ORIGINAL RESEARCH PAPER

Volume-7 | Issue-5 | May-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ANTIMICROBIAL RESISTANCE PATTERN OF ISOLATES FROM INTENSIVE CARE UNIT IN A TERTIARY CARE HOSPITAL IN NORTH-EAST INDIA

Microbiology

Dr. Krossnunpuii*	PGT, Department of Microbiology, RIMS, Imphal, Manipur *Corresponding Author
Dr. Ksh. Mamta Devi	Associate Professor, Department of Microbiology, RIMS, Imphal, Manipur
Dr. S Damrolien	Demonstrator, Department of Microbiology, RIMS, Imphal, Manipur
Dr K. Tharbendra Sharma	PGT, Department of Microbiology, RIMS, Imphal, Manipur
Dr Swarnatrisha Saha	PGT, Department of Microbiology, RIMS, Imphal, Manipur
Prof. Kh. Sulochana Devi	Professor & Head, Department of Microbiology, RIMS, Imphal, Manipur

ABSTRACT

BACKGROUND: Because of their underlying illnesses and high use of antibiotics among ICU patients, colonization with multidrug resistant organism is very common. This study aim to find out the antimicrobial resistance pattern in ICU.

OBJECTIVE: To determine the prevalence of aerobic bacterial pathogens among ICU patients and to see their antimicrobial resistance pattern in RIMS Hospital, Imphal, Manipur.

MATERIALS AND METHODS: A retrospective study was conducted on samples from ICU for three years (November 2014 - October 2017). Samples were subjected to isolation and identification by standard methods. AST was done by Kirby Bauer disc diffusion method following the latest CLSI guidelines.

RESULTS: Out of 501 samples evaluated, 208 (41.5%) were culture positive for aerobic pathogenic bacteria. From the 208 culture positive samples, a total of 224 aerobic pathogens were isolated. Among gram negative, *E. coli* (25.89%) was the most common isolates, followed by Klebsiella spp. (18.75%) and Pseudomonas spp. (18.75%). Among gram positive, *Staphylococcus aureus* (9.38%) was the most common isolates followed by Enterococcus spp. (7.59%). The isolates showed high resistance to Cephalosporins. MRSA was found in 61.90% and MRCONS in 77.78%.

CONCLUSION: As ICU isolates showed high resistance to antimicrobial agents, judicious use of antimicrobial agents and regular monitoring of pattern of resistance among common pathogens is important to prevent further increase in resistance.

KEYWORDS

ICU, antimicrobial resistance pattern, prevalence, aerobic bacterial pathogens.

INTRODUCTION:

ICU caters to patients with severe and life threatening illnesses and injuries. Compared with an average patient, ICU patient has 5 to 10 folds higher risk of nosocomial infection and although ICUs generally comprise 5% of all hospital beds, they account for 20-25% of all nosocomial infections.[1] Factors like increasing use of invasive devices, immunosuppressive drugs and status as well as irrational use of antibiotic therapy in ICUs are all contributing for the same.[2] The ICUs are an area of considerable antibiotic use in which antibiotic-resistant organisms are prevalent. The ICU has even been defined as a factory for creating, disseminating, and amplifying antimicrobial resistance.[3] Antimicrobial resistance is on the rise and is a serious threat to global public health. AMR surveillance helps us to generate information by providing a baseline data on pattern of microorganisms in the hospital and their susceptibility pattern which helps to choose the appropriate antimicrobials.[4] The pattern of organisms causing infections and their antibiotic resistance pattern vary widely from one country to another; as well as from one hospital to other and even among ICUs within one hospital.[5] This study was undertaken with the objective to determine aerobic bacterial pathogens and their antimicrobial resistance pattern among intensive care unit patients in RIMS Hospital, Imphal, Manipur which is a tertiary care hospital in North East India.

MATERIALS AND METHODS:

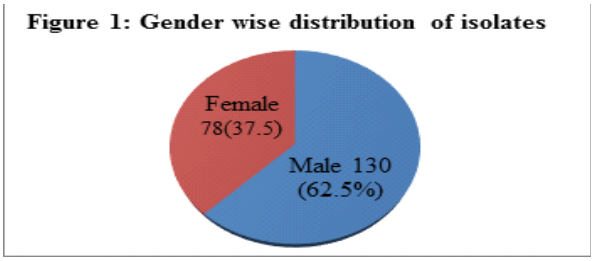
This was a retrospective study, conducted in the Department of Microbiology, RIMS from data collected from laboratory record. The study period was 3 years from November 2014 to October 2017. Samples were inoculated on to Blood agar (BA) and MacConkey's agar (MA) for pus, blood, sputum, aspiration etc and CLED agar for urine and are incubated at 37°C for 24 hrs in aerobic condition. Identification was done by standard microbiological techniques like gram staining, motility testing by hanging drop, and various biochemical tests such as catalase, coagulase, oxidase, citrate utilization, urease, indole, and TSI etc. Antibiotic sensitivity testing was done by Kirby Bauer's disc diffusion method on Mueller Hinton agar and interpreted as per the latest CLSI guidelines. The antimicrobial agents tested were- Gentamicin (10µg), Ceftazidime (30µg), Cefatazidime+Clavulanic acid (30/10µg), Ciprofloxacin (5µg), Nitrofurantoin (300µg), Norfloxacin (10µg), Imipenem (10µg), Piperacillin+Tazobactum(100/10µg), Colistin (10µg) for gram negative bacterial isolates. For Staphylococci and Streptococcus spp., Vancomycin (30µg), Erythromycin (5µg), Linezolid (30 µg), Norfloxacin (10µg), Cefoxitin (30µg), Gentamicin (10µg), Clindamycin (10 µg), Ciprofloxacin (5µg), Nitrofurantoin (300µg), Penicillin (10units) were tested. For Enterococci- High Level Gentamicin (120µg) were employed. Few isolates were subjected to ViTEK2 system.

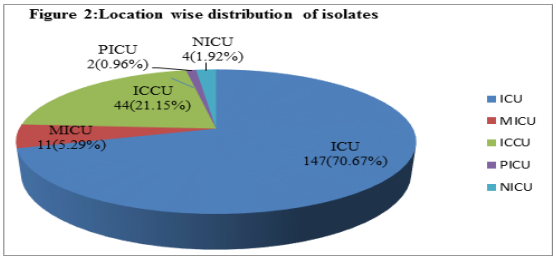
STATISTICAL ANALYSIS:

Data were entered and analyzed using Microsoft Excel. Sample wise and age wise distribution of isolates were expressed in numbers while type of isolates/gender/type of ICUs and drug resistance pattern were analyzed and expressed as percentages.

RESULTS:

In this study, total sample received was 501, out of which total sample positive was 208 (41.52%) and total number of isolates was 224. Isolates from female patients constituted 37.5% (78) whereas isolates from male patients constituted 62.5% (130) (figure 1).





Maximum number of isolates were from Intensive Care Unit (ICU)-147(70.67%), followed by Intensive Cardiac Care Unit (ICCU)-44 (21.15%), Medical Intensive Care Unit (MICU)-11(5.29%), Neonatal Intensive Care Unit (NICU)-4(1.92) and Paediatric Intensive Care Unit (PICU)-2(0.96%) (figure 2). When age wise distribution of isolates were calculated, maximum number of isolates were seen in the age group of 51-60 (43 isolates), followed by 61-70 (41 isolates). (figure 3).

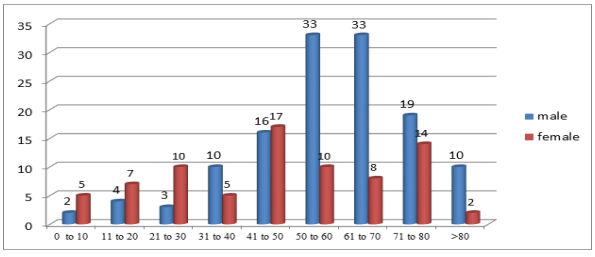


Figure 3: Age wise distribution of isolates

Maximum number of isolates were obtained from tracheal aspirates - 64 isolates , followed by Urine – 62, Blood – 22 and Pus – 12. (figure 4).

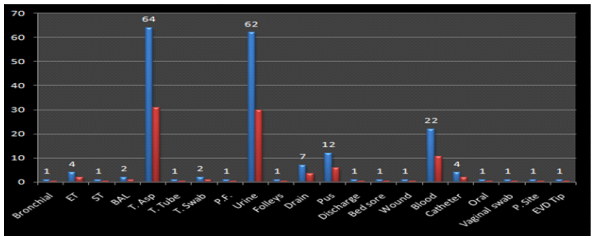


Figure 4: Sample wise distribution of isolates

E. coli was the most common isolates - 58 isolates (25.89%), followed by Klebsiella spp. & Pseudomonas spp.- 42 isolates each (18.75%), Acinetobacter spp. - 26 isolates (11.61%), Staphylococcus aureus - 21 isolates (9.38%), Enterococcus spp.- 17 isolates (7.59%), CONS-9 isolates (4.2%). (figure 5).

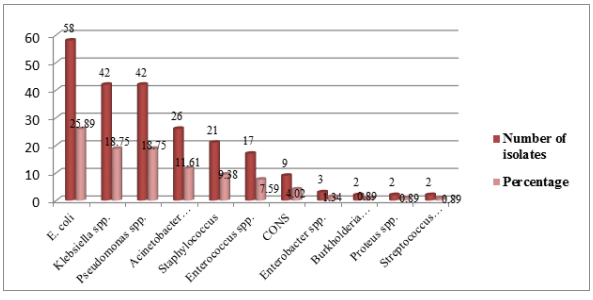


Figure 5: Isolates number and percentage

Gram negative isolates comprises of 78.13%, of which *E.coli* was the most common isolates followed by Klebsiella spp. and Pseudomonas spp.. Gram positive isolates comprises of 21.87% of all the isolates, of which *Staphylococcus aureus* was the most common isolates, followed by Enterococcus spp. (table 1 & 2).

Table 1: Gram positive isolates

Gram Positive Isolates	Number of Isolates	Percentages
Staphylococcus aureus	21	9.38%
Enterococcus species	17	7.59%
CONS	9	4.02%
Streptococcus species	2	0.89%
TOTAL	49	21.87%

Table 2: Gram negative isolates

Gram Negative Isolates	Number of Isolates	Percentages
E.coli	58	25.89%
Klebsiella species	42	18.75%
Pseudomonas species	42	18.75%
Acinetobacter species	26	11.61%
Enterobacter species	17	1.34%
Burkholderia cepacia	2	0.89%
Proteus species	2	0.89%
TOTAL	189	78.12%

Among the *Staphylococcus aureus* isolates, Methicillin Resistant *Staphylococcus aureus* was found in 61.9%. 47.6% of the isolates also showed resistance to Ciprofloxacin. None of the *Staphylococcus aureus* isolates showed resistance to Vancomycin, Linezolid and Nitrofurantoin. Norfloxacin was highly resistant among the Enterococcus spp. isolates (58.9%) and none of the isolates showed resistance to Vancomycin. Among the Coagulase negative Staphylococcus (CONS) isolates, Methicillin Resistant Coagulase negative Staphylococcus (MRCONS) was seen in 77.8% of the isolates. Ciprofloxacin and Erythromycin resistance was observed in 44.4% ofthe isolates each. None of the isolates showed resistance to Vancomycin, Nitrofurantoin and Gentamicin. Among the *E.coli* isolates, high level of resistance was seen to Cephalosporins (> 60%) and Ciprofloxacin (56.9%) and Imipenem was the most sensitive drug. Among Klebsiella spp. isolated, high level of resistance was seen to Cephalosorins(> 59%), Gentamicin,

Ciprofloxacin & Piperacillin+Tazobactum (>45%) and Nitrofurantoin and Imipenem was the most sensitive drugs. The Acinetobacter spp. isolates showed high level of drug resistance- >70% to Cephalosporins, Gentamicin-57.9%, Ciprofloxacin-53.8%. 50% of the isolates showed resistance to Piperacillin+Tazobactum as well. Colistin was the most sensitive drug (100%). Among the isolates of Pseudomonas spp., resistance to Piperacillin+Tazobactum was seen in 45.2% and the most sensitive drug was Colistin (Table 4 & 5).

Table 4: Percentage of Drug Resistance Pattern of Gram Positive Isolates

Organisms	VA	ERY	LZ	NX	FOX	GEN	CD	CIP	NIT	HLG	P
Staphylococcus aureus (21)	0	28.6 (6)	0	19.1 (4)	61.9 (13)	23.8 (5)	23.8 (5)	47.6 (10)	0	NT	38.1 (8)
CONS (9)	0	44.4 (4)	11.1 (1)	11.1 (1)	77.8 (7)	0	22.2 (2)	44.4 (4)	0	NT	44.4 (4)
Enterococcus species (17)	0	35.3 (6)	23.5 (4)	58.8 (10)	IR	IR	IR	17.7 (3)	29.4 (5)	35.3 (6)	41.2 (7)
Streptococcus species (2)	0	50 (1)	0	NT	NT	NT	50 (1)	0	NT	NT	0

VA- Vancomycin, ERY- Erythromycin, LZ- Linezolid, NX- Norfloxacin, FOX- Cefoxitin, GEN- Gentamicin, CD- Clinamycin, CIP- Ciprofloxacin, NIT- Nitrofurantoin, HLG- High level gentamycin, P-Penicillin, NT- Not tested, IR- Intrinsic resistance.

Table 5: Percentage of Drug Resistance Pattern of Gram Negative Isolates

Organisms	GEN	CAC	CAZ	CIP	NIT	NX	IPM	PIT	CL
E.coli (58)	31.0 (18)	63.8 (37)	65.5 (38)	56.7 (33)	6.9 (4)	13.8 (8)	5.2 (3)	25.9 (15)	NT
Klebsiella species(42)	47.6 (20)	59.5 (25)	61.9 (26)	45.2 (19)	4.76 (2)	19.1 (8)	7.1 (3)	45.2 (19)	NT
Pseudomonas species(42)	23.8 (10)	38.1 (16)	35.7 (15)	28.6 (12)	9.5 (4)	7.1 (3)	16.7 (7)	45.3 (19)	2.4 (1)
Acinetobacter species(26)	57.7 (15)	73.1 (19)	76.9 (20)	53.8 (14)	11.5 (3)	19.2 (5)	38.5 (10)	50 (13)	0
Enterobacter species(3)	66.7 (2)	66.7 (2)	33.3 (1)	33.3 (1)	NT	NT	0	33.3 (1)	NT
Proteus species(20)	0	50 (1)	100 (2)	0	IR	0	0	0	NT
Burkholderia cepacia(2)	IR	NT	NT	50 (1)	100 (20)	50 (1)	IR	100 (2)	NT

GEN- Gentamicin, CAC- Ceftazidime+Clavulanic acid, CAZ- Cefatazidime, CIP- Ciprofloxacin, NIT- Nitrofurantoin, NX- Norfloxacin, IPM- Imipenem, PIT- Piperacillin+Tazobactam, CL- Colistin. NT-not tested. IR- intrinsic resistance.

DISCUSSION:

Out of the 501 samples received, 208(41.52%) cases yielded positive culture. Of the 208 positive cases, 195(93.75%) showed pure aerobic growth and 13(6.25%) mixed aerobic growths, giving a total of 224 isolates. Male:Female ratio was 1:0.6(130:78). Maximum number of samples were in the age group of 51-70 years (84, 40.38%).

Most isolates were recovered from respiratory specimen followed by urinary tract and blood which is in accordance with a study done by Jamshidi *et al*, 2009.[6] Among the respiratory specimens, Pseudomonas spp. was the most common isolates (31) followed by Klebsiella spp. (22) and *E.coli* (21). *E.coli* (24) was the most common isolates from Urine sample and *Staphylococcus aureus* (9) from blood samples.

More gram negative (78.13%) were isolated as compared to gram positive (21.87%) which was in accordance with a study done by Sudhamani *et al*, 2015 and Sheth *et al*, 2012.[7,8] In a study by Khan *et al*, 2012, the frequency was 15% & 85% for gram positive and gram negative bacteria respectively.[9]

E.coli (58, 25.89%) was the most predominant gram negative isolate, followed by Klebsiella spp. and Pseudomonas spp. (42, 18.75% each). An almost similar findings were observed in a study by Kumar *et al*, 2017 in India & Akter *et al*, 2014 in Bangladesh.[10,11] Among the 58 *E.coli* isolates, 37(63.79%) were resistant to Ceftazidime+Clavulanic acid, and 38(65.52%) were resistant to Ceftazidime. 60.3% were resistant to Ceftazidime in a study done by Wikaningtyas *et al*, 2015 in Indonesia.[12] Imipenem was the most sensitive drug, 3(5.17%) isolates showed resistance to this drug.

Among the 21 *S.aureus* isolates, 13(61.90%) were Methicillin Resistant *Staphylococcus aureus* (cefoxitin disc diffusion) and 10(47.62%) isolates showed resistance to Ciprofloxacin. *S.aureus* were more sensitive to drugs like Vancomycin, Linezolid and Nitrofurantoin (100% each). our findings in regards to Vancomycin are similar with Sheth *et al*, 2012 where as a study by Hamishehkar *et al*, 2016 found MRSA in 87.5% and Sudhamani *et al*, 2015 found MRSA in 25% of all *S.aureus* isolates.[7,8,13] Among 9 CONS, Methicillin Resistant Coagulase Negative Staphylococcus was found in 7 which comprises of 77.78% of all the CONS isolates.

Out of 17 Enterococcus spp. isolated, 10(58.82%) showed resistance to Norfloxacin; 6 isolates (35.29%) showed resistance to Erythromycin and High Level Gentamicin. Vancomycin was the most susceptible drug for this group (100%).

Among the 42 Pseudomonas spp. isolates, 19(45.24%) showed resistance to Piperacillin+Tazobactum and 15(35.7%) isolates showed resistance to Ceftazidime. In a study done by Golia *et al*, 2016, Piperacillin+Tazobactum resistance was found in 8.92%.[14] 57-63.3% resistance to Ceftazidime was found in a study by Mohanasoundaram, 2011 in Salem India.[15]

The most susceptible drug for Pseudomonas spp. and Acinetobacter spp. was Colistin; 97.62% and 100% respectively. Acinetobacter spp. showed high level of drug resistance to Ceftazidime+Clavulanic acid (73.1%), Ceftazidime (76.9%), Gentamicin (57.7%), Ciprofloxacin (53.8%) and also to Piperacillin+Tazobactum (50%). Kaur *et al*, 2016, found 100% resistant to Ceftazidime, Gentamicin (93.7%), Ciprofloxacin (91.6%) & Piperacillin+Tazobactum 95.8%.[16]

CONCLUSION:

Bacterial isolates from our ICU showed high to moderate level of drug resistance to antibiotics. Development of drug resistance is the major issue while dealing with patients in ICU settings. Our data indicated an alarming pattern of antibiotic resistance among the majority of ICU isolates. Thus, strategies to prevent the emergence of multidrug resistant bacteria in ICU must be implemented. The implementation of antibiotic policy, development of antibiotic stewardship programs in hospitals, proper functioning of Hospital Infection Control Committee and strict adherence to hand washing practices, standard precautions, rational use of antibiotics, and national and local antibacterial surveillances and control programs are the need of the hour to fight emergence of these resistant strains. This study will prove beneficial for the clinicians in knowing the present microbiological profile predominant in our hospital ICU and the antibiotic sensitivity patterns will guide them in adopting the best empirical antibiotic policy for the critical patients.

ACKNOWLEDGEMENT:

The authors would like to thank all direct and indirect participants in this study, the laboratory technicians and department staffs as well as our colleagues for their help and support.

FUNDING: No funding received for the study.

REFERENCES:

1. Günseren F, Mamikoğlu L, Oztürk S, Yücesoy M, Biberoğlu K, Yulug˘ N, et al. A surveillance study of antimicrobial resistance of gram-negative bacteria isolated from intensive care units in eight hospitals in Turkey. J Antimicrob Chemother. March 1999; 43(3):373–8.
2. Mohammadi-mehr M, Feizabadi MM. Antimicrobial resistance pattern of gram-negative bacilli isolated from patients at ICUs of army horpital in Iran. Iranian J Microbiol. 2011;3(1):26-30.
3. Carlet J, Ben AA, Chalfine A. Epidemiology and control of antibiotic resistance in the intensive care unit. Curr Opin Infect Dis. August 2014;17(4):309-16.
4. Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India. National treatment guidelines for antimicrobial use in infectious diseases. National centre for disease control. version 1.0 2016:1-64. Retrieved from: http://www.ncdc.gov.in/writereaddata/linkimages/AMR_ guideline7001495889.pdf.
5. Barai L, Fatema K, Ashraful Haq J, Omar Faruq M, Areef Ahsan ASM, Golam Morshed MAH, et al. Bacterial profile and their antimicrobial resistance pattern in an intensive care unit of a tertiary care hospital in Dhaka. Ibrahim Med. Coll. J. 2010;4(2):66-9.
6. Jamshidi M, Javadpour S, Eftekhari T, Moradi N, Jomehpour F. Antimicrobial resistance pattern among intensive care unit patients. Afr J Microbiol Res. October 2009;3(10):590-4.
7. Sudhamani, Devnikar AV, Mali SH and Parvangada BM. Bacteriological spectrum and antimicrobial resistance pattern in a multidisciplinary intensive care unit. National Journal of Laboratory Medicine. October 2015;4(4):28-32.
8. Sheth KV, Patel TK, Malek SS and Tripathi CB. Antibiotic sensitivity pattern of bacterial isolates from the intensive care unit of a tertiary care hospital in India. Trop J Pharm Res. December 2012;11(6):991-9.
9. Khan M.A. Bacterial spectrum and susceptibility patterns of pathogens in ICU and IMCU of a secondary care hospital in kingdom of Saudi Arabia. International Journal of Pathology. 2012; 10(2):64-70.
10. Kumar M, Sharma AK, Sherwal BL and Neha. Antimicrobial resistance pattern of bacterial isolates from ICU patients in tertiary care hospital. Int J Med Res Prof. 2017;3(2): 364-8.
11. Akter T, Murshed M, Begum T, Nahar K, Duza SS and Shahnaz S. Antimicrobial resistance pattern of bacterial isolates from intensive care unit of a tertiary care hospital in Bangladesh. Bangladesh J Med Microbiol. 2014;8(1):7-11.
12. Wikaningtyas P, Sigit JI, Sukandar EY and Gunawan I. Profile of antibiotic resistance and usage pattern in ICU of private hospital in Bandung, Indonesia. Int J Pharm pharm Sci. January 2015;7(2):160-2.
13. Hamishehkar H, Shadmehr P, Mahmoodpoor A, Mashayekhi SO and Entezari-Maleki T. Antimicrobial susceptibility patterns among bacteria isolated from intensive care units of the largest teaching hospital at the northwest of Iran. Braz J Pharm Sci. July/September 2016; 52(3):403-12.
14. Golia S, Suhani, Manasa S and Jyoti. Isolation of *Pseudomonas aeruginosa* from various clinical isolates and it antimicrobial resistance pattern in a tertiary care hospital. Int J Curr Microbiol App Sci. 2016;5(3):247-53.
15. Mohanasoundaram KM. The antimicrobial resistance pattern in the clinical isolates of Pseudomonas aeruginosa in a tertiary care hospital; 2008-2010 (A 3 year study). J Clin Diagn Res. June 2011;5(3):491-4.
16. Kaur A, Singh S, Gill AK, Kaur N and Mahajan A. Isolation of *Acinetobacter baumannii* and its antimicrobial resistance pattern in an intensive care unit (ICU) of a tertiary care hospital. International Journal of Contemporary Medical Research. June 2016;3 (6):1794-6.