Original Article

Bacteriological Profile and Antimicrobial Sensitivity Pattern of Clinical Isolates from Patients Attending Tertiary Care Hospital

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ABSTRACT

Background: Bacteriological infection plays vital role in determining the outcome as well as cost and duration of hospital stay for patients. Therefore a regular surveillance of important bacterial isolates and their susceptibility pattern is mandatory. So the present study was undertaken to find out bacterial pathogens causing infection in patients attending at our tertiary care hospital and to know drug sensitivity pattern of isolates.

Methods: The study was carried out in the Department of Microbiology, Indian Institute of Medical Science and Research during the period from July 2015 to February 2016. A total 8189 clinical samples (urine, blood, sputum, pus etc.) were collected and processed for culture, identification as per standard recommended procedures and antibiotic susceptibility testing were carried out on isolates as per Clinical Laboratory Standard Institute (CLSI) guidelines.

Result: 2976 different types of bacterial pathogens were isolated. The prevalence of gram negative bacilli were 70.83% and gram positive bacilli were 29.17%.The commonest pathogen isolated was Escherichia coli 33.09%, followed by Staphylococcus aureus 26.27%, Klebsiella spp 23.85% and nonfermenters 10.68 % (Pseudomonas aeruginosa and Acinetobacter Spp). Most of gram negative bacilli were resistant to commonly used drugs such as cotrimaxozole, ciprofloxacin and were sensitive to carbepenems. whereas Gram positive bacteria shown resistant to erythromycin, cotrimaxozole and to some extent cefoxitin.

Conclusion: The present study reveals microbiological profile in patients attending our hospital. Regular surveillance help in implementing better therapeutic strategies to reduce morbidity and mortality associated in patients in health care facility.There is, in general resistance amongst gram negative bacilli to commonly used drugs and shown good sensitivity to carbepenems and aminoglycosides. Resistance among gram positive is not acute, although the Methiciilin resistance staphylococcus aureus (MRSA) incidence is increasing in our setup.

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Introduction

In spite of vast advances made by medical science, bacterial infection remains major cause of concern. Throughout the world bacterial infections are one of leading cause of morbidity, mortality, responsible for increased health care cost and accounts for major burden on patients and public health system of any country. [1,2]

The increased risk of bacterial infection is further compounded by rising trends of antibiotic resistance in commonly implicated organisms all over the world. [3] Antibiotic resistance among bacteria is becoming more and more serious problem throughout the world. This is particularly true in the case of members of *Enterobactericae* group like *Escherichia coli* and *Klebsiella Spp* and non-fermenter group of bacteria such as *Pseudomonas Spp* and *Acinetobacter Spp*. [3,4]

Increasing resistance among gram positive organisms is also matter of concern and high rates of methicillin resistance *Staphylococcus aureus* (MRSA) in clinical samples have been noted. Similarly resistance to glycopepetides antibiotics such as vancomycin and teicoplanin among clinical isolates of *Enterococci Spp*is also increasing. [5,6]

The pattern of bacteria causing infections and their antibiogram vary widely from one country to another,as well as from one hospital to other and even among ICUs with one hospital. [2,4,5] There also appears to be a significant lack of studies highlighting susceptibility patterns of locally prevalent organisms.

Knowledge of predominantly isolated bacterial microorganisms and their sensitivity to available drugs is of immense value to the rational selection of antimicrobial agents and for development of appropriate antibiotic policies.

Therefore, the present study was undertaken to identify prevalence of common bacterial isolates and their antimicrobial susceptibility pattern of various clinical samples from patients attending tertiary care hospital.

Materials and Methods

Study Design: The present study is prospective type of study and was carried out at Department of Microbiology, Indian Institute of Medical Sciences Badnapur , Jalna, Maharshtra a tertiary care hospital after approval from institutional ethics committee.

The study was carried out during the period of July 2015-February 2016; a total 8189 clinical samples were evaluated. The clinical samples received from various departments of the hospital were included in the study.

Collection of Sample and Processing : The samples collected were mainly urine, blood, pus, sputum, CSF, throat swab, stool, tracheal aspirate and other body fluids such as pleural fluid and ascetic fluid. The samples were then sent immediately to the microbiology laboratory for culture and sensitivity. Standard operating procedures were used to collect samples.

The samples were then inoculated on Nutrient agar, Blood agar and MacConkey's agar plates and incubated aerobically at 37° C temperature for 24 hours. Growth was processed according to standard microbiological techniques which includes Gram staining, colony characteristics and biochemical properties. [7,8]

Antimicrobial Sensitivity Testing: Criteria for antimicrobial sensitivity testing was carried out as per Clinical Laboratory standard institute (CLSI). [9] Antimicrobial sensitivity testing was done on Muller Hinton Agar (MHA) by Kirby Bauer's disc diffusion method . Commercially available discs (Hi-media) were used. Concentration of discs used were Erythromycin (15 mcg), Vancomycin (30mcg), Co-trimoxazole (25mcg), Ciprofloxacin (5mcg), Linezolid (30mcg), Ampicillin (30mcg), Piperacillin+Tazobactum (100/10mcg), Ceftazidime (30 mcg), Amikacin (30 mcg), Ofloxacin (5mcg), Gentamicin (10mcg) & high level (30mcg) ,Furazolidone (300mcg), Azetronam (30mcg), Chloramphenicol (30mcg),and Imipenem (10mcg).

Nitrofurantoin (300mcg) was used in case of urine isolates.

Methicillin resistance in *Staphylococcus aureus* (MRSA) was tested using Muller Hinton Agar with Cefoxitin disc (30mcg) by Kirby-bauer disc diffusion methods as per CLSI guidelines. [9]

Suspected extended- spectrum beta lactamases (ESBLs) producing Enterobactericae were confirmed by double disk synergy test as per CLSI guidelines.[9]

Staphylococcus aureus (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used as quality control throughout the study for culture and antimicrobial susceptibility testing.

Statistical Analysis: The data was analysed and evaluated on the basis of percentage values and the result were presented in the form of tables and figures. Microsoft excel was used for the interpretation of these results.

Results

During 8 month study period, 8189 clinical samples were analysed. 2976 (36.37%) organisms were isolated. Of all positive samples, 1956 (65.7%) were the organisms

isolated from hospitalised patients while 1020 (34.3%) organisms were isolated from those who attended out-patients department.Total number of organisms isolated from various clinical samples are shown in Table.1

2108 (70.83%) were gram negative isolates and 868 (29.17%) were gram positive isolates. Most common bacteria isolated were *E.coli/ Klebsiella spp* 1687(56.68%) followed by *Staphylococcus aureus* 782(26.27%) and non-fermenters 318 (10.68%) (*Pseudomonas aeruginosa / Acinetobacter spp*). Table 2 & 3

The detailed bacteriological profile and their antibiogram from various clinical samples can be seen from table 4,5,6,7&8. The prevalence of multidrug resistance organisms is shown in table 9

Table 1: Number Organisms isolated from various clinical samples (n-2976).

Sr. No.	Clinical samples	Number of organisms isolated	Percentage%
1.	Urine	1370	46.01
2.	Blood	369	12.48
3.	Pus swab	876	29.43
4.	Sputum	181	6.08
5.	Others	180	6.04
	Total	**2976**	**100**

Others- CSF, throat swab, stool, pleural fluid, ascetic fluid, tracheal aspirate

Table 2 Gram Negative Isolates (n-2108).

Sr.No.	*Gram negative isolates*	Total numbers	Percentage%
1.	*E.coli*	985	46.72
2.	*KlebsiellaSpp*	710	33.68
3	*Pseudomonas aeruginosa*	198	9.39
4	*Acinetobacterspp*	120	5.69
5	*Salmonella typhi/ S.paratyphi A*	51	2.41
6	*Proteus*	44	2.08
Total		**2108**	**100**

Table 3 Gram positive Isolates (n-868).

Sr. No.	*Gram positive isolates*	Total numbers	Percentage%
1.	*Staphylococcus aureus*	782	90.09
2.	*Enterococci spp*	69	7.94
3	*Streptococcus pneumonia*	12	1.38
4	*Streptococcus pyogens*	5	0.58
Total		**2108**	**100**

Table 4.Urinary Tract Infections (UTI) – Antibiogram Microbiology data (n=1370)

Most common pathogens	Prevalence%	Antibiotic sensitivity
Ecoli (n=822)	60	Imipenem (100%), Ofloxacin/ Ceftazidime (83%), Gentamicin (74%) Amikacin/ Nitrofurantoin(60%) Ciprofloxacin (54%), Cotrimaxozole(40%)
Klebsiellaspp (n=383)	28	Imipenem (100%), Ceftazidime (92%),Ofloxacin(78%), Gentamicin (70%) Amikacin/Nitrofurantoin (64%) Ciprofloxacin(58%), Cotrimaxozole(50%)
Staphylococcus aureus (n=137)	10	Linezolid (100%),Vancomycin (96%), Cefoxitin(64%), Gentamicin (60%), Erythromycin(58%), Cotrimaxozole/ Nitrofurantoin (40%)
Pseudomonas aeruginosa (n=14)	1	Imipenem (92%),Pipercillin-tazobactam(85%),Amikacin(78%) Ceftazidime/ciprofloxacin (64%),Azetronam/ Cotrimaxozole (50%)
Enterococci (n=08)	0.5	Linezolid/ Vancomycin (100%), Ampicillin(87%), Erythromycin (75%) Gentamicin (high level)(62%), Nitrofurantoin(50%)
Proteus/Acinetobacter (n=07)	0.5	Imipenem (100%), Ofloxacin(85%), Gentamicin(71%) Amikacin /Nitrofurantoin (57%),Ceftazidime/ciprofloxacin(42%) Cotrimaxozole(28%)

Table 5: Blood Stream Infections (BSIs) – Antibiogram Microbiology data (n=369).

Most common pathogens	Prevalence%	Antibiotic sensitivity
Staphylococcus aureus (n=181)	49	Linezolid/ Vancomycin (100%), Cefoxitin(77%), Gentamicin(69%), Erythromycin(56%), Cotrimaxozole/ Ciprofloxacin(50%)
Klebsiellaspp (n=77)	21	Imipenem (100%), Ofloxacin(90%), Gentamicin(80%) Amikacin (62%) Ceftazidime /Ciprofloxacin(54%), Cotrimaxozole(45%)
Salmonella typhi/Para typhi A (n=51)	14	Ceftrioxone(100%),Ofloxacin(88%),Amoxycillin(82%), Cotrimoxazole(80%), chloramphenicol(76%), furazolidone(62%)
Pseudomonas aeruginosa / Acinetobacter (n=41)	11	Imipenem/ Pipercillin-tazobactam (100%), Amikacin(85%) Ceftazidime/ciprofloxacin(78%), Azetronam/ Cotrimaxozole (50%)
Enterococci (n=18)	5	Linezolid/ Vancomycin (100%), Ampicillin(88%), Gentamicin (high level)(83%), Erythromycin(66%) Nitrofurantoin(50%)

Table 6: Skin & soft tissue infection- Antibiogram Microbiology data (n=876).

Most common pathogens	Prevalence %	Antibiotic sensitivity
Staphylococcus aureus(n=351)	40	Linezolid(100%), Vancomycin(92%), Gentamicin(72%), Cefoxitin(63%), Erythromycin (56%) Cotrimaxozole/ Ciprofloxacin(50%)
Ecoli /Klebsiellaspp (n=245)	28	Imipenem (100%), Ofloxacin(88%), Gentamicin(78%) Amikacin (72%) Ceftazidime /Ciprofloxacin (64%), Cotrimaxozole(51%)
Pseudomonas aeruginosa (n=122)	14	Polymyxin B (100%)Imipenem(95%), Pipercillin-tazobactam (83%) , Amikacin(77%), Ceftazidime (73%),Ciprofloxacin(69%), Azetronam/ Cotrimaxozole (50%)
Proteus spp / Acinetobacter (n=113)	13	Imipenem (100%), Ofloxcin(92%), Gentamicin(85%) AmikaciN(82%),Ceftazidime/ciprofloxacin (71%) Cotrimaxozole(48%)
Enterococci (n=43)	5	Linezolid/ Vancomycin (100%), Ampicillin(93%), Gentamicin (high level)(90%), Erythromycin(50%)

Table 7: Lower respiratory tract infection (pneumonia) – Antibiogram Microbiology data (n=181).

Most common pathogens	Prevalence%	Antibiotic sensitivity
Klebsiella pneumonia and other *enterobacteriaceae* (n=103)	57	Imipenem (98%), Oflaxcin(92%), Gentamicin (85%), Ceftazidime(82%) Amikacin /Ciprofloxacin(77%), Cotrimaxozole(49%)
Staphylococcus aureus(n=41)	23	Linezolid(100%), Vancomycin(90%), Gentamicin (78%), Cefoxitin(50%), Erythromycin(43%), Cotrimaxozole/ Ciprofloxacin(27%)
Pseudomonas aeruginosa/ Acinetobacter (n=24)	13	Polymyxin B (100%),Imipenem(95%), Pipercillin-tazobactam (83%) Amikacin(75%), Ceftazidime (70%), Ciprofloxacin(69%), Azetronam/ Cotrimaxozole (62%)
Streptococci pneumonia (n=12)	7	Linezolid /Cetriaxone (100%), Ofloxacin(83%), Amoxy-clavulante(75%), Erythromycin(43%)

Table8: Bacteriological profile of other potential infectious clinical samples- Antibiogram(Thorat swab, pleural fluid, ascetic fluid, CSF, stool, tracheal aspirate) Microbiology data (n=180).

Most common pathogens	Prevalence%	Antibiotic sensitivity
Staphylococcus aureus (n=72)	40	Linezolid(100%), Vancomycin(97%), Gentamicin(90%), Cefoxitin(75%), Erythromycin(69%), Cotrimaxozole/ Ciprofloxacin(50%)
E.coli and other *enterobacteriaceae* (n=57)	32	Imipenem (98%), Oflaxcin(92%), Gentamicin (85%), Ceftazidime(82%) Amikacin /Ciprofloxacin(77%), Cotrimaxozole(49%)
Pseudomonas aeruginosa/ Acinetobacter (n=45)	25	Polymyxin B/ Imipenem (100%), Pipercillin-tazobactam (94%) Amikacin(83%), Ceftazidime(80%),Ciprofloxacin(75%), Azetronam/ Cotrimaxozole (50%)
Streptococcus pyogens(n=5)	3	Ceftriaxone/Ofloxacin/Vancomycin(100%), Erythromycin(80%), Penicillin(80%)

Table 9: MDRO pattern

Sr.No.	Organisms	Total numbers	MDRO	Percentage%
1.	E.Coli/ Klebsiellaspp	1687	310 (ESBL- producers)	18.37
2	Staphylococcus aureus	782	282 (MRSA)	36.06

MDRO- multi drug resistant organisms.

Discussion

The microbial pathogens, as well as their antibiotic sensitivity patterns may change from time to time and place to place. The overuse and misuse of antibiotic is leading to emergence of resistance. Hospital antibiogram are commonly used to help guide empiric antimicrobial treatment and are important component of detecting and monitoring trends in antimicrobial resistance.

In the present study the most common microorganism isolated were *Escherichia coli* (33.09%), *Staphylococcus aureus* (26.27%), *Klebsiella spp* (23.85%) and nonfermenter (10.68%) (*Pseudomonas aeruginosa* and *Acinetobacter Spp*) similar findings were seen in studies carried out by many researchers.[10,11,12,13]

Amongst gram negative bacilli *Escherchia coli* was dominant pathogen isolated from urine and skin & soft tissue infections whereas *Klebsiella pneumonia* was mostly isolated from lower respiratory tract infections and blood stream infection. While amongst gram positive bacteria *Staphylococcus aureus* was dominant pathogen isolated in blood stream infections, urinary tract infection and from other potentially infectious samples followed by *Enterococci Spp,* similar trends were seen in studies carried out by K Yadav et al.[11]

The study showed a very high percentage of resistance among organisms to betalactam antibiotics, combination of betalactam/ betalactamase inhibitors. Most of gram negative bacteria shown resistance to cotrimaxozole, ciprofloxacin, and to less extent to amikacin, whereas most of the uropathogens shown 50% resistant to nitrofuantoin, similar findings regarding drug resistance pattern were observed by other researchers .[5,12,13] The present study also highlights that gram negative bacilli were 95-98% sensitive to carbepenems, and incidence of carbepenems resistance is very low in our setup as oppose to increasing trend of carbepenems resistance shown by other researchers. [14,15] Most of the non fermenter (*Pseudomonas aeruginosa* and *Acinetobacter Spp*) shown 100% sensitivity to polymyxin B and showed excellent sensitivity to carbepenems and pipercillin-tazobactam.

In case of gram positive bacteria most of isolates especially *Staphylococcus aureus* were sensitive to vancomycin & linezolid and 50% of resistance was shown to ciprofloxacin, cotrimaxozole and erythromycin. Most of *Enterococci Spp* were 100% sensitive to vancomycin/linezolid, followed by high level gentamicin and ampicillin and 50% resistant was seen with erythromycin. Our sensitivity pattern was in concordance with studies carried out by many other researchers though in their study resistant pattern to vancomycin was on higher side as compared to our study.[14,15]

Our study also found out that around 18% Enterobactericae isolates were ESBL producers which is in concordance with other studies. [12,13] Around 36.06% were detected as methicillin resistant *Staphylococcus aureus* (MRSA) which is on slightly higher side, though many other studies shown that overall prevalence of MRSA ranges from 14-45%.[15,16]

Conclusion

The most common microorganism isolated were *Escherichia coli* (33.09%), *Staphylococcus aureus*

(26.27%), *Klebsiella spp* (23.85%) and nonfermenters (10.68%) (*Pseudomonas aeruginosa* and *Acinetobacter Spp*).Antimicrobial resistance pathogens in any hospital settings is major deterrent to patient outcome, increasing duration of patient stay as well as expense. Strict infection control measures like universal precaution, simple hand washing, rational use of antibiotics and strictly adhering to antibiotic policies are necessary for decreasing drug resistance in hospital.

Fortunately, in our study almost all gram negative bacteria were sensitive to carbepenems and also retained useful susceptibility to third generation cephalosporins and aminoglycosides whereas in case of gram positive bacteria all were sensitive to vancomycin/ linezolid though 36.06% were MRSA.

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Competing Interests
None Declared

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