

STUDY OF PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIAL ISOLATES IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: Many human illnesses are bacterial in origin which can be treated with appropriate antibiotics and selection of these is mostly based on culture and sensitivity. The problem of antimicrobial resistance has burgeoned throughout the world both in inpatients and outpatients. We must work together to preserve the power of antimicrobials so as to use these effectively in treating diseases. The aim of the study is to find out the prevalence of infection and sensitivity pattern among bacterial pathogens in a tertiary care hospital.

Methods: This retrospective study was carried out in Vinayaka Missions Medical College and Hospital, Karaikal, Puducherry (U. T) to study the culture and sensitivity pattern of clinical isolates from blood, urine, sputum, wound, ear/throat swab for one year (June 2012- June 2013). The positive cultures and their antibiotic susceptibility testing were performed under the guidelines of Clinical and Laboratory Standard Institute (CLSI). The lab data from Microbiology department were utilized and filled in a proforma and analyzed.

Results: Out of 788 samples, 296 were culture positive. Isolated bacteria were mostly Gram negative organisms (GNO) of which Klebsiella (41.55%) was commonly followed by Pseudomonas (15.20%), Escherichia coli(4.05%) and Proteus (3.71%). Among Gram positive organisms (GPO) Staphylococcus (35.47%) was common. Imipenem (100%), Gentamycin (90.20%), Amikacin (89.14%), were the antimicrobials most sensitive for GNO, while Ceftriaxone (100%), Cefotaxime(100%),Nitrofurantoin(96.15%) and Linezolid(92.13%) were most sensitive for GPO. Widespread resistance to Nalidixic acid (99%),Ampicillin (85.90%), Cefuroxime (75%) is seen in both groups, while Cefazolin(79.25%) and Norfloxacin(83%) was noted for GNO, resistance to Amoxicillin with clavulanic acid (81.66%) and Imipenem(55.55%) was noted for GPO.

Conclusion: The technical abilities to detect novel, resistant mechanisms and to overcome the microbial resistance has to be improved. Antibiotic policies agreed among clinicians, microbiologists and pharmacologists will guide good prescribing, provide maximum coverage for treating infections and ensure antibiotic cycling.

Keywords: Surveillance, Prevention, Drug resistance, Antibiotic cycling.

INTRODUCTION

Antimicrobial resistance is a modern warfare against trillions upon trillions of microorganisms which is constantly evolving [1]. Antimicrobial drugs includes all agents that work against a variety of microorganisms such as bacteria, viruses, fungi and parasites. Antimicrobial resistance is when bacteria or other microbes become resistant to the effects of a drug after being exposed to them [2]. Each antimicrobial agent is injurious only to a certain segment of the microbial world. So, for a given antibacterial agent there are some species of bacteria that are susceptible and others are not. Bacterial species insusceptible to a particular drug are naturally resistant. Species that were once sensitive but eventually become resistant to it are said to have acquired resistance. Acquired resistance affects a subset of strains in the entire species; this explains the different resistance pattern of the same species in different location [1].

Antibiotics were too widely, too cheaply and indiscriminately used worldwide [3]. In India, the main reason for development of antimicrobial resistance could be due to irrational use of antibiotics, over the counter availability of higher / broader antimicrobial agents, higher prevalence of infection and poor monitoring of antibiotic susceptibility surveillance in hospitals [4]. If resistant strains spreads widely, important group of drugs lose their efficacy and would render many infections untreatable [5]. Apart from medical consequences of antibiotic resistance, there is direct cost to society. Newer drugs are costlier and are difficult to manufacture. Hence prevention of resistance outbreaks makes the health care society a spendthrift [3], thus costing the nation a huge sum of money. The problem of resistance is global and need to be tackled on that scale [3]. The intention of the study is to find out the prevalence of common infection and sensitivity / resistance pattern among

bacterial pathogens in a tertiary care hospital situated in a coastal area of Karaikal, Puducherry.

MATERIALS AND METHODS

This retrospective study was carried out in the Department of Pharmacology along with Department of microbiology, Vinayaka Missions Medical College and Hospital, Karaikal-09, Puducherry, U. T. The permission to the study was taken from the Principal of the institution. The data comprising a total of 788 samples collected over a period of one year (June 2012 to June 2013) was taken. Isolates from clinical specimen such as urine, pus, blood, sputum, wound, ear / throat swabs, and peritoneal fluid sample were analyzed. The positive cultures and their antibiotic susceptibility testing were performed under the guidelines of Clinical and Laboratory Standard Institute (CLSI).

The isolates were subjected to antibiotic susceptibility testing by disc diffusion technique after positive culture. The lab data from Microbiology department were utilized and filled in a proforma and analyzed. The Antimicrobial agents used and their drug content in disc are shown in the Table1.

RESULTS

Study population

During the study period, total of 788 samples were received for culture and sensitivity test in the Department of Microbiology in Vinayaka Missions Medical College and Hospital, Karaikal. From the total of 788 specimen obtained, 296 (37.56%) were cultured positive and remaining 492(62.4%) were cultured negative. Out of 296 positive samples, 156 (52.7%) are from male patient and 140 (47.29%) are from female patient.

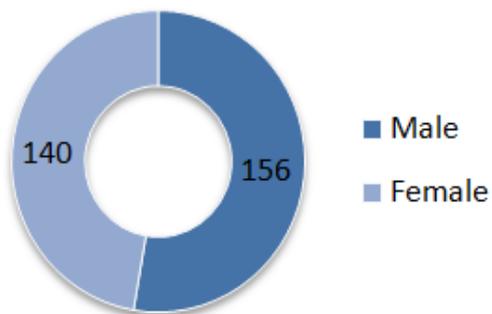


Fig. 1: Represents gender wise distribution of cultural isolates

Stratification based on age indicates more samples are from the age group of 31-40, 41-50 & 51-60.

Prevalence & Characteristics of infection

Out of 296 positive samples, based on microscopy and culture characters 191 were Gram Negative isolates and 105 were Gram positive isolates.

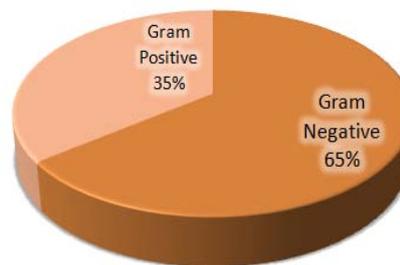


Fig. 2: Represents Gram staining and cultural characteristics of cultural isolates.

Table 1: Antimicrobial agents used and their drug content in the disc

Symbol	Anti microbial agent	Disc content
AK	Amikacin	30 mcg
CF	Ceftazidime	30 mcg
AC	Amoxycillin/Clavulanic acid	30 mcg
CIP	Ciprofloxacin	5 mcg
CPM	Cefepime	30 mcg
CN	Cephoxitin	30 mcg
CXM	Cefuroxime	30 mcg
CZ	Cefazolin	30 mcg
IPM	Imipenem	10 mcg
MR	Meropenem	10 mcg
NX	Norfloxacin	10 mcg
NIT	Nitrofurantoin	300 mcg
OF	Ofloxacin	5 mcg
CTX	Cefotaxime	30 mcg
CFM	Cefixime	5 mcg
CTR	Ceftriaxone	30 mcg
CD	Clindamycin	2 mcg
GEN	Gentamycin	10 mcg
LZ	Linezolid	30 mcg
TEI	Teicoplanin	30 mcg
VA	Vancomycin	30 mcg
CEP	Cephalothin	30 mcg
NA	Nalidixic acid	30 mcg
LE	Levofloxacin	5 mcg
R	Rifampicin	5 mcg
AMX	Amoxycillin	20 mcg
AMP	Ampicillin	10 mcg
CTR	Ceftriaxone	30 mcg

Table 2: Represents Age wise distribution of cultural isolates in this study.

Age	Positive cultural samples
0-10	12
11-20	15
21-30	32
31-40	58
41-50	59
51-60	55
61-70	44
71-80	16
81-90	4
90-100	1

The most predominant Genus/Species in Gram Negative isolates are Klebsiella - 121 (41.55%), Pseudomonas - 45 (15.20%), Escherichia coli - 12 (4.05%) & Proteus vulgaris - 11 (3.71%). Similarly the

most predominant Genus/Species in Gram Positive isolates is Staphylococcus - 105 (35.47%).

^organism of least /negligible count was not included in this study as certain infections are uncommon and rare.

The positive isolates are obtained from following samples: Urine (112*), Pus (116*), Sputum (41*), Blood (8*), Ear swab (7*), Throat swab (1*), Peritoneal fluid (1*) & Wound (10*).

*indicates no. of positive samples obtained from each type of sample.

The above fig. 3 indicates that Klebsiella is the most common causative organism of urinary tract infection (UTIs) and Staphylococcus being the most common causative organism of pyogenic infections.

Antibiotic Resistance

Antibiotic resistance pattern revealed that the majority of bacterial isolates were resistant to multiple anti-bacterial agents.

Widespread resistance to nalidixic acid (99%), Ampicillin (85.90%), Cefuroxime (75%) is seen in both Gram negative isolates and Gram positive isolates. Widespread resistance to Cefazolin(79.25%) and Norfloxacin(83%) was noted for Gram negative isolates and widespread resistance to Amoxicillin with clavulanic acid (81.66%) and Imipenem(55.55%) was noted for Gram positive isolates

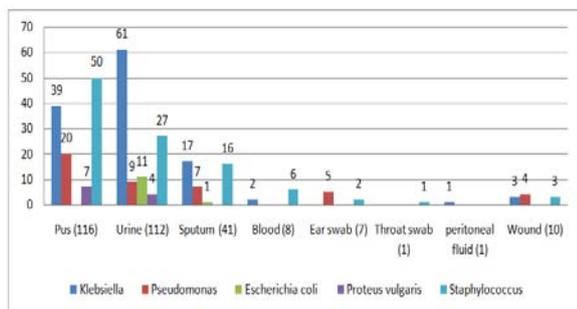


Fig. 3: Represents distribution of isolates, based on type of sample and organism isolated and cultured

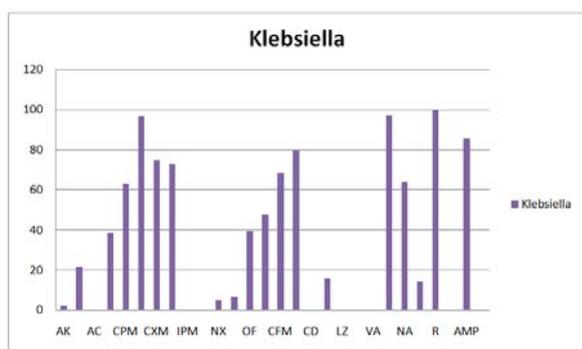


Fig. 4: Resistance pattern of Klebsiella isolates

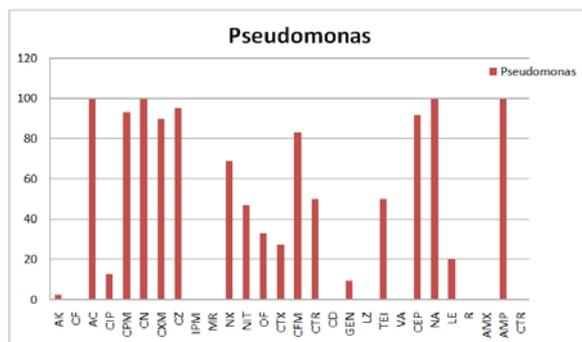


Fig. 5: Resistance pattern of Pseudomonas isolates

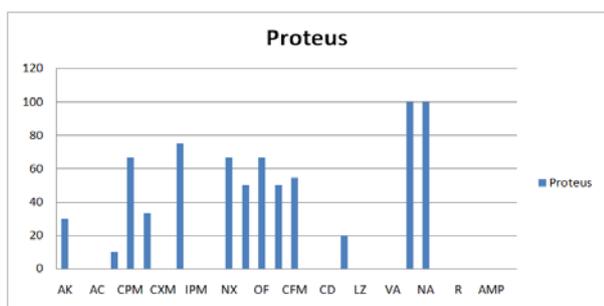


Fig. 6: Resistance pattern of Proteus isolates

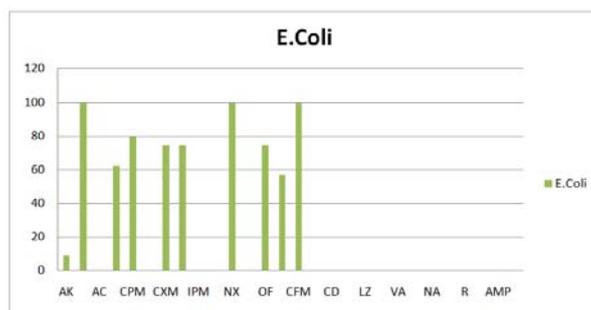


Fig. 7: Resistance pattern of E. coli isolates

Antibiotic Sensitivity

Antibiotic sensitivity pattern indicates the effective drugs to manage multidrug resistant bacterial infections in patients.

Imipenem (100%), Gentamycin (90.20%), Amikacin (89.14%), were most sensitive for Gram Negative isolates, while Ceftriaxone(100%), Cefotaxime(100%), Nitrofurantoin (96.15%) and Linezolid (92.13%) were most sensitive for Gram positive isolates.

DISCUSSION

The discovery of antibiotics has revolutionized the management of infectious diseases more efficiently and timely. As by the famous saying “Every invention and discovery has its own downside”, the bacteria are developing resistance to those antibiotics, becoming recalcitrant and troublemaker to treatment.

In this study, appalling results were obtained about the Sensitivity/Resistance pattern of microbes to antibiotics. The number of positive isolates was 296 out of 788 samples with an infection rate of 37.56%. This is quite higher compared to a other study by Mehta A et al [6]. which showed an infection rate of 20%. The age wise distribution of clinical isolates of positive cultures showed that most of the patients were of the 31-50 years of age. This is similar to Rajat Rakesh M et al [4], Balan K et al [7] and Rashid A et al [8].

The maximum clinical isolates obtained were pus samples followed by urine and sputum samples. This is in line with Balan K et al [7] and Shenoy S et al [9].

On evaluating the common bacterial isolates, Klebsiella was found to be the commonest among urine isolates followed by Staphylococcus aureus, Escherichia coli and Pseudomonas which is comparable to Bajaj JK et al [10]. Klebsiella is most commonly isolated from urine sample which is similar to other studies by Kaushal V Sheth et al [5], Bajaj JK et al [10] and Iffat Javeed et al [11].

Among the wound/swab isolates, Klebsiella was the predominant gram negative bacteria followed by Pseudomonas and E. coli. This is similar to the study done at the same institution, on Surgical Site Infections by Ramesh A et al, 2012 [12]. This result is in contrast to Girish M. B et al [13] where E. coli was the predominant gram negative bacteria.

The isolates from pus samples showed staphylococcus aureus was the commonest organism which is comparable to the studies by Vibha B. Tiwari et al [3] and most other articles. This is in contrast to Sumita Rajeevan et al [14].

Among sputum samples, Klebsiella is more common than Staphylococcus aureus and Pseudomonas. Predominance of Klebsiella over other isolates is similar to Kaushal V Sheth et al [5] and Sriram S et al [15] and is in contrast to Vibha B. Tiwari et al [3].

Among the blood samples isolated Staphylococcus aureus was the predominant followed by Klebsiella and E. coli, comparable to studies by Iffat Javeed et al [11], Sumita Rajeevan et al [14], Vanitha Rani N et al [16], Amita Jain et al [17] and Shreshtha et al [18]. A few positive cultures with Proteus vulgaris were obtained in few pus /urine isolates.

Gram negative isolates and their resistance pattern: The microbes which were positively cultured in our study was Klebsiella, Staphylococcus aureus, Pseudomonas, Escherichia coli, Proteus

vulgaris. Similar to Kaushal V Sheth et al [5] and Raval PN et al [19] Gram negative bacteria predominate Gram positive cocci in our study. On observing the resistance pattern-

Table 3: Resistance pattern of cultural isolates

	Proteus	Pseudomonas	Klebsiella	E. coli	Staphylococci
AK	30	2.57	1.754	9.09	8.602
CF		0	21.43	100	19.04
AC		100			81.66
CIP	10	12.9	38.28	62.25	13.15
CPM	66.66	93.34	62.86	80	50
CN	33.33	100	96.87		
CXM		90	75	75	66.66
CZ	75	95.45	72.98	75	39.13
IPM	0	0	0	0	55.55
MR					
NX	66.66	69.23	5	100	27.27
NIT	50	47.06	6.451	0	3.85
OF	66.66	33.34	39.62	75	41.67
CTX	50	27.28	47.61	57.14	
CFM	54.55	83.33	68.49	100	16.167
CTR	0	50	80		
CD		0			20.77
GEN	20	9.375	15.51	0	30
LZ		0	0		7.86
TEI		50			20.89
VA					15.78
CEP	100	91.66	97.36		
NA	100	100	64		100
LE		20	14.28		30
R			100		44.45
AMX					54.55
AMP		100	85.72		72.72
CTR					0

Table 4: Sensitivity pattern of cultural isolates

AMA	Proteus	Pseudomonas	Klebsiella	E. Coli	Staphylococcus
AK	70	97.43	98.24	90.9	91.39
CF		100	78.57	0	80.95
AC		0			18.33
CIP	90	87.09	61.72	37.5	86.84
CPM	33.33	6.66	37.14	20	50
CN	66.66		3.125		
CXM		10	25	25	33.33
CZ	25	4.54	27.02	25	60.86
IPM	100	100	100	100	44.44
MR					
NX	33.33	30.769	46	0	72.72
NIT	50	52.94	93.54	100	96.15
OF	33.33	66.66	60.37	25	58.33
CTX	50	72.72	52.38	42.85	100
CFM	45.45	16.66	31.5	0	83.33
CTR	100	50	20		
CD		100			69.23
GEN	80	90.62	84.48	100	89.33
LZ		100	100		92.13
TEI		50			79.1
VA					84.21
CEP	0	8.33	2.63		
NA	0	0	36		0
LE		80	85.71		70
R			0		55.55
AMX					45.45
AMP		0	14.28		27.27
CTR					100

Klebsiella showed a high level of resistance to Cephalothin (97.36%), Cefoxitin (96.87%), Ceftriaxone (80%), Cefuroxime (75%), Cefazolin (72.9%) and Ampicillin (30%). The resistance

shown by Klebsiella was also observed in other studies Kaushal V Sheth et al [5], Iffat Javeed et al [11], Li-Yang Hsu et al [20], Amin A et al [21].

Pseudomonas was resistant to Ampicillin (100%), Nalidixic acid (100%), Amoxicillin with Clavulanic acid (100%), Cefoxitin (100%), Cefazolin (95.45%), Cefepime (93.34%) and Cephalothin. This is similar to Iffat Javeed et al [11]. Resistance to Cefepime was also observed by Mohanasundaram KM et al [22] and Basanti Pathi et al [23].

Escherichia coli was resistant to Cefixime (100%), Nalidixic acid (100%), Ceftazidime (100%), Cefipime (80%), Cefuroxime (75%), Cefazolin (75%) and Ofloxacin (75%). This was also identical to Li-Yang Hsu et al [20], Mangaiarkkarasi. A et al [24] and Oteo J et al [25].

Proteus was resistant to Cephalothin (100%), Nalidixic acid (100%), Cefaclin (75%), Cefipime (66%), Norfloxacin (66%), Ofloxacin (66%).

Most gram negative were resistant to first and second generation Cephalosporins and resistance to third generation Cephalosporins is now emerging which is due to Beta lactamase activity by the bacteria. This was similar to the study of Maksun Radji et al [26] and Vera Vlahović-Palčevski, [Croatia] et al [27]. Vera Vlahović-Palčevski et al [27] quotes that third or fourth generation Cephalosporins and Amoxiclav were most misused antibiotics.

Gram negative isolates and their sensitivity pattern

Antibiotics which retained their usefulness and showed less resistance to these gram negative isolates in our study were Imipenem (0% /0% /0%), Amikacin (1.754% /2.57% /9.09%) and Gentamicin (15.51% /9.37% /0%) against Klebsiella, Pseudomonas and E. coli respectively. This is similar to Kaushal V Sheth et al [5], Balan K et al [7], Iffat Javeed et al [11], Girish M. B et al [13], Amin A et al [21], R. Shyamala et al [28], Nathisuwan S et al [29] and Muhammad Naeem et al [30]. According to a study conducted by Hasan AS et al [31], Amikacin and cefotaxime is still effective against Gram negative bacterial infections. In another study conducted by Saghir H et al [32], Imipenem was the most effective drug against Gram negative strains. This is also in line with Kaushal V Sheth et al [5], Mehta A et al [6], Sumita Rajeevan et al [14], Sriram S et al [15] and Gaurav Dalela et al [33].

Specifically, *Pseudomonas aeruginosa* was sensitive to Aminoglycosides, Fluoroquinolones. This effectiveness is also reported by Kaushal V Sheth et al [5], Basanti Pathi et al [23].

Similarly *E. coli* was sensitive to Gentamicin (100%), Imipenem (100%), Nitrofurantoin (100%) and Amikacin (100%). Sensitivity of *E. coli* to Imipenem was also reported by Mangaiarkkarasi. A et al [24], Syed Mustaq Ahmed et al [34]. Similarly Sensitivity to Nitrofurantoin was also reported by Mangaiarkkarasi. A et al [24], Gaurav Dalela et al [33], Bonten M et al [35], and to Amikacin was also reported Mangaiarkkarasi. A et al [24], Mutate AJ [36].

Gram positive isolate and its resistance pattern

Staphylococcus aureus shows resistance to Amoxicillin with Clavulanic acid (81.66%) and Ampicillin (72.72%) and Imipenem (55.55%). Resistance to Ampicillin was also noted by Shrestha S et al [18]. Few of *Staphylococcus aureus* isolates about 15.78% of total 19, shows a low resistance to Vancomycin similar to Vanitha Rani N et al [16], Li-Yang Hsu et al [20] and M. Mehdinejad et al [37].

Gram positive isolate and its sensitivity pattern

Regarding effectiveness of antibiotics for *Staphylococcus aureus* Linezolid, Amikacin, Nitrofurantoin and Vancomycin were much effective. This sensitivity of *Staphylococcus aureus* to Linezolid was observed similarly by Sriram S et al [15] and Raval PN et al [19]. Sensitivity of *Staphylococcus aureus* to Vancomycin was observed similarly by Vanitha Rani N et al [16] and Raval PN et al [19]. Sensitivity to Amikacin was noted by Sriram S et al [15].

CONCLUSION

The results of the present study highlights the alarming development of resistance to almost all the drugs with a very few exception of drugs used in this study. Non empirical /inappropriate

antibiotic use contributes to the emergence of antimicrobial resistance in bacteria both Gram negative and Gram positive organisms. In developing countries, antibiotics are prescribed for 44-97% of patients in hospital, often inappropriately. This was reported earlier by Raval PN et al [19], Vera Vlahović-Palčevski et al [27], Hu S et al [China] [38], Ansari F et al [Iran] [39], Maksun Radji et al [40] and Baktygul Kambalaliev et al [Kyrgyz Republic] [41].

Shockingly, India has one of the highest rates of Gram negative bacillary resistance in the world according to Vibha B. Tiwari et al [3], Mathai D et al [42]. This is due to over reliance on broad spectrum antibiotics, which is because of diagnostic uncertainty among physicians and non-empirical or inappropriate use of antimicrobial agents. Optimal selection of antibacterial agents would decrease the emergence of resistance and would reduce the pharmacy expenditure. Existing and future medical community, health care professionals, Government should address this emerging problem and curb this at its root or else a scenario of pre antibiotic era in near future is inevitable.

Limitation of the study

Antibiotic disc sensitivity test may vary with hospital settings, where infection rate depends on environment and locality, type of infection, its control practices and antibiotic use. Thus, these factors would limit the applicability of this finding to other locality/hospital settings.

CONFLICT OF INTERESTS

We have no conflict of interests.

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