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An in-vitro study on the prevalence of antibiotic resistance by different aerobic bacteria isolated from various clinical samples

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Abstract

Introduction: The antimicrobial resistance by organisms to commonly used antibiotics is increasing day by day. This is a major challenge to clinicians for treating patients. The aim of the study was to find out the overall prevalence of drug resistant bacterial isolates among the clinical isolates and to identify the degree of resistance by each organisms.

Materials and Method: The study was conducted in the department of Microbiology DM WIMS medical college for a period of 4 months starting from Jan-April 2017.The clinical samples were cultured and bacterial strains were identified. The antibiotic susceptibility profile of different bacterial isolates was studied.

Results: A total of 3985 samples were processed. 372 samples were identified with 395 drug resistant bacterial isolates. Escherichia coli was the most common organism isolated and most of the isolates were ESBL producers. Among the Gram positive isolates, Vancomycin, linezolid and clindamycin were the most susceptible antibiotics whereas among the gram negative isolates the most susceptible drug were Piperacillin/tazobactem, Amikacin and imipenem.

Conclusion: The present study reports suggests that antibiotic selection for empirical treatment should be based on drug sensitivity test results and it is evident that the drug resistance are more commonly seen in antibiotics and this has become a great challenge in treating pyogenic infections.

Keywords: Antibiotic resistance, ESBL, Aerobic bacteria, MDR, MRSA

Introduction

Emergence of antimicrobial resistance to different antibiotics by various pathogenic bacteria poses a major threat to patients and also it is a growing public health concern.[1,2] The widespread use of antibiotics has lead to the emergence of resistant human pathogens.[3] Multidrug resistant (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.[4] Extended spectrum beta-lactamases (ESBL) are enzymes that confer resistance to most beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam. Infections with ESBL-producing organisms have been associated with poor outcomes.[5] Metallo-beta-lactamase New Delhi Metallo-beta-lactamase-1(NDM-1) is an enzyme that makes bacteria resistant to a broad range of beta-lactam antibiotics. These include the antibiotics of the carbapenem family, which are the mainstay for the treatment of antibiotic-resistant bacterial infections. Methicllin-resistant *Staphylococcus aureus* (MRSA) MRSA can be defined as the strains of *S. aureus* that are resistant to the Isoxyl Penicillin such as methicillin, oxacillin and flucloxacillin. The aim of the study was to find out the overall prevalence of drug resistant strains among the clinical isolates and to identify the degree of resistance by each organisms.

Materials and Method

The present study was carried out in the Department of Microbiology, DM WIMS Medical College, Wayanad, Kerala for a period of 4 months starting from January to April 2017. A total of 3985 samples were processed. Out of this 372 samples were identified with drug resistant bacteria, which were from 148 each from urine and pus, 54 isolates from respiratory samples and 22 isolates from blood samples. Antibiotic sensitivity testing was done by Kirby Bauer's disk diffusion method on Muller Hinton agar. Standard antibiotics like penicillin-G (10 units), ampicillin (10 mcg), amoxyclav (20/10 mcg), piperacillin/tazobactum (100 /10 mcg), oxacillin (1 mcg), vancomycin (30 mcg), ceftriaxone (30 mcg), cefotaxime (30 mcg), ceftazidime (30 mcg), cefepime (30 mcg), imipenem (10 mcg), aztreonam (30 mcg), ciprofloxacin (5 mcg), levofloxacin (5 mcg), co-trimoxazole (1.25/23.75 mcg) gentamycin (10 mcg), amikacin (30 mcg), clindamycin (2 mcg) and erythromycin (15 mcg) were tested and different drug resistance like Methicillin resistance, ESBL, MBL and MDR strains were identified as per the CLSI guide lines 2012.[10] American Typing Culture Collection isolates of E. coli (ATCC-25922), S. aureus (ATCC- 25923) and Pseudomonas aeruginosa (ATCC 27853) were used as a reference strain in the laboratory. Results obtained were analyzed by counts and percentages using MS Excel, 2007 version.

Result

A total of 395 drug resistant isolates which includes MRSA, ESBL, MDR and MBL were identified. Out of the 395 isolates 242 were ESBL producers, 22 were MDR, 18 were MBL, 73 were MRSA, and 40 samples showed significant growth of MRCoNS. Among these

drug resistant bugs majority of the isolates were Escherichia coli, 157 (40%), 73 (18.25%) were MRSA, 68(17%) were Klebsiella, 40 (10%) were MRCoNS, 21(5%) were Pseudomonas, 18(5%) were Acinetobacter, 6(2%) were Citrobacter, 2(0.50%) were non fermenting gram negative bacteria and 1(0.25%) was identified as Proteus *vulgaris*.(Table 1). These isolates were from different clinical samples and the majority of drug resistant organisms were identified from Urine and exudates, i.e., 148 isolates each were from urine and exudate, 54 from sputum and 22 from blood.(Table 2) Out of the 148 drug resistant isolates from urine samples 122 were ESBL, 7 were MBL, 9 were MDR, 4 were MRSA and 6 were MRCoNS. And the drug resistant isolates from exudate noted were 64 ESBLs, 13 MBL, 7 were MDR, 56 were MRSA and 8 were MRCoNS. (Fig. 1)

Table 1

Bacterial Isolates	Number of Isolates	Percentage
E.coli	157	40%
MRSA	73	18.25%
Klebsiella	68	17%
MRCoNS	40	10%
Pseudomonas	21	5%
Acinetobacter	18	5%
Enterobacter	9	2%
Citrobacter	6	2%
GNNF	2	0.50%
Proteus	1	0.25%
Total	395	100

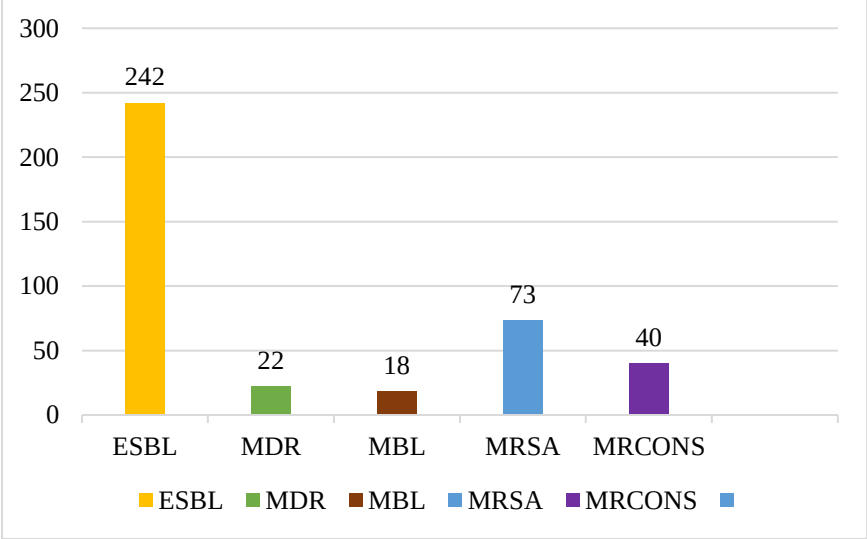

Fig. 1: Details of Drug resistant isolates

Table 2: Sample wise distribution of resistant isolates

Sample	Organism	ESBL	MBL	MDR
Urine	*E.coli*	100	2	1
	Klebsiella	19	2	6
	Pseudomonas	4	2	0
	Acinetobacter	2	0	1
Exudate	*E.coli*	32	0	0
	Klebsiella	12	4	3
	Pseudomonas	10	2	0
	Acinetobacter	3	1	2
Sputum	*E.coli*	6	0	0
	Klebsiella	18	1	3
	Pseudomonas	1	0	0
	Acinetobacter	5	1	2

Among the 372 drug resistant isolates, most of them were from female patients, 198 (53%) were from female and 174(47%) were from males, yielding a male: female ratio of 3.3: 2.9. The Department wise distribution of pus samples revealed that surgery dept. was the highest contributors (37.21%), followed by Orthopaedics (27.5%), Gynae & Obs. (12.7%), Medicine (9.80%), Skin (8.3%) and ENT (4.52%) departments. The antibiotic sensitivity testing revealed that most of the commonly used antimicrobials against gram positive and gram negative organisms are showing resistance to cephalosporins, gentamicin, ciprofloxacin, penicillin G,amoxyclav. The Antibiogram of Gram Positive cocci revealed that the Vancomycin (100%) was the most susceptible drug followed by Linezolid (76.92%). Gram Negative Bacilli are susceptible to Imipenam (83%) and Piperacilline+Tazobactum(82%).

Discussion

In the present study a total of 372(18.3%) samples were identified with Drug resistant bacterial pathogens. In a study conducted by Revathy Saravanan and Vinod Raveendran 2011 out of 999 samples, 125 (12.5%) showed significant growth of organisms exhibiting resistance to either single or multiple drugs.(6)

In this study it was observed that the infection rate in females (53%) was higher than males (47%) and majority of infected patients belongs to the age group of 60-79 (40%), followed by the age group of 40-59(26%), and least in the age group of 80-99(7%). This may be due to the weak immune status of the patients due to progressing age and are more vulnerable to infections.

In the present study, Cefotaxime and Cefuroxime showed 97% resistance to Enterobacteriaceae and cefoperazone sulbactam 31.22% resistance, gentamicin 51%, ciprofloxacin 68% and norfloxacin which is used for urinary isolates showed 73% resistance. Amikacin showed 70% sensitivity to Enterobacteriaceae, followed by Netilmycin 31%. Out of 73 MRSA identified, Pencillin G showed 93% resistance to MRSA followed by Amoxyclav 90% vancomycin and linezolid showed 100% sensitivity to MRSA. In a study conducted by Harijinder Kumar and R.P. Singh found 2015 that as high as 22/23 (95.65%) of the *E. coli* isolates were sensitive to imipenem. In another study conducted by Ganguly NK *et al.,* 2011 MRSA showed high resistance to co-trimoxazole and ampicillin/amoxicillin followed by quinolones.(8) Vancomycin and amikacin were found to be 100% effective on MRSA and 100% sensitivity has been reported by another study by Tiwari HK *et al.,* 2012.(9)

Conclusion

Changing antimicrobial resistance pose challenge in treating these conditions. Appropriate and judicious selection of antibiotic by using antibiotic sensitivity data would limit the emerging drug resistant strains in the future to treat these clinical conditions successfully. Our study thereby will guide the clinician in choosing appropriate antibiotics which not only contribute to better treatment but there judicious use will also help in preventing emergence of resistance to the drug which are still sensitive. The present study reports suggests that antibiotic selection for empirical treatment should be based on drug sensitivity test results. In most of the cases the sources are from hospital and most of the affected were in the age group of 60-79. There is also an urgent need to develop a new combination of therapeutic agent for the effective managemaent of UTI. The lack of knowledge about the consequence of inappropriate use of antibiotics by the consumers, misuse and overuse of antibiotics over the counter selling of antibiotics without the prescription are identified as an important cause for the emergence of antimicrobial resistance.

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