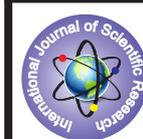


## To identify the antimicrobial sensitivity and resistance pattern of Bacterial isolates among clinical samples from the ICU patients – A prospective study



### Medical Science

**KEYWORDS:** - Gram positive bacteria, Gram negative bacteria, Antibiotic sensitivity pattern, Antibiotic resistance pattern, Mueller Hinton Agar.

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### ABSTRACT

**Introduction:** Resistance to antimicrobial agents (AMR) has resulted in increased morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increased costs is not a simple undertaking, there is little doubt that emerging antibiotic resistance is a serious global problem. The present write-up enumerates various methods for detecting the resistance of common bacteria to various antimicrobial agents as available today. Antibiotic therapy is usually given to protect from infection against various organisms, however, routine use of empirical treatment has resulted in widespread antibiotic resistance and development of antibiotic resistant genes. **Objective:** To identify the antimicrobial sensitivity and resistance pattern of bacterial isolates among clinical samples from the ICU patients. **Materials & Methods:** All the clinical specimens submitted to microbiology laboratory were processed as per standard microbiological procedures and antibiotic sensitivity tests were performed on the isolates as per guidelines of clinical and laboratory standards institute (CLSI). **Result:** A total of 250 organisms were isolated from all clinical specimens, out of which 113 were Gram positive and 137 were Gram negative bacteria. Amikacin showed highest degree of sensitivity among Gram positive bacteria as well as in Gram negative bacteria. Gram positive bacteria showed highest degree of resistance to penicillin and erythromycin, whereas, Gram negative bacteria showed highest degree of resistance to Cefuroxime. **Conclusion:** High frequency of resistance against commonly used antibiotics such as penicillin and cefuroxime as shown in the present study indicates a serious problem in the treatment of infections by gram positive and negative organisms. Therefore continuous surveillance is needed and treatment based on antibiogram report is essential.

### Introduction

Antibiotic resistance among bacteria is becoming more and more serious problem throughout the world. It is said that evolution of bacteria towards resistance to antimicrobial drugs, including multidrug resistance, is unavoidable because it represents a particular aspect of the general evolution of bacteria that is unstoppable. 1. Antibiotic resistance emerges commonly when patients are treated with empiric antimicrobial drugs. To overcome these difficulties and to improve the outcome of serious infections in our institutions, monitoring of resistance patterns in the hospital is needed. 2. A number of studies have been carried out in the west to monitor antimicrobial resistance at national level. The academic and educational value of these studies is particularly useful for microbiologists and infectious disease clinicians. The data collected from these studies are useful in improving antimicrobial use in those communities(3-6).

The discovery of antimicrobial agents had a major impact on the rate of survival from infections. However, the changing patterns of antimicrobial resistance caused a demand for new antibacterial agents. Antimicrobial resistance is a well-known clinical and public health problem (7-8). Bacterial antimicrobial drug resistance is a worldwide problem that is exacerbated by the diminishing number of new antimicrobial drugs in the pharmaceutical pipeline(9-11). This is an emerging public health problem, especially in hospitals of the newly industrialized countries of Asia and the Pacific (12).

Hospital infections, a severe public health issue, are widespread and have high economic and social impact (13). Most infections may be related to unbalanced microbiota and host defense mechanisms, but undoubtedly hospital environments are a great source of potentially pathogenic microorganisms (14). Several bacteria are associated to nosocomial infections, mainly representatives of Gram negative rods from the Enterobacteriaceae family (GNR), nonfermenting Gram-negative rods (NFGNR), Gram positive cocci Staphylococcus, especially coagulase-negative species (CNS) and Enterococcus (ENT) (15). Antimicrobial resistance turns into a complex both ecological and clinical problems when considering the genetic variability in microorganisms. Its contention is one of the greatest challenges of the 21st century, and originates appeals from several international Health Organizations asking for regional data in bacterial

susceptibility patterns, especially for strains of nosocomial circulation (16). Microorganisms may be associated to several biological materials in the hospital environment such as floors, walls, ceiling, doors, windows, electro-electronic equipment and specific hospital articles in use for assistance to patients (17). Thus, the quality of cleaning services is an important condition in the prevention and control of microbial spread, as well as the type of disinfectants used to diminish risks of cross infections during healthcare assistance (18). The most commonly used chemical agents in the nosocomial environment for high level of disinfection are glutaraldehyde, the association of peracetic acid/hydrogen peroxide (0.5 to 2%) and sodium hypochlorite (1%). For medium level of disinfection the products generally used are sodium hypochlorite (0.3 to 0.5%), iodofors, phenol derivatives, 70% ethyl alcohol and 92% isopropyl alcohol. Quaternary ammonium compounds and low concentration sodium hypochlorite(0.2%) are used for low level cleaning and disinfection (19).

### Materials & Methods

A hospital based prospective study was conducted from August. 2016 to December 2016, and various clinical specimens, such as, urine, pus, sputum, blood, synovial fluid, bone, high vaginal swab and ear swab, submitted to the department of microbiology in Heritage institute of medical sciences were included in the study.

All the collected specimens were cultured on blood agar and MacConkey agar and incubated aerobically at 37°C for 24 – 48 hours, but in case of urine the culture was done on Cystiene lactose electrolyte deficient agar (CLED) and the plates were incubated for 24 hours at 37°C aerobically. Growth on culture plates were identified by culture characteristics, gram's staining and standard biochemical test.(20) The antimicrobial susceptibility test was performed on Mueller Hinton agar (Blood agar in case of Streptococcus pyogenes) by Kirby Bauer Disc diffusion method (21), and zone diameters in millimetres were recorded after incubation at 37°C for 24 hours as per guidelines of clinical and laboratory standards institute (CLSI) using antibiotic discs (HiMedia Laboratories, India) such as, amikacin (30µg), gentamicin (10µg), clindamycin (2 µg), levofloxacin (5 µg), chloramphenicol (30 µg), cefoxitin (30 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), pristinamycin (15 µg), tobramycin (10 µg), erythromycin (15 µg), netilmicin (30 µg), penicillin (10 units), co-

trimoxazole (1.25/23.75 µg), ampicillin (10 µg), high strength gentamicin (120 µg), high strength streptomycin (300 µg), vancomycin (30 µg), linezolid (30 µg), piperacillin (100µg), piperacillin/tazobactam (100/10µg), ceftazidime (30µg), cefotaxime (30µg), ceftriaxone.

**RESULTS**

A total of 500 samples were included in the study, out of which 250 showed positive bacterial growth and 750 were negative for any bacterial growth. Out of 250 isolated organisms, 113 were gram positive and 137 were gram negative bacteria,

**Table 1: Distribution pattern of isolated organisms according to specimens**

ORGANISMS ISOLATED	SAMPLES TESTED								TOTAL
	Urine	Pus	Sputum	Blood	Synovial Fluid	Bone	High Vaginal Swab	Ear Swab	
<i>Staphylococcus aureus</i>	8	54	4	-	4	-	4	5	79
<i>Staphylococcus epidermidis</i>	-	6	-	-	-	-	-	-	6
<i>Streptococcus pyogenes</i>	-	3	2	-	-	-	-	-	5
<i>Enterococcus spp.</i>	12	11	-	-	-	-	-	-	23
<i>Escherichia coli</i>	33	16	-	-	-	-	2	-	51
<i>Pseudomonas aeruginosa</i>	4	4	-	2	-	2	-	12	24
<i>Klebsiella spp.</i>	12	18	-	-	-	-	-	3	33
<i>Citrobacter spp.</i>	13	4	-	-	-	-	-	-	17
<i>Acinetobacter spp.</i>	8	4	-	-	-	-	-	-	12
<b>TOTAL</b>	90 (36%)	120 (48%)	6 (2.4%)	2 (.8%)	4 (1.6%)	2 (.8%)	6 (2.4%)	20 (8%)	<b>250</b>

**Table 2: Antibiotic sensitivity pattern of gram positive isolates**

ANTIBIOTICS TESTED	ORGANISMS (n=113)							
	<i>Staphylococcus aureus</i> (n=79)		<i>Staphylococcus epidermidis</i> (n=6)		<i>Streptococcus pyogenes</i> (n=5)		<i>Enterococcus spp.</i> (n=23)	
	S	R	S	R	S	R	S	R
Amikacin	69	10	4	2	NT	NT	NT	NT
Clindamycin	57	22	4	2	5	0	NT	NT
Doxycycline	59	20	4	2	5	0	22	12
Levofloxacin	51	28	3	3	5	0	NT	NT
Chloramphenicol	42	37	5	1	NT	NT	19	4
Cefoxitin	52	27	NT	NT	NT	NT	NT	NT
Ofloxacin	40	39	4	2	4	1	NT	NT
Ciprofloxacin	42	37	6	0	NT	NT	NT	NT
Gentamicin	42	37	6	0	NT	NT	NT	NT
Pristinamycin	54	25	3	3	NT	NT	18	5
Tobramycin	56	23	5	1	NT	NT	NT	NT
Netilmicin	38	41	5	1	NT	NT	NT	NT
Erythromycin	34	45	1	5	NT	NT	18	5
Penicillin	58	21	1	5	1	4	15	8
Co-trimoxazole	45	34	3	3	NT	NT	NT	NT
Ampicillin	45	34	3	3	4	1	18	5
High strength Streptomycin	NT	NT	NT	NT	NT	NT	22	1
High strength Gentamicin	NT	NT	NT	NT	NT	NT	20	3
Vancomycin	NT	NT	NT	NT	NT	NT	22	1
Linezolid	79	0	6	0	NT	NT	23	0

**Table 3: Antibiotic sensitivity pattern of gram negative isolates**

ANTIBIOTICS TESTED	ORGANISMS (n=137)									
	<i>Escherichia coli</i> (n=51)		<i>Pseudomonas aeruginosa</i> (n=24)		<i>Klebsiella spp.</i> (n=33)		<i>Citrobacter spp.</i> (n=17)		<i>Acinetobacter spp.</i> (n=12)	
	S	R	S	R	S	R	S	R	S	R
Amikacin	35	16	24	0	28	5	10	7	8	4
Doxycycline	16	35	NT	NT	26	7	9	8	9	3
Levofloxacin	19	32	22	2	28	5	11	6	6	6
Cefoxitin	28	23	NT	NT	12	21	13	4	NT	NT
Ofloxacin	15	36	18	6	13	20	12	5	NT	NT
Ciprofloxacin	12	39	15	9	18	15	13	4	6	6
Gentamicin	38	13	19	5	29	4	14	3	7	5
Ampicillin	25	26	NT	NT	NT	NT	15	2	NT	NT
Cefotaxime	16	35	NT	NT	17	16	11	6	4	8

Ceftazidime	13	38	18	6	14	19	12	5	8	4
Colistin	NT	NT	24	0	NT	NT	NT	NT	NT	NT
Imipenem	34	17	21	3	17	16	13	4	5	7
Cefaclor	19	32	NT	NT	17	16	14	3	NT	NT
Cefixime	19	32	NT	NT	18	15	16	1	NT	NT
Cefuroxime	8	43	NT	NT	20	13	12	5	NT	NT
Aztreonam	26	25	21	3	21	14	13	4	NT	NT
Piperacillin-Tazobactam	26	25	24	0	24	9	12	5	6	6
Fosfomycinβ	49	2	22	2	NT	NT	NT	NT	NT	NT

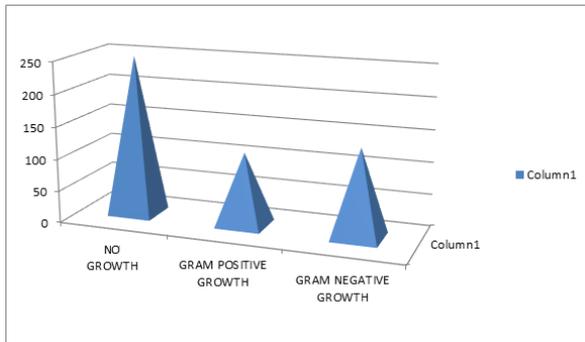


Figure 1: Distribution of positive and negative growth in all clinical specimens.

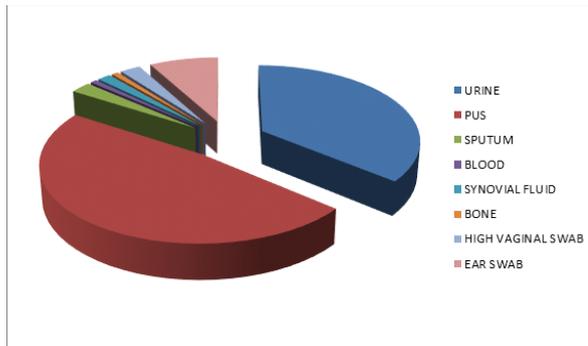


Figure 2: Distribution of various specimens which were showing positive growth.

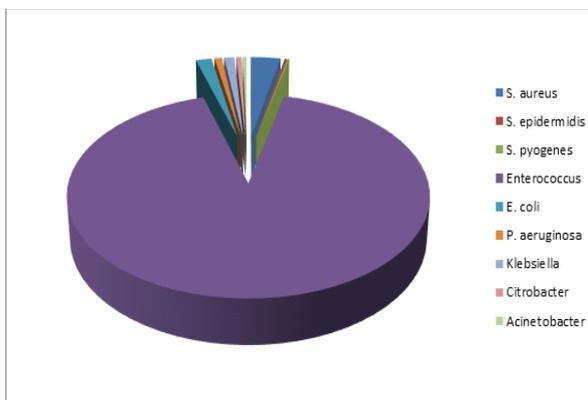


Figure 3: Distribution of various isolated organisms from clinical samples.

**Discussion**

The discovery of antibiotics revolutionized the management of infectious diseases. However, the overuse and misuse of antibiotics is leading to the emergence of resistance to these lifesaving drugs. Resistance due to adulteration of the antibiotics has also been reported. The microbial pathogens, as well as their antibiotic sensitivity patterns may change from time to time and place to place.

Hospital antibiograms are commonly used to help guide empirical antimicrobial treatment and are an important tool for detecting and monitoring trends in antimicrobial resistance.(22,23) Keeping this in mind the present study was done to evaluate the sensitivity and resistance pattern of various clinical isolates.

A total of 1000 samples were submitted in the microbiology laboratory from the all department including ICU, out of which 113 (45.2%) showed positive bacterial growths. In the present study maximum clinical isolates were from pus (48%) followed by urine (36%). The prevalence of negative rods was higher (54.8%) than the gram positive cocci (45.2%). These findings are not similar to those of other worker who also reported higher growth of gram positive bacteria (51%) as compared to gram negative bacteria (49%) from clinical samples.(24-26)

Amongst the gram negative isolates in our study most of them were found to be sensitive to amikacin, piperacillin-tazobactam and imipenem and maximum resistance was shown to ceftazidime .This finding is similar to another study which also showed maximum sensitivity of gram negative bacteria to amikacin, piperacillin-tazobactam and imipenem.(27-28)

In the present study, all the Pseudomonas aeruginosa isolates were found to be 100% sensitive to amikacin, piperacillin-tazobactam, followed by sensitivity to imipenem, ceftazidime and colistin. This is similar to another study which showed highest sensitivity of Pseudomonas aeruginosa isolates to amikacin followed by ceftazidime , however, in contrast to our study, they reported 100% sensitivity to imipenem.(29)

In our study the most prevalent gram positive bacteria was Staphylococcus aureus (69.9 %) followed by Enterococcus species (20.3%), which is comparable to another study done previously.(30)

In the present study it was found that most of the isolates of Staphylococci were highly sensitive to amikacin. Amongst other tested drugs doxycycline showed high sensitivity among gram positive bacteria, whereas, most of them were found to be resistant to Erythromycin .This finding is similar to another study which also reported high susceptibility of Staphylococcus to amikacin.(31)

**Conclusion**

High frequency of resistance against commonly used antibiotics such as Penicillin and Cefuroxime as reported in the present study indicates a serious problem in the management and treatment of infections caused by gram positive and negative organisms. To overcome this problem of drug resistance, continuous surveillance is needed and treatment based on antibiogram report is essential.

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