A Study Of Bacteriological Profile And Their Antibiotic Susceptibility Patterns Of Isolated Organisms From ICU's With Special Reference To Multi-Drug Resistant Organisms

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Abstracts: Background and Aim**:** Microbes are notorious for rapidly develop drugs resistant due to gene transfer and spontaneous mutation. So their continues surveillance of antibiogram pattern is necessary to detect muti-drugs resistant organisms to improve patients outcome admitted in ICUs. Objective is to detect bacterial organisms causing infection in different ICUs and to know their antibiotic resistance pattern. Methodology**:** Total 602 different samples were collected from different ICUs and processed for culture, bacterial identification and antibiotic susceptibility testing done according to CLSI recommendation. Results: Out of total 602 samples, 248 (41.02%) were culture positive. The number of isolated Gram negative and Gram positive organisms were 196 (79.03%) and 52 (20.97%) respectively. Most common isolated of Gram negative organism was *Pseudomonas spp*. 58(23.38%), followed by *Acinetobacter spp*. 46 (18.55%). While, most common isolated gram positive cocci was coagulase *negative staphylococcus spp.* 32 (12.95%), followed by *Enterococcus spp* 8 (3.24%). From total 196 isolates of *gram negative,* 71.43% were MDROs, 7.14% were XDROs. Out of total 52 gram positive isolates, 40.38% were MDRO, 3.85% were XDRO. Conclusion**:** Routine Microbiological surveillance helps to guide in implementing better antibiotic policies to improve patient's morbidity and mortality suffering from multi-drug resistant infections in ICUs. [Patel B NJIRM 2016; 7(3):25 - 29]

Key Words: Bacterial contamination, mobile phones, pens.

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Introduction: Microbes have ability to survive in unfavourable environment by gene transfer or spontaneous mutation. This property helps them to develop resistance against antibiotics.[1] Nowadays Multi-drug resistance microorganisms and nosocomial infections caused by them are major challenge for clinician and are enlisted in major causes for morbidity as well as mortality. Intensive care units (ICUs), is one of such sources of multi-drug resistant organisms and nosocomial infections.[2] the patient in the ICU has a 5 to 7 times higher risk of a nosocomial infection compared with the other patient. This is due to long duration of hospital stay, impaired immunity, various lifesaving procedures, cross infections, colonization of resistance microorganism and exposure to multiple antibiotics.[3, 4]

Infections frequently encountered with drug resistant organisms are ß lactamase, Methicillin resistance Staphylococcus aureus (MRSA), Extended spectrum ß lactamase (ESBL), Metallo ß lactamase (MBL) and carbapenemase producing organisms. So, antibiotic resistance is major concern across the world including India thus Surveys of the prevalence and susceptibility patterns of bacterial isolates are influential for optimum empirical therapy of infections in critically ill patients. In such situations, microbiologist play an important role for prevention and treatment of nosocomial infections caused by multi drug resistant organisms.so, purpose of this study was to find out organism causing infections in ICU patients and to know isolated organisms resistance pattern.

Material and Methods: A total of 602 samples of patients admitted in two ICUs during the period of June 2011 to November 2011 were collected. Samples were collected with sterile aseptic precautions and processed for culture and isolate identification, this was done by standard methods.[5] For culture, Samples were inoculated on Nutrient agar, Blood agar and Mac conkey agar under proper aseptic measures.

All cultured agar media were incubated at 37^0C for 24 hours in aerobic environment, but Blood agar also incubated with presence of 5% CO_2 .After incubation, growth of microorganisms were confirmed by physical appearance of growth on different agars, gram stain from growth and various biochemical reactions.[5] Antibiotic susceptibility testing of all isolates were done on Muller Hinton agar with modified Kirby Bauer disc diffusion method according to CLSI recommendations.[6] After incubation, zone diameter of growth inhibition were measured and it interpreted in susceptible, intermediate or resistant.[6] detection of ß

lactamase, Methicillin resistance Staphylococcus aureus (MRSA), Extended spectrum ß lactamase (ESBL), Metallo ß lactamase (MBL) and carbapenemase production were done separately.[5] On basis of, an international expert proposal for interim standard definitions for acquired resistance isolates, organisms categories as Multidrug resistant (MDR), extensively drug resistant (XDR) and pandrug-resistant (PAN) bacteria: [7]

Results:

Table 1: Details of different clinicalsamples from different ICUs with number of organisms isolated

Samples	Total samples	No. of Positive organisms (n=248)
Blood	173	74(42.77)
Pus	9	6(66.67)
Swab	138	52(37.68)
Urine	179	49(27.37)
Drain	10	9(90)
Resp. Samples	93	58(62.36)
Types of ICUs		
Medical ICU	438	136(31.05)
SICU	107	78(72.90)
Neuro ICU	57	34(59.64)

Table 1 shows out of 602 samples growth obtained in 248 (41.02%) samples.

Table 2: Distribution of various organisms from different clinical samples

Organism	Blood	Respiratory samples	Swab	Urine	Drain	Pus	Total
Pseudomonas spp.	5	13	14	22	2	2	58 (23.38%)
Klebsiella spp.	12	23	5	2	2	0	44 (17.74)
Citrobacter spp.	0	0	2	0	1	2	5 (2.02%)
Acinetobacter spp.	11	11	18	4	0	2	46 (18.55%)
E.coli	7	6	7	13	5	2	40 (16.13%)
Proteus spp.	0	0	2	0	0	0	2 (0.81%)
Providencia spp.	0	1	0	0	0	0	1 (0.4%)
Staph.aureus	2	0	5	0	0	0	7 (2.82%)
Coagulase negative Staphylococcus spp.	32	0	0	0	0	0	32 (12.90%)
Enterococcus spp.	2	0	0	6	0	0	8 (3.23%)
Streptococcus spp.	3	0	0	2	0	0	5 (2.02%)

From total 248 positive samples, Table 2 shows distribution of various organisms from different samples.

In present study organism isolation rate was 41.02%. In gram positive organisms, most common isolated gram positive cocci was 32 (12.95%) *coagulase negative staphylococcus spp.* from, followed by *Enterococcus spp.* 8 (3.24%) and most common gram negative bacilli isolated was 58(23.38%) *Pseudomonas spp.*, followed by *Acinetobacter spp.* 46(18.55%) and *Klebsiella spp.* 44(17.74%).

Table 3 shows Resistance pattern of *pseudomonas spp.* isolates. From total 58 isolates of *Pseudomonas spp*., all were sensitive to colistin (100%) and tigecycline(100%). Out of 58 (100%) isolates, 44(75.86 %) comprised of MDROs. About 13.79% isolates were carbapenemase enzyme producer. *Pseudomonas spp.* isolates were most sensitive to were most sensitive to Colistin (100%), followed by Tigecycline (100%), Carbapenem- Imipenem (86.21%), Quinolones-Levofloxacin (55.17%), β-lactam + β- lactam inhibitor (50%). They were least sensitive to Aminoglycosides-Gentamicin (12.07%). Out of total 58 (100%) isolates, 62.06% were MDROs, 13.79% were XDROs and none of them were PDROs

Table 3: Drug resistance pattern of *Pseudomonas spp.* isolates

Antibiotics	**Resistance pattern (%) of *Pseudomonas spp.*(n=58)**
Piperacillin	43(74.14%)
Ceftazidime	47(81.03%)
Cefepime	26(44.83%)
Ticarcillin-clavulinic acid	29(50%)
Pip.-Tazobactam	29(50%)
Cefepime-Tazobactem	29(50%)
Gentamicin	51(87.93%)
Netilmicin	46(79.31%)
Amikacin	43(74.13%)
Levofloxacin	26(44.83%)
Aztreonam	30(51.72%)
Imipenem	8(13.79%)
Colistin	0(0%)
Tigecycline	0(0%)

Second most common organism isolated was *Acinetobater spp.*

Table 4: Drug resistance pattern of *Acinetobacter spp*. And Enterobacteriaceae

Antibiotics	*E.coli* (n= 40)	*Klebseilla spp.* (n= 44)	*Acinatobacter spp.* (n= 46)
Cefaclor	35 (87.5%)	38(86.36%)	-
Cefotaxime	32 (80%)	35(79.54%)	43 (93.48%)
Ceftizoxime	31 (77.5%)	35(79.54%)	43 (93.48%)
Cefipime	29 (72.5%)	34(72.27%)	41 (89.13%)
Ampi.-sulbectum	-	-	15 (32.61%)
Amox.-clavulinic acid	17 (42.5%)	17(38.64%)	-
Pip.-Tazobactam	15 (37.5%)	17(38.64%)	33 (71.74%)
Ceftriaxone-Tazobactem	15 (37.5%)	17(38.64%)	33 (71.74%)
Gentamicin	34 (85%)	34(72.27%)	43 (93.48%)
Amikacin	31 (77.5%)	25(56.82%)	31 (65.21%)
Levofloxacin	2 (5%)	2(4.54%)	10 (21.74%)
Tetracycline	29 (72.5%)	32(72.73%)	37 (80.43%)
Chloramphenicol	21 (52.5%)	33(75%)	-
Trimethoprim-sulfamethoxazole	29 (72.5%)	39(88.64%)	34 (73.91%)
Imipenem	0 (0%)	0(0%)	1(2.17%)
Polymyxin B	0 (0%)	0(0%)	0(0%)

Table 4 shows Resistance pattern of *Acinetobacter spp.* isolates. From total 46 isolates of *Acinetobacter spp.* They were most sensitive to colistin (100 About 2.17% isolates were carbapenemase enzyme producer. *Acinetobacter spp.* isolates were most sensitive to were most sensitive to Colistin (100%), followed by Carbapenem- Imipenem (97.83%), Quinolones-Levofloxacin (78.26%), β-lactam + β-lactam inhibitor-Ampicillin sulbactum (67.39%). They were least sensitive to Cephalosoprins group of drugs. Out of total 46 (100%) isolates, 30 (65.22)% were MDROs, 4 (8.70)% were XDROs and none of them were PDRO.

Among Enterobacteriaceae group, most common isolate was *Klebsiella spp.* (17.81%) followed by *Escherichia coli* (16.13%) and others as shown in Table-2. Table- 4 also shows resistance pattern (%) of *Klebsiella spp. & E.coli.* In Enterobacteriaceae group, they were most sensitive to Polymyxin B (100%- except proteus spp., intrinsic resistance), followed by Imipenem (100%) and fluroquinolones (95.46%). 39.13% were ESBL producers and none of them were produced carbapenemase enzyme. Out of total 92 (100%) isolates, 78.26% were MDROs, 5.43% were XDROs and none of them were PDRO.

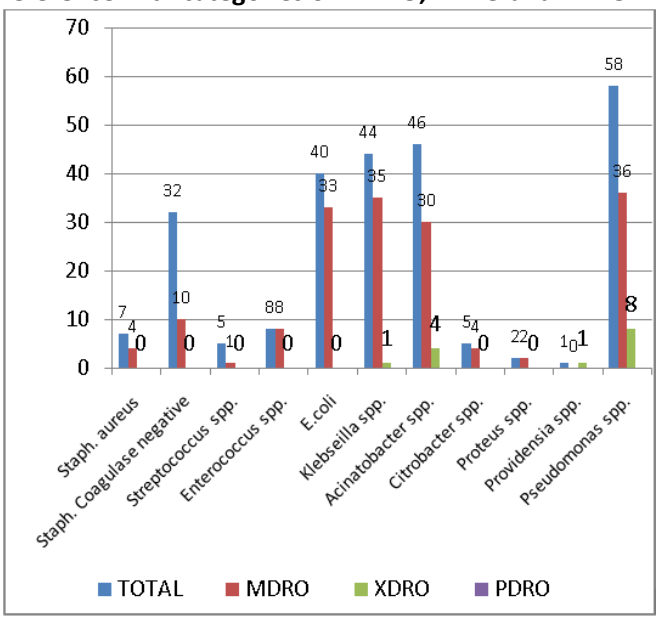
Details regarding resistance pattern of various gram positive organisms is depicted in Table-5. Amongst gram positive organism most common was *Coagulase negative staphylococcus spp.* (12.90%), followed by *Enterococcus spp.* (3.23%) and *Staphylococcus aureus* (2.82%). All gram positive isolates were 100% sensitive to Vancomycin and Linezolid. *Coagulase negative staphylococcus spp. (CONS)* were most sensitive to vancomycin and linezolid followed by Fluroquinolones-Levofloxacin (93.75%), Aminogycosids (84.38%) and least sensitive to Cephlosporin group drug-

Table 5: Drug resistance pattern of Gram positive organisms

Antibiotics	*Staph. Aureus* (n=7)	*Staph. Coagulase negative* (CONS) (n=32)	*Streptococus spp.* (n=5)
Ampicillin	5 (71.43%)	12 (37.5%)	3 (60%)
Oxacillin	3 (42.86%)	5 (15.62%)	1 (20%)
Cefaclor	5 (71.43%)	27 (84.37%)	3 (60%)
Ampicillin-sulbactum	3 (42.86%)	5 (15.62%)	1 (20%)
Gentamicin	1 (14.28%)	5 (15.62%)	1 (20%)
Tetracycline	1 (14.28%)	7 (21.87%)	1 (20%)
Erythromycin	3 (42.86%)	18 (56.25%)	1 (20%)
Clindamycin	1 (14.28%)	11 (34.37%)	1 (20%)
Chloramphenicol	2 (28.57%)	6 (18.75%)	1 (20%)
Ciprofloxacin	2 (28.57%)	5 (15.62%)	1 (20%)
Levofloxacin	0 (0%)	2 (6.25%)	0 (0%)
Trimethorprim-sulfamethoxazole	3 (42.86%)	18 (56.25%)	1 (20%)
Vancomycin	0 (0%)	0(0%)	0 (0%)
Linezolid	0 (0%)	0(0%)	0(0%)

Cefaclor (15.63%).While *Staphylococcus aureus* were most sensitive to Levofloxacin (100%), followed by Aminoglycosides (85.72%) and Chloramphenicol(71.43%). 3 isolates (42.86%) were found to be *Methicillin resistance Staphylococcus aureus (MRSA)*. Out of total 52 (100%) isolates, 40.38% were MDRO, 3.85% were XDRO and none of them were PDRO.

Chart 1 MDRO pattern of isolated organism in reference with categories of MDRO, XDRO and PDRO.



Discussion: In recent years, infection in ICU settings is becoming major problem as it is associated with high rate of mortality particularly due to multi-drug resistant strains. They are an important source for septicaemia, pneumonia and urinary tract infections. Reporting of such isolates is increased in last few years due to use of broad spectrum antibiotics.[8] hence, this study was carried out to assess this problem in our hospital. In present study organisms isolation rate was 41.02%, study by Anurag Ambroz singh [10] in 2015 isolated 94.65 % & 4.73% gram negative and gram positive organisms respectively. Frequent isolation of gram negative organisms may be due to their wide prevalence in the hospital environment. In addition, their frequent resistance to antibiotics may play a role in their persistence and spread.

Finding of Isolation of *pseudomonas spp*. (23.48%) as most common pathogen is comparable with study by Beena patel et al[12], in 2013 & Amit Varaiya et al, in which they found 25 % prevalence.[9] This result can be justified by ability of pseudomonas to produce biofilm and moreover they can survive in unexpected places, making them resistance to multiple antibiotics. In the current study, high level of resistance was observed to Aminoglycosides – Gentamicin(87.93%) against the most common isolate *pseudomonas spp*, while study by Anurag Ambroz singh [10] in 2015 showed highest resistance of *pseudomonas spp.* to ciprofloxacin (84.78%).

Present study isolated *Acinetobacer spp.* as second most common pathogen amongs gram negative organism with highest resistance to cephalosorins (95.56%). Study by Beena patel et al[12], in 2013 Isolated it as third most common organism with highest resistance level to cepahlosporins (100%). Among Enterobacteriaceae group, They were most sensitive to Polymyxin B (100%- except *proteus spp*., intrinsic resistance), followed by Imipenem (100%) and fluroquinolones (95.46%)[11].

Concerning the Gram-positive bacteria, the predominant isolate was *Coagulase negative staphylococcus spp. which* represented 12.95 % of the total bacterial isolates, followed by *Enterococcus spp.* (3.24%) while study by Beena patel et al[12] showed *Staphylococcus aureus* (4%) as most common gram positive organism followed by CONS (3%). Regarding the Gram-positive bacteria, all isolates of *Staphylococcus aureus*, *CONS* and *Enterococcus spp.* showed most sensitivity to vancomycin (100%) & Linezolid (100%). So these drugs can be used in life threatening infections caused by gram positive MDROs.

Conclusion: Non fermenters are most common class of organisms isolated in our ICUs The high frequency of multidrug resistant bacteria in ICUs suggests that we need to prescribe broad-spectrum antibiotics more wisely to reduce pressure on sensitive strains. It is recommended that when physician get antibiogram of isolated organism, physician should deescalate empirical therapy to narrow spectrum antibiotic therapy. The drug of choice in MDROs remain Carbapenems and higher quinolones for all gram negative bacteria and Glycopeptides and oxizolidinones class antibiotics for Gram positive organisms.

Tigecycline is highly effective against Gram- positive bacteria and Gram-negative (except Proteus spp.), it should be used judiciously. The mainstay to control of

antibiotic-resistant pathogens in the ICU is rigorous adherence to infection control guidelines and prevention of antibiotic misuse. Strictly following antibiotic policies result in reduced drug costs and due to continuous changes in antibacterial susceptibility patterns, periodical antibacterial sensitivity assessment in ICUs should be mandatory. *Emphasis was laid on various infection control measures such as* adequate hand washing techniques, aseptic measures for all procedures, antibiotic cycling and health education for the health personnel.

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