



ANTIBIOTIC RESISTANCE PROFILE OF CLINICALLY SIGNIFICANT BACTERIA ISOLATED FROM VARIOUS CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL IN CHANDRAPUR

Microbiology

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ABSTRACT

Our study was aimed to generate data on prevalence of antibiotic resistance among clinically significant bacteria isolated from patients at a tertiary care hospital in Chandrapur, Maharashtra, India. Various clinical samples were processed for culture, identification and AST by conventional method. Among 265 different isolates, 54.3% were Gram positive and 45.6% were Gram negative. The commonest pathogen isolated was *Staphylococcus aureus*, followed by *E. coli*. Among *S. aureus*, 51.9% were MRSA. Most of Gram positive bacteria were resistant to co-trimoxazole and ciprofloxacin while most Gram negative bacilli were resistant to ampicillin, amoxyclav, co-trimoxazole and cephalosporins. Least resistance was for amikacin. The study showed a high rate of MRSA infection along with in general resistance amongst gram positive and negative isolates to commonly used drugs underlining need of continuous surveillance, antimicrobial stewardship and strict infection control program to restrict the ongoing resistance.

KEYWORDS

Antibiotic resistance, bacterial pathogen, MRSA

INTRODUCTION

Throughout the world bacterial infections are a leading cause of morbidity and mortality, resulting in increased health care cost on patients and public health system.^[1,2] Antibiotic resistance is a major clinical hindrance in treating infections caused by pathogenic microorganisms.^[3] In recent years, multidrug resistant organisms like methicillin-resistant *Staphylococcus aureus* (MRSA) and the extended-spectrum β -lactamase (ESBL) producing bacteria have been reported to be responsible for treatment failures in humans as the physicians have limited options left for treatment of such cases.^[4] The rise and spread of resistant bacteria has become a major threat to public health and a challenge to both science and medicine.^[5]

Earlier, the problem of antibiotic resistance was primarily a concern for nosocomial infections. But now, even community acquired infections are caused by organisms with high levels of antibiotic resistance.^[5] According to a recent report, such multi-drug resistant community acquired infections can be a cause of significant morbidity.^[6] Earlier, such drug resistant organisms were said to infect mainly patients with identifiable risk factors or profound immunosuppression. But now, such infections are being reported in seemingly normal healthy persons.^[7]

It is noteworthy that the antibiotic resistance is not a static phenomenon and hence a regular updating of antibiogram is very essential for the judicious use of antibiotics. Moreover, the antibiotic susceptibility test contributes directly to patient care, and has a significant impact on the prudent usage of antibiotics. Thus, the acquaintance of knowledge on the current susceptibility pattern is imperative for the physicians for choosing the appropriate antibiotics and for developing the appropriate treatment protocols as well.^[8]

The trend of antibiotic resistance pattern in bacteria in India is similar to the global epidemic. Several published reports from different parts of India have consistently revealed a dangerously high level of resistance to all the common antibiotics among all groups of clinically important pathogens.^[7,9] The patterns of organisms causing infections and their antibiotic resistance pattern vary widely from one region to another; as well as from one hospital to other and even among different wards within one hospital.^[2,10] In this study, we aimed to generate data on the prevalence of antibiotic resistance among clinically significant bacteria isolated from patients treated at a tertiary care hospital in Chandrapur.

MATERIALS AND METHODS

A retrospective cross sectional study was conducted for a period of 2 years from January 2017 to December 2018 in Microbiology department of a tertiary care hospital in Chandrapur. Depending on the clinical suspicion laboratory samples like pus, urine, sputum, swab, body fluids (e.g. cerebrospinal fluid, ascitic fluid, pleural fluid), blood and stool were collected from the patients. A total of 581 non-repetitive samples were included in this study. The clinical samples were obtained aseptically. Only one sample from each patient was included. Only bacterial infections were studied in detail in present study.

Isolation & identification of pathogenic bacteria

For isolation of bacterial pathogens, all the samples were inoculated on the MacConkey agar, blood agar, and chocolate agar. Urine samples were inoculated on CLED (cystine lactose electrolyte- deficient) agar. Agar plates were incubated at 37°C for 18 to 24 hours under aerobic conditions. If an organism was grown, colony morphology on different media was recorded. Gram stain was performed from the suspected isolated colonies for initial identification. Finally, standard biochemical tests were performed to identify the bacteria of interest. If more than two organisms were isolated from any specimen, it was considered contaminated and rejected.

Antibiotic susceptibility test of bacterial isolates

Antibiotic susceptibility test was performed using Kirby-Bauer disc diffusion technique recommended by the Clinical and Laboratory Standards Institute (CLSI).^[11] Antibiotic tested for Gram-positive bacteria were penicillin (10 μ g), gentamicin (10 μ g), erythromycin (15 μ g), doxycycline (30 μ g), ciprofloxacin (5 μ g), co-trimoxazole (25 μ g), vancomycin (30 μ g), and linezolid (30 μ g). Antibiotics that were tested against Gram negative bacteria included ampicillin (10 μ g), amoxyclav (20/10 μ g), piperacillin+tazobactam (100/10 μ g), co-trimoxazole (25 μ g), ciprofloxacin (5 μ g), cefotaxime (30 μ g), ceftazidime (30 μ g), cefepime (30 μ g), gentamicin (10 μ g), amikacin (30 μ g), and meropenem (10 μ g). Nitrofurantoin (300 μ g) was tested only for 33 urinary isolates. The susceptibility patterns were reported as sensitive or resistant. The "intermediate resistant" term was avoided. The data were tabulated and analyzed using descriptive statistics and the results were expressed.

RESULTS

In this study, a total 581 samples were analyzed which included pus 260 (44.8%), blood 153 (26.3%), urine 86 (14.8%), CSF 16 (2.8%),

vaginal swab 12 (2%), and the rest miscellaneous samples which included sputum, throat swab, wound swab, central line catheter tip, pleural fluid, peritoneal fluid, knee aspirate, tissue, etc. Out of 581 samples, 249 (43%) showed significant bacterial growth. (Table 1) Remaining 332 samples either had no organisms grown or had insignificant growth (urine samples). Among the specimens with growth of pathogenic bacteria, pus ranked first followed by urine and blood samples. The frequency of isolated pathogenic bacteria was very low in case of CSF. Out of 249 samples with growth, 233 (93.6%) showed single isolate whereas 16 (6.4%) showed two isolates.

Table 1: Samples profile and rate of positive culture from different samples

Samples	Number of samples (%)	Samples yielding bacterial growth (%)
Pus	260 (44.8%)	166(28.6%)
Blood	153(26.3%)	21(3.6%)
Urine	86(14.8%)	38(6.5%)
CSF	16(2.8%)	1(0.2%)
Vaginal swab	12(2%)	5(0.9%)
Miscellaneous	54(9.3%)	18(3.1%)
Total	581(100%)	249 (43%)

Total of 265 different isolates were obtained in which, 144(54.3%) were Gram positive bacteria and 121(45.6%) were Gram negative bacteria. Table 2 shows the organisms isolated from different clinical specimens. Gram-positive isolates were more frequent as compared to gram-negative isolates and included *Staphylococcus aureus* (48.7%), coagulase negative *Staphylococci* (2.6%), *Enterococcus* species (2.3%), *Streptococcus pneumoniae* (0.4%) and *Streptococcus spp* (0.4%). Of the gram negative organisms (n=121), 68 (25.6%) were *Escherichia coli*, 20 (7.5%) were *Klebsiella species*, 18 (6.8%) were *Pseudomonas aeruginosa*, and 6 (2.3%) were *Acinetobacter spp* and *Proteus spp* each.

The commonest organism isolated from all samples was *Staphylococcus aureus* (48.7%), followed by *E.coli* (25.7%). The most common isolate from pus and blood was also *S.aureus* while *E. coli* was found to be the commonest pathogen from urine samples followed by *S.aureus*. Among the vaginal swab samples, *E.coli* was the most commonly isolated pathogen.

Antibiotic resistance pattern of Gram positive isolates is as per Table 3.

Table 2: Pattern of organisms isolated from various samples

Organism	Pus	Blood	Urine	CSF	Vaginal swab	Miscellaneous	Total
<i>Staph aureus</i>	106	13	07	-	-	03	129 (48.7%)
<i>E. coli</i>	40	01	20	-	05	02	68 (25.6%)
<i>Klebsiella</i>	11	03	02	-	01	03	20 (7.5%)
<i>Pseudomonas</i>	09	01	02	-	-	06	18 (6.8%)
<i>CoNS</i>	03	03	-	-	-	01	07 (2.6%)
<i>Enterococcus</i>	04	-	02	-	-	-	06 (2.3%)
<i>Acinetobacter</i>	03	-	03	-	-	-	06 (2.3%)
<i>Proteus</i>	04	-	02	-	-	-	06 (2.3%)
<i>Citrobacter</i>	01	-	-	-	-	02	03 (1.1%)
<i>S. pneumoniae</i>	-	-	-	01	-	-	01 (0.4%)
<i>Streptococcus</i>	-	-	-	-	-	01	01 (0.4%)
Total	181	21	38	01	06	18	265 (100%)

Table 3: Antibiotic resistance pattern for Gram positive isolates

Antibiotics	Resistance (%) among Gram positive isolates (n = 144)					
	MRSA	MSSA	CoNS	<i>Enterococcus</i>	<i>Streptococcus</i>	<i>S. pneumoniae</i>
Penicillin	-	-	-	33.33	100	100
Gentamicin	38	6.89	14.28	-	-	-
Erythromycin	54.9	44.8	42.8	50	0	0
Doxycycline	42.25	34.48	28.57	66.6	-	0
Ciprofloxacin	77.46	56.9	42.8	66.6	-	-
Co-trimoxazole	87.32	70.68	71.42	-	-	0
Vancomycin	0	0	0	0	0	0
Linezolid	0	0	0	0	0	0
Nitrofurantoin	75	66.6	-	50	-	-

Among 106 *S.aureus* isolates, 55 (51.9%) were methicillin-resistant *Staphylococcus aureus* (MRSA) and 51 (48.1%) were methicillin-sensitive *Staphylococcus aureus* (MSSA). Gentamicin resistance among staphylococci ranged from 6-38%. Ciprofloxacin resistance varied from 42—78% for different species. Highest resistance (70-88%) was seen against co-trimoxazole. In present study, all of the gram positive isolates including *S. aureus* and *Enterococcus spp.* showed sensitivity against vancomycin and linezolid. *Streptococcus spp.* and *Streptococcus pneumoniae* isolates were found to be resistant against penicillin and sensitive to erythromycin, vancomycin and linezolid. Table 4 shows the antibiotic resistance pattern of Gram negative isolates.

Table 4: Antibiotic resistance pattern for Gram Negative isolates

Antibiotics	Resistance (%) among Gram negative isolates (n = 121)					
	<i>E. coli</i>	<i>Klebsiella</i>	<i>Pseudo monas</i>	<i>Acinetobacter</i>	<i>Proteus</i>	<i>Citrobacter</i>
Ampicillin	85.3	-	-	-	83.3	100
Amoxicillin + Clavulanic acid	70.5	75	-	-	50	100
Piperacillin + Tazobactam	27.9	30	22.2	50	16.6	33.3
Co-trimoxazole	67.64	90	-	66.6	66.6	66.6
Ciprofloxacin	60.29	55	61.1	66.6	50	66.6
Cefotaxime	67.64	70	-	50	66.6	100
Ceftazidime	-	-	50	50	-	-
Cefipime	60.29	65	50	50	66.6	100
Gentamicin	17.64	55	27.7	33.3	33.3	0
Amikacin	7.35	25	16.6	33.3	16.6	0
Meropenem	26.47	35	16.6	33.3	0	33.3
Nitrofurantoin	25	50	-	-	0	-

High amount of resistance was noted to ampicillin, amoxy-clav, co-trimoxazole and cephalosporins. In our study 50 to 100% resistance was noted to cephalosporins. For ciprofloxacin resistance ranged from 50-66.6%. In gram-negative isolates, amikacin was the most effective antibiotic (0-25% resistance), followed by meropenem (0-35% resistance).

For *E.coli*, amikacin (7%) followed by gentamicin (17%) showed the least resistance. *Klebsiella* isolates showed high level of resistance against all antibiotics. For *Pseudomonas aeruginosa*, lowest resistance was seen for amikacin (16.6%) and meropenem (16.6%). *Acinetobacter sp.* was found most sensitive to gentamicin, amikacin and meropenem.

DISCUSSION

In our study, Gram positive isolates were more than Gram negative isolates. This is in contrast to various studies where gram negative isolates were more common than gram positive isolates.^[10,12,13,14] This study showed *S. aureus* as the most common isolate followed by *E. coli*, *Klebsiella* and *Pseudomonas* in descending order. Abebe *et al*^[15] also reported *Staphylococci* as most common isolate. Many other authors have reported *E.coli* as the predominant isolate in their studies.^[7,8,16] Studies by Kotgire S.A. *et al*^[10] and Javeed *et al*^[13] revealed *E.coli* as commonest isolate followed by *Staphylococci*, *Klebsiella* and *Pseudomonas* in descending order. In a study from Marathwada, *E.coli* followed by *Acinetobacter spp* was the predominant pathogen.^[17] Study by Kumburu *et al*^[18] revealed *Proteus spp* as the most common bacterial isolate. This suggests that the bacterial spectrum differs from place to place.

Among the gram positive isolates, least resistance was seen to vancomycin and linezolid which is similar to many other studies.^[7,10,13,19,20,21] Vancomycin is the drug of choice for MRSA while linezolid is oral antibiotic that is reserved for resistant staphylococcal infection. MRSA isolates showed resistance against most antibiotics including co-trimoxazole, ciprofloxacin, nitrofurantoin, erythromycin and doxycycline with multidrug resistance being higher in MRSA as compared to MSSA. Similar finding was reported in other studies.^[19,20,22] In present study co-trimoxazole resistance among staphylococci ranged from 70-88% which is higher than that reported by Nazneen *et al.* (59%) and Zoubi *et al* (16.8%).^[17,21] Study by Mamtara *et al*^[20] revealed 60.8% and 35.4% co-trimoxazole resistance among MRSA and MSSA respectively. In a study done by Paul R. *et al*^[7] 81% and 87% co-trimoxazole resistance was noted for *Staphylococcus aureus* and CoNS respectively.

In present study *Enterococcus* spp. showed 33.3%, 50% and 66.6% resistance to penicillin, erythromycin and ciprofloxacin respectively. It is similar to the findings of Mamtara *et al*^[20] and Nazneen *et al*^[17]. While Paul R. *et al*^[7] has reported 100% resistance to penicillin, macrolid as well as fluoroquinolones. Although increasing prevalence of VRE infection is being reported in India, no VRE was isolated in our study.^[23] Nazneen *et al*^[17] has reported 33% resistance to linezolid among *Enterococcus* which is very high compared to our findings.

One isolate of *Streptococcus* spp. and *Streptococcus pneumoniae* each was found to be resistant against penicillin. Increasing numbers of penicillin resistant streptococci are being reported from India.^[24]

Among gram negatives, most were found resistant to ampicillin, amoxy-clav, co-trimoxazole and ciprofloxacin while least resistance was exhibited to amikacin similar to the results of many studies.^[8,10,13,14,25] Restricted use of amikacin because of the cost and parenteral administration may be the contributing factor for less resistance.

Ciprofloxacin resistance was seen in 60% of *E.coli*, 66% of *Acinetobacter*, 61% of *Pseudomonas* and 55% of *Klebsiella* spp in our study. Similar results were observed in a study by Hossam MA *et al*^[26] while higher resistance was reported by Dutta S. *et al*^[8] and Nazneen *et al*^[17]. The increased use of fluoroquinolones might have resulted in increased resistance to them.

The present study showed a very high percentage of resistance among Gram negative organisms to cephalosporin antibiotics. Similar findings were reported by Paul R. *et al*^[7], Javeed *et al*^[13] and Sankarankutty J *et al*^[14]. High incidence of resistance to cephalosporins noted in our study may be due to the increasing emergence of ESBL producing organisms following increased usage.

The resistance to carbapenems in our study was similar to those reported by Nazneen *et al*^[17] (16%-33%) which was lower than that reported by Paul R. *et al*^[7] but higher than the findings depicted by Dutta S. *et al*^[8], Kotgire S. *et al*^[10] and Sankarankutty J. *et al*^[14].

Our resistance pattern was in concordance with studies carried out by many other researchers though in their studies resistance to nitrofurantoin in uropathogens was on lower side as compared to our study.^[8,25] Sankarankutty J. *et al*^[14] have reported nitrofurantoin resistance comparable to our study.

High frequency of resistance to different classes of antibiotics was observed for both gram positive and gram negative bacteria in the present study. Most of the isolates from our study showed resistance against two or more of the commonly used antibiotics. Such a situation creates difficulties in treating patients with multi drug resistant organisms. Increasing antibiotic resistance in bacteria leads to increased use of last line antimicrobial agents which are associated with more side effects and much higher costs. Since the bacterial profile and antibiotic resistance patterns may change from time to time, there is a continuous need of surveillance in our set up to get updated knowledge of this changing trend.

CONCLUSION

Antimicrobial resistance is a major deterrent to favourable patient outcome by increasing overall morbidity and mortality, lengthening duration of hospital stay as well as health care expense. Reduction of the same is both challenge and goal for health care providers around the globe. Strict infection control measures, formulation of antibiotic policy as well as surveillance activities have become imperative. A continuous monitoring and regular generation of data on aetiological agents and their antimicrobial susceptibility patterns are important measures. Our study will prove to be of great significance in understanding the current trend of resistance pattern of common bacterial pathogen and in developing the antibiotic policy.

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Conflicts of interest

There are no conflicts of interest.

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