%)	4 (100%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Cefixime	516	149 (44.61%)	30 (8.98%)	155 (46.41%)	136 (74.73%)	9 (4.95%)	37 (20.33%)
Levofloxacin	587	48 (12.12%)	17 (4.29%)	331 (83.59%)	19 (9.95%)	2 (1.05%)	170 (89.01%)
Ciprofloxacin	604	57 (15.54%)	20 (4.75%)	344 (81.71%)	19 (9.79%)	1 (0.52%)	174 (89.69%)
Vancomycin	395	0 (0.00%)	6 (1.53%)	387 (98.47%)	0 (0.00%)	0 (0.00%)	2 (100%)
Amikacin	154	0 (0.00%)	0 (0.00%)	86 (100%)	1 (1.47%)	0 (0.00%)	67 (98.53%)
Gentamycin	454	7 (2.12%)	1 (0.30%)	322 (97.58%)	6 (4.65%)	0 (0.00%)	123 (95.35%)
(Piperacillin + Tazobactam)	208	0 (0.00%)	1 (0.73%)	136 (99.27%)	1 (2.44%)	0 (0.00%)	40 (97.56%)
Co Trimoxazole	11	0 (0.00%)	5 (100%)	0 (0.00%)	3 (50%)	3 (50%)	0 (0.00%)
Meropenem	1	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (100%)
Linezolid	402	13 (3.27%)	6 (1.51%)	379 (95.23%)	1 (25%)	0 (0.00%)	3 (75%)
Amoxicillin + Clavulanate	631	22 (5.05%)	6 (1.38%)	408 (93.58%)	111 (56.92%)	9 (4.62%)	75 (38.46%)
Erthromycin	7	0 (0.00%)	1 (14.29%)	6 (85.71%)	0 (0.00%)	0 (0.00%)	0 (0.00%)

Gram Negative organisms showed high sensitivity to VA and MEM (100%), very good sensitivity to CIP (89.69%), LVX (89.01%), CRO (87.79%) and CAZ (82.6%), while the gram-negative organisms were highly resistant to CFM 74.73%, CXM (59.73%), AMC (56.93%) and Co-Trimoxazole (50%). Sensitivity pattern of the most prevalent Gram positive *(Staphylococcus aureus)* and Gram negative *(Pseudomonas spp.)* is given in table 4.

Table 4: Antimicrobial Susceptibility Pattern of Prevalent Gram Positive and Gram Negative Bacteria Isolated

Gram Positive Staphylococcus Aureus		*Gram Negative Pseudomonas Spp.*	
Antimicrobial Drug Sensitivity	**n (%)**	**Antimicrobial Drug Sensitivity**	**n (%)**
Amikacin	86 (100)	Cefoperazone + Sulbactam	20 (100)
Ceftazidime	8 (100)	Amikacin	55 (98.18)
Cefoperfazone +Sulbactam	8 (100)	Piperacillin+ Tazobactam	34 (97.06)
Cefoperazone	3 (100)	Levofloxacin	151 (97.03)
Piperacillin+ Tazobactam	136 (99.37)	Ceftazidime	156 (95.51)

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Vancomycin	387 (98.47)	Gentamycin	111 (95.49)
Gentamycin	315 (97.52)	Ciprofloxacin	164 (90.24)
Linezolid	379 (95.47)	Ceftriaxone	162 (87.04)
Amoxicillin +Clavulanate	401 (93.71)	-	-
Ceftriaxone	365 (91.71)	-	-
Levofloxacin	324 (83.29)	-	-
Cefuroxime	92 (82.88)	-	-
Ciprofloxacin	337 (81.40)	-	-
Drug Resistance		**Drug Resistance**	
Cefixime	327 (45)	Cefixime	154 (75.32)
		Cefuroxime	124 (61.29)
		Amoxicilin + Clavalunate	164 (58.54)

DISCUSSION

The current study revealed a predominance of Gram-Positive organisms to the tune of 69.02% (n=441) with a sensitivity as shown in table 3, of 100% with Cefoperazone+Sulbactam, Ceftazidime, Cefoperazone, and Amikacin, while this group was highly resistant to Cefixime. In another local study, Kabeer S et al noted a high sensitivity of Gram-positive organisms to Vancomycin (100%) followed by amikacin (94.87%) [10]. In the current study, Gram Negative organisms which accounted for 30.98% (n=198) of cultures, showed excellent sensitivity to Vancomycin and Meropenum (100%), very good sensitivity to Ciprofloxacin (89.69%), Levofloxacin (89.01%), Ceftriaxone (87.79%) and Ceftazidime (82.6%), while they were highly resistant to Cefixime (74.73%), Cefuroxime (59.73%), Amoxicillin+Clavulanate (56.93%) and Co Trimoxazole (50%). In contrast, Kabeer et al., [10], reported that susceptibility was high to Sulbactam/ Cefoperazone (96.46%), Piperacillin+Tazobactam (96.1%). Here, in contrast to our study Gram-negative organisms showed very poor sensitivity to Ceftriaxone (38.94%), Chloramphenicol (38.05%), Cefotaxime (31.86%), Amoxicillin+Clavulanic acid (30.09%) and Cefuroxime (23.01%). Current study revealed that the dominating organism was *Staphylococcus aureus* (67.92%) followed by *Pseudomonas* (26.13%). Similarly, in a Nepalese study by Dechen, *Staphylococcus aureus* was predominant with overall sensitivity to Amoxicillin (53.84%), Cloxacillin (53.84%), Ciprofloxacin (46.15%), Gentamicin (46.15%), and Cephalosporin (46.15%) and resistance to Erythromycin, Tetracycline, Co-Trimoxazole and Norfloxacin [17]. In contrast in a local study *Pseudomonas Spp*. was the predominant organism followed by *Staphylococcus aureus* [10, 12]. Similarly, in an Ethiopian study by Hailu *et al.*, [18] the predominating organism was *Pseudomonas* (29.7 %) followed by Staphylococcus aureus (26.3 %) with a high level of resistance to Amoxicillin+Clavulanic acid, Ampicillin, and Penicillin.In our study, *Pseudomonas* was resistant to Amoxicillin+Clavulanate (58.54%), Cefuroroxime (61.29%), and Cefixime (75.32%), while *Staphylococcus aureus* which was predominant organism was sensitive to Amoxicillin+Clavulanate (93.47%) and most other drugs but resistant to Cefixime (45%)(Table 4). In another local study by Marium *et al.*, [11] in CSOM cases, the predominating organism was *Staphylococcus aureus* 30 (65.2%) followed by *Pseudomonas* (15.2%), *Proteus mirabilis* (13.1%) and *Escherichia coli* (6.5%) which were sensitive to Ceftriaxone (89.2%) followed by Ofloxacin (82.6%), Cefotaxime (69.6%), Cephradine (63.1%), Augmentin (60.9%), Erythromycin (52.2%), Ampicillin (2.9%) and Cephalexin (26.1%) with the least sensitivity to Cefixime (39.2%), which was also found to be least sensitive in our study. Abdullah *et al.*, (13) in a study on infected ears, noted that the most effective antimicrobials for empirical treatment were Pipericillin+Tazobactam, Cefoperazone+Sulbactam, Imipenam, and Fosfomycin. Whereas Ciprofloxacin and Amoxicillin+Clavulanate showed intermediate sensitivity, and the majority of the bacterial isolates were indifferent to Cotrimoxazole, Cefixime, Lincomycin, Doxycycline and Polymyxin B. According to Elies *et al.*, [19], *Pseudomonas aeruginosa* is a bug that should be well covered by antimicrobial therapy. According to them though, Ciprofloxacin and Ceftazidime are widely used however, Ciprofloxacin resistance increased, while Ceftazidime sensitivity is unchanged. Also, Afolabi *et al.*, found *Pseudomonas aeruginosa* to be the commonest middle ear pathogen which has sensitivity in favor of ciprofloxacin (20). In a local study by Arshad *et al.*, *Staphylococcus aureus* and Pseudomonas were equally prevalent in otitis externa and showed excellent sensitivity to Imipenem, Enoxacin, Ciprofloxacin, and Ofloxacin but resistance to Co-Trimoxazole, Amoxicillin, and Erythrocin [21]. In a prospective randomized study in cases of COM with *Pseudomonas aeruginosa* as the commonest pathogen, Khanna *et al.*, noted no role of culture and sensitivity in initial management and proposed broad spectrum antimicrobials keeping culture and sensitivity reserved for failed cases [22]. However, it is advisable to study resistance profiles and fluctuations to ensure suitability of empirical treatment [23]. On the other hand,

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Deyno *et al.*, [24] recommended avoiding irrational use of antibiotics and delay resistance. They noted *S. aureus* having resistance to Cloxacillin (97%) and Vancomycin (74.2%). According to Mane *et al.*, [25], since antibiotics are freely available especially with availability without prescription, and antibiotics are used just to relieve symptoms, irrational use is a norm and therefore, the emergence of drug resistance is very frequent and this also makes partially resistant organism to flourish. The same is the situation in this country. Mane also recommended the institution of antibiotic treatment for ear infections following culture sensitivity studies. This shows diversity in the prevalence and antibiotic sensitivity indicating a growing public health issue and valid reason to conduct the study.

CONCLUSIONS

It was concluded with the present study that in our environment, the diversity in prevalence and growing resistance of bacterial isolates justifies culture and sensitivity studies prior to antibiotic prescription. However, since ENT infections are predominated by *Staphylococcus aureus* and *Pseudomonas Spp*, when unavoidable the empirical therapy should consider covering these organisms. This study highlights the need for periodic surveillance studies and reassessment of policies on antibiotics use.

Authors Contribution

Conceptualization: GS
Methodology: NI
Formal analysis: SARN
Writing-review and editing: GS, JA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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