MICROBIAL PROFILE AND ANTIBIOTIC RESISTANCE PATTERN OF THE BACTERIAL ISOLATES IN A TERTIARY CARE PSYCHIATRY HOSPITAL

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ABSTRACT: BACKGROUND: Antibiotic resistance is a challenge for effective management of infections as it increases the morbidity, mortality and costs of treating infectious diseases.
AIMS: This study was aimed to obtain the profile of the bacterial isolates and their antibiotic resistance pattern.
SETTINGS AND DESIGN: It is a cross sectional study carried out in a tertiary care psychiatry hospital in India.
MATERIALS AND METHODS: Isolation and identification of the isolates were done by standard methods. Susceptibility patterns were checked by Kirby Bauer disc diffusion method.
STATISTICAL ANALYSIS USED: Statistical analysis was done by using SPSS 16.0 version to calculate the frequencies as well as for cross tabulation.
RESULTS: Significant bacterial growth observed in 43(25.6%) samples, of which 39(90.7%) showed resistant to at least one of the antibiotics used and 36(83.7%) were multi-drug resistant. Gram negative organism accounted for the 25(58.14%) of total significant isolates, Escherichia coli being the highest (76%) in this group. Among multi-drug resistant (MDR) isolates E.coli was the highest (44.4%) and imipenem resistance was also observed in 1(5.3%) of 19 E.coli isolates. Among the 43 isolates 18(41.86%) were Gram positive with Streptococcus spp. showing incidence of 41.7% among the total MDR isolates.
CONCLUSION: Increasing incidence of MDR strains seen in the population requires continuous monitoring and a restricted use of antibiotics to keep a check on resistance pattern, for effective treatment plan.

KEYWORDS: Microbial Profile, Drug Resistance, Carbapenem Resistant, Gram positive and Gram Negative Bacteria.

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INTRODUCTION: Antibiotics play a crucial role in the reduction of mortality and morbidity from diseases and have revolutionized the treatment process of common bacterial infections. The progressively increasing incidence of bacterial resistance toward common antibiotics in developing countries is becoming a critical area of concern. Irrational and increased practice of consuming inappropriate non-prescribed antibiotics in the treatment of diseases could lead to increased threat in patients with high mortality and morbidity caused by antimicrobial resistant bacteria.[1-5] Antibiotic resistance stands as a major clinical obstacle in treating infections caused by common bacterial pathogens.[6] The unpredictability of this increased upshots have led to speculations such as 'end of the antibiotic era', 'crisis of modern medicine' and the application of the chaos theory to medicine but still some doctors are in the hope of the possibilities of controlling or even reducing the spread of antibiotic resistance.[7–12]

Antimicrobial resistance is a growing concern and over the last decade, bacterial have emerged resistant to all commonly used front line antimicrobial agents. The emergences of multi-drug resistance (MDR) were evidenced mainly due to the production of enzymes, such as pencillinases, cephalosporinases, carbapenemases and extended spectrum β-lactamases (ESBLs). Organisms such as carbapenemase producing enterobacteriaceae (CPE) have very high levels of resistance, with capability to break down all β-lactam agents including carbapenems and make them ineffective. Carbapenem drugs- imipenem, meropenem, ertapenem and doripenem are the last resort antibiotics for the treatment of ESBL producing enterobacteriaceae.[13,14]

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Current knowledge on the hospital microbial profile and antibiogram is mandatory in solving the problem of antimicrobial resistance in hospitals. This study aimed to identify the different bacterial isolates obtained from varied samples and to determine the incidence of drug resistance among the clinical isolates.

MATERIALS AND METHODS:

Study Design and Collection of Specimen: This study was a cross sectional study conducted during the time period of January 01, 2014 to December 31, 2014. A total of 168 clinical specimens including post operative swab, throat swab, vaginal swab, sputum, pus, stool, urine and wound swab samples were processed.

Isolation and Identification of Microorganisms: For isolation of desired pathogens, all the specimens were inoculated on the MacConkey agar, blood agar, chocolate agar, and mannitol salt agar media plates, followed by overnight incubation at 37^0C. Chocolate agar plates were incubated in CO_2. Identification of the bacteria of interest was performed by series of biochemical tests following standard methods including the triple sugar iron agar (TSI) test, indole motility test, citrate utilization test, catalase test, oxidase test, coagulase test etc.[15]

Assay of Bacterial Antibiotic Susceptibility Pattern: All the bacterial isolates of interest were subjected to antibiotic susceptibility testing to different antibacterial agents by standard Kirby-Bauer disc diffusion method[16] using commercially available discs (Hi-media, Mumbai, India) as per Clinical and Laboratory Standards Institute (CLSI) guidelines.[17] The quality control of the antibiotic discs was done by using ATCC strains of E Coli 25922 and S. aureus 25923. The MDR categorizations were done based on the interim standard proposed by Magiorakos et al.[18] The antibiotics tested were as classified in table 1.

Data Collection and Analysis: All the data obtained were entered in the SPSS 16.0 version worksheet and all the analysis was done using the software. Microsoft word and Excel has been used to generate figure and tables.

RESULTS:

Types of the specimens received: A total of 168 non-duplicate samples received throughout the study period of which urine specimens (n=142) accounted for the highest in number followed by sputum (n=7), throat swab (n=7), stool (n=4), pus (n=4), wound swab (n=1), post operative wound swab (n=1), vaginal swab (n=1) and fungal sample (n=1).

Microbial Growth Status of the Samples: Of total 168 samples 99 samples showed no growth, 14 insignificant growths, 4 mixed growths, 7 normal floras and 1 sample gave Candida spp. Only 43 samples gave significant bacterial growth.

Microbial profile of pathogens: The most frequently encountered isolates from all samples were E. coli (44.2%), Streptococcus spp. (23.2%), Staphylococcus aureus (16.3%), Citrobacter spp. (4.7%), Klebsiella spp. (4.7%), Pseudomonas aeruginosa (2.3%), Moraxella catarrhalis (2.3%) and Micrococcus spp. (2.3%) (Figure 1). E. coli was found to be the most common bacterial pathogen (63.33%) from urine samples followed by Staphylococcus aureus (10%), Streptococcus spp. (6.67%), Klebsiella spp. (6.67%), Citrobacter spp. (6.67%), Pseudomonas aeruginosa (3.33%), and Micrococcus spp. (3.33%). All the isolates from throat swab and sputum samples were Streptococcus spp. except one Moraxella catarrhalis in sputum. Vaginal swab, wound swab and pus showed the growth of Staphylococcus aureus as the only predominant pathogen isolate Table 2.

Antibiotic Resistance Patterns Among the Bacterial isolates: As per our stated objective of determining the antibiotic resistance pattern, a series of data was collected both for Gram negative and Gram positive bacteria (Tables 3 and Table 4). In our study, 90.7% isolates were found to be resistant against at least one of the antibiotics. This resistance pattern among Gram positive and Gram negative bacteria was 94.4% and 88.0% respectively. MDR accounted for 83.7% of the total bacterial isolates, higher among the Gram positives (84%) compared to Gram negatives (83.3%). Among total 25 Gram negative isolates 23 (92%) were enterobacteriaceae, MDR prevalence was found to be 19 (82.6%), also E. coli isolate obtained from one urine sample was found to be resistant to imipenem accounting for 5.3% carbapenem resistance prevalence rate among MDR enterobacteriaceae.

DISCUSSION: Significant bacterial growth was observed in 25.6% of the total samples received, near about similar to a recent study.[19] Resistant to at least one of the commonly used antibiotic was detected in 90.7% of the isolates which is very high figure compared to 70% as shown by a similar study in Bangladesh.[20] The overall prevalence of MDR was 83.7% against 62.8% shown by earlier report.[19] This may be due to small sample size or difference in the kind of study site.

MDR prevalence among enterobacteriaceae was 82.6%, nearly similar to the studies in Ethiopia (85.5%,87.4%) [21,22] while higher than the studies in Nepal (40.1%, 64.04%) [23,24]. This variation in the prevalence rate could be due to increased incidence of MDR strains with time, difference in study period and study population. All the E. coli isolates were from urine sample constituting the majority (63.3%) among the uropathogens slightly more compared to similar study from North India (56.8%).[25] Of these 84.2% E. coli were MDR which is similar to a study from South India (82.6%).[26]

Among 23 enterobacteriaceae isolates 1 (4.35%) were found to be carbapenem resistant, similar to other studies in India (5.4%)[27] and Bangladesh(4.8%).[28] In the mean time, another study in India is seen to have reported

carbapenem resistant prevalence rate of up to 12.9%.[29] This difference in the prevalence of carbapenem resistance enterobacteriaceae in various studies may be due to increased trends in the utilization of carbapenems and other broad spectrum antibiotics, traditional practices, transfer of patients from the place of high incidence to another. Among the two isolates of Klebsiella spp. one was found to be MDR and all isolates of Citrobacter spp., Pseudomonas aeruginosa and Moraxella catarrhalis are found to be MDR. These later findings are not in concordance with the other studies,[19,30-32] as because of small study sample, targeted patient group basically having psychiatric complaints and study period.

The incidence of Gram positive organism was reported to be 41.9% which is consistent with other studies that have shown the higher incidence of Gram negative organism compared to that of Gram positive.[33-36] Among Gram positive organism S. aureus accounted for 38.9 %, Streptococcus spp. 55.55% and Micrococcus spp. 5.55%, of which 83.33% of Gram positive isolates were MDR, very huge difference in figures among the isolates (Table 3) compared to previous findings.[35-37] This variation may be due to the small sample population as well as due to limited number of visits with related complaints as the study was carried out in a psychiatric setting.

CONCLUSION: Compared to previous studies our study reported high incidence of mono drug and multi-drug resistant isolates. This study was restricted in a specific population, there might be various limitations as in study sample, site, size of sample, study period, etc. which might have led to such variations. Thus it is suggestive to conduct such study in a large population on regular basis so as to assess the epidemiology and antibiotic susceptibility patterns of hospital isolates to guide clinicians in their choice of empirical therapy. Furthermore, research plans should focus on the genetic makeup of all MDR strains to understand more about their genes mutations and the effects on antibiotics resistance.

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Name of the Group	Name of the antimicrobials
Aminoglycosides	Amikacin(30µg), Gentamicin(10µg).
Cephalosporins	Cefuroxime(30 µg), Cefotaxime(30µg), Cepfodoxime(10µg), Ceftriaxone(30µg), Cefixime(5 µg), Cephalexin(30 µg), Ceftriaxone/sulbactum(45µg)
Fluoroquinolones	Ciprofloxacin(5µg), Levofloxacin(5µg), Prulifloxacin(5µg), Ofloxacin(5µg)
Penicillins	Amoxycillin/clavulunate(30 µg),Amoxycillin(10µg), Ampicillin/sulbactum(20µg), Ampicillin(10µg), Piperacillin/tazobactum(110µg),Oxacillin(1µg)
Macrolides	Azithromycin(15µg), Erythromycin(15µg)
Carbapenems	Imipenem(10µg)

Lincosamides	Clindamycin(2µg)
Sulphonamides	Cotrimoxazole(25µg)
Nitrofurans	Nitrofurantoin(300µg)

Table 1: Classification of antimicrobial agents used in the study

Sl. No.	Sample Type	Isolated Bacterial Strains							
		E. coli	Klebsiella spp.	Citrobacter spp.	P.aeruginosa	M.catarrhalis	S.aureus	Strept. spp.	Micrococcus spp.
1	Urine	19(63.3%)	2(6.7%)	2(6.7%)	1(3.3%)	0(.0%)	3(10.0%)	2(6.7%)	1(3.3%)
2	Sputum	0(.0%)	0(.0%)	0(.0%)	0(.0%)	1(25%)	0(.0%)	3(75.0%)	0(.0%)
3	Throat swab	0(.0%)	0(.0%)	0(.0%)	0(.0%)	0(.0%)	0(.0%)	5(100%)	0(.0%)
4	Pus	0(.0%)	0(.0%)	0(.0%)	0(.0%)	0(.0%)	2(100%)	0(.0%)	0(.0%)
5	Wound swab	0(.0%)	0(.0%)	0(.0%)	0(.0%)	0(.0%)	1(100%)	0(.0%)	0(.0%)
6	Vaginal swab	0(.0%)	0(.0%)	0(.0%)	0(.0%)	0(.0%)	1(100%)	0(.0%)	0(.0%)

Table 2: Sample Wise Distribution of Bacterial Isolates

Sl. No.	Organism	Multi-drug resistant(MDR)	Mono-drug resistant(MR)	Non-Resistant(NR)	Total
1	Escherichia coli	16 (84.2%)	1(5.3%)	2(10.5)	19
2	Klebsiella spp.	1 (50%)	-	1(50%)	2
3	Citrobacter spp.	2(100%)	-	-	2
4	Pseudomonas aeruginosa	1(100%)	-	-	1
5	Moraxella catarrhalis	1(100%)	-	-	1
6	Staphylococcus aureus	4(57.1%)	2(28.6%)	1(14.3%)	7
7	Streptococcus spp.	10(100%)	-	-	10
8	Micrococcus spp.	1(100%)	-	-	1
Total		**36(83.7%)**	**3(7%)**	**4(9.3%)**	**43**

Table 3: Distribution of drug resistant bacterial isolates

Sl. No.	Resistance Category	Bacterial Group		Total
		Gram Negative	Gram Positive	
1	Multi-drug resistant(MDR)	21(58.3%)	15(41.7%)	**36**
2	Mono-drug resistant(MR)	1(33.3%)	2(66.7%)	**3**
3	Non- Resistant(NR)	3(75%)	1(25%)	**4**
Total		**25(58.1%)**	**18(41.9%)**	**43**

Table 4: Cross tabulation of resistance category and the bacterial groups

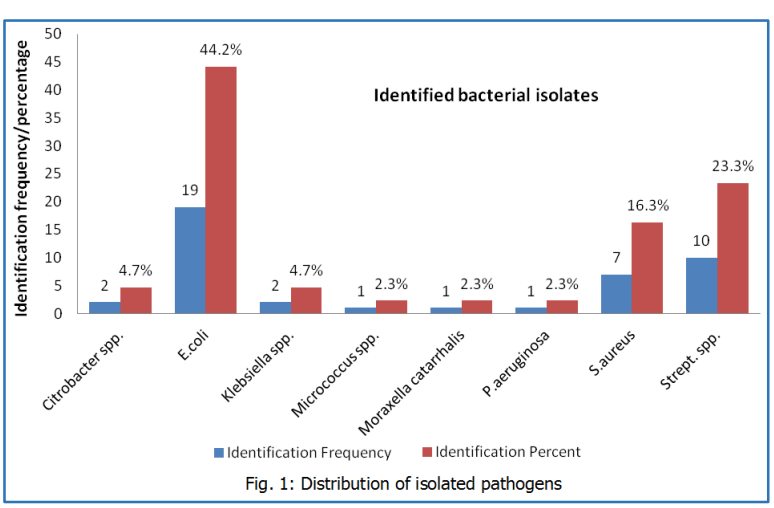

Fig. 1: Distribution of isolated pathogens