Bacteriological ProfileAnd Antimicrobial Susceptibility Pattern Among Patients Admitted InMICU at A Tertiary Care Hospital.

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Abstract
Background:MICUarefrequentlocations for multidrug resistant germs since the majority of the patients aretakinggreaterdosesofantibiotics.Thedifferencebetweenlifeanddeathinbloodstreaminfectionscanbemadebyearlydetectionandeffectivetreatment[14].Bloodculture is a precise indicator of bacteraemia, and antimicrobial susceptibilitytesting is very important in determining the best antibiotic to use.
Methods: *Study was conducted in tertiary care hospital, during the period from January 2021 to June 2022. Ethical clearance was received from the Ethical Committee of institution .The study is Prospective and Observational.Total 408 sample from Medicine MICU were received in Bacteriology section, Microbiology Department having suspected bloodstream infection (BSI). Their bacteriological profile and antimicrobial susceptibility pattern was studied.*
Results: *Total 508 samples of clinically suspected cases of BSI were received. Majority of subjects belong to 31 – 40 years age with male preponderance. Total 390 (76.77%) samples have not shown any growth of organism i.e. samples were sterile. Total 118 (23.23%) showed growth of various pathogens in the samples received from MICU. In majority of samples 36(30.50%), Staphylococcus aureus was found followed by Klebsiella pneumonia 25(21.18%). Most of the organism were resistant to routinely used antibiotics.*
Conclusion: *Broad spectrum antibiotics must be prescribed to all MICU patients suspected of BSI.*
Key Words: Bacteria, susceptibility, MICU

I.   Introduction

Critically sick individuals are hospitalised to the hospital's MICU and ICU; these patients are more likely to develop blood stream infections. For these individuals, blood stream infections are a major cause of mortality and morbidity[1] . In the first month of hospitalisation, about 7% of patients in ICU[2] have blood stream infections .Blood stream infections are linked to a high mortality rate of between 40 and 60 percent and a three times increased risk of dying in the hospital, which lengthens hospital stays, necessitates the use of numerous antibiotics, and
necessitates regular diagnostic tests[3,4].

ICU and MICU are frequent locations for multidrug resistant germs since the majority of the patients are taking greater doses of antibiotics. The difference between life and death in bloodstream infections can be made by early detection and effective treatment[5]. Blood culture is a precise indicator of bacteraemia, and antimicrobial susceptibility testing is very important in determining the best antibiotic to use[6].

Bloodstream infection patients admitted in ICU have primary disease like endocarditis, CAP (Community acquired pneumonia). Gram positive aerobic bacteria (Staphylococcus aureus, Enterococcus), and Gram negative aerobic bacteria(Enterobacteriaceae, Pseudomonas aeruginosa), are most commonly isolated from the patients in ICU[7] .

The study was done for identifying the organism, microbial characterisation and antibiotic susceptibility pattern. Finding of study would be of great help in formulating antibiotic policy for MICU patients of hospital.

II.   AIMS AND OBJECTIVES

Primary Objective: To study bacteriological profile of bloodstream infections (BSI) in MICU.

III.   Materials & Methods

Study was conducted in tertiary care hospital, during the period from January 2021 to June 2022. Ethical clearance was received from the Ethical Committee of institution .The study is Prospective and Observational .Total 408 sample from Medicine MICU were received in Bacteriology section, Microbiology Department having suspected bloodstream infection (BSI). Samples were collected from patient having at least one criteria like :

1. Fever >380c or
2. Hypothermia ,
3. Rigors and Chills and
4. Increased heart rate.

Inclusion criteria:

1. All Blood cultures from suspected cases of BSI patient admitted in MICU
2. Recognized pathogens isolated from blood cultures .
3. Patients having at least one signs or symptoms of BSI like fever>38 degree C
or hypothermia ,Rigors ,chills &increased heart rate.

Exclusion criteria:

1. Surgical ,Neonatal intensive care unit and Pedriatic patients
2. Common skin contaminated isolates from blood cultures .
3.Fungus and Anaerobes

Sample Collection:[8,9]

Blood was drawn from suspected cases of BSI using aseptic precautions. Blood was collected from the peripheral vein (Median cubital vein) as well as from thehub of the central line catheter.

Volume of blood sample required [10, 11]

For adults : 10ml of blood was put in 50 ml of brain -heart infusion broth .
For children : 5ml of blood was put in 20 ml of brain -heart infusion broth .

Number of Blood Samples:

2 Blood Specimens were drawn,from other two sites – that is peripheral one andother from central line catheter(if present).
Blood collected in Brain-heart infusion broth bottles ,was also checked forits seal.
Send with complete filled laboratory requisition form.

Processing of Blood Cultures:

• The blood culture bottles were quickly transferred to microbiologylaboratory. After receiving sample blood in BHI broth , bottles were keptin incubator at 370C for 48 hours or appearance of turbidity , Haemolysis,gas production ,pellicle formation, puffballs ,clotting which wereindicated for microorganisms. Following this ,they were subjected to Gram staining and culture.

All samples were inoculated on Blood agar(BA)and Mac Conkey agar(MAC).• Plates of both Blood agar and Mac conkey agar are incubated overnightat,37 0C aerobically.Any growth was identified by colony morphology and Gram staining fromthe agar plates .

Isolates Identification[12]

The organism were identified by colony morphology on different media usedsuch as Blood agar, MacConkey agar and by using different biochemical tests i.e. by conventional methods.According to standard microbiological methods ,following test were performed

• Gram stain method
• Catalase test
• Coagulase test
• Oxidase test
• Motility test
• Indole test
• Methyl red test
• Voges Proskauer test
• Citrate utilisation test
• Urease production test

• Triple sugar test

Antimicrobial Susceptibility Testing[9,13]

Antimicrobial susceptibility testing of isolated organism were carried bymodified Kirby-Bauer Disc diffusion method on Mueller Hinton Agar. Using 0.5McFarland as the turbidity standard as per CLSI guidelines 2021.A few coloniesof the microorganism ,need to be tested were suspended and emulsified .The suspension was then diluted, with saline solution to match0.5Mcfarland standard .The bacterial suspension then was streaked evenly in 3planes on the surface of the plates containing Muller -Hinton Agar with a sterilecotton swab ,then the disc were placed on the agar with flamed forceps and gentlypassed down to confirm contact with medium .Disc were evenly placed andplates were immediately incubated.

Commercially available disc(HI-Media) with known potency were usedsensitivity was performed using control strains tested for efficacy againststandard American Type Culture Collection .(ATCC) bacteria ofStaphylococcus aureus ATCC 25923,E.coli ATCC 25922,Pseudomonas aeruginosa ATCC 27853,Klebsiella pneumoniaeATCC700603, Methicillin resistant Staphylococcus aureus (MRSA) ATCC43300.

Quality control for MHA was tested by E, faecalis ATCC2912 as general quality control laboratory procedure.The Zone inhibition was calculated and reported ,after the overnight incubationdone. Interpreted as sensitive or resistant according to CLSI guidelines.

IV. Results

Prospective and observational study, done for all the clinically suspected casesof BSI,admitted in ICU and MICU of tertiary care hospital during January2021 to June 2022 were included .During this period a total of 508 samples ofclinically suspected cases of BSI were processed .All the samples weresubjected for bacteriological investigations.

Table no.1 Shows Age – wise distribution of BSIs patient .

Age (Years)	Male		Female		Total	
	No. of patient	Percentage(%)	No. of patient	Percentage(%)	No. of patient	Percentage(%)
12-20	31	6.12	27	5.31	58	11.43
21-30	46	9.08	40	7.84	86	16.92
31-40	101	19.85	83	16.42	184	36.27
41-50	38	7.59	33	6.37	71	13.96
51-60	31	6.12	27	5.31	58	11.36
61-70	21	4.16	17	3.43	38	7.59
71-80	6	1.22	04	0.74	10	1.96
>80	2	0.49	01	0.24	3	0.73
Total	276	54.33	232	45.67	508	100

Table no 3 shows that among 508 patients with BSIs .The maximum number were inthe age of31-40years is 184 (36.27%), followed by 21-30years age group is 86(16.92%), 41-50years age group were 71(13.96%),51-60 years age group were58 (11.36%).So the maximum patients were from age group 31-40 years, and leastcases were find in the age group of more than and equal to 80 years(0.73%). Majority of subjects were males 276 (54.33%) and females were 232 (45.67%).

Table no.2 Shows distribution of Samples and organisms in MICU

Organism on growth	No.	Percentage
No pathogen growth	390	76.77
Pathogen Growth		
1.Staphylococcus aureus	36	30.50%
2.*Acinetobacter baumannii*	23	19.49%
3.*Escherichia coli*	09	7.62%
4.*Streptococcus spp*	01	0.84%
5.*Klebsiella pneumonia*	25	21.18%
6.*Enterococcus*	13	11.01%
7.*Pseudomonas aeruginosa*	09	7.62%
8.*Proteus*	01	0.84%
9.*Enterobacter*	01	0.84%
Total	**118**	**100 %**

Total 508 samples were received from MICU. Out of it, 390 (76.77%) samples have not shown any growth of organism i.e. samples were sterile. Total 118 (23.23%) showed growth of various pathogens in the samples received from MICU. In majority of samples 36(30.50%), Staphylococcus aureus was found followed

by Klebsiella pneumonia 25(21.18%), Acinetobacter baumannii23(19.49%), Enterococcus 13 (11.01%), Escherichia coli 09 (7.62%), Pseudomonas aeruginosa 09 (7.62%) and Streptococcus spp, Proteus, Enterobacter each in one sample.

Table no.3 Shows **antibiotic resistant pattern of Gram-Negative organisms:**

Gram-negative organism	No of isolates	AMIKACIN	IMIPENEM	MEROPENEM	PIPERACILLIN-TAZOBACTUM	GENTAMICIN	CIPROFLOXACIN	CEFEPIME	CEFAZOLIN	AZTREONAM	CEFTAZIDIME	CEFOTAXIME	CEFUROXIME	AMPICILLIN	LEVOFLOXACIN	TOBRAMYCIN	TETRACYCLINE	MINOCYCLINE	AMPICILLIN-SULBACTUM	CLOTRIMOXAZOLE	COLISTIN
Klebsiellapneumoniae	30	66.7	78.8	75.8	81.8	66.7	87.9	87.9	90.9	NT	NT	90.9	90.9	NT	90.9	NT	NT	NT	NT	72.7	100
Acinetobacterbaumannii	29	69.2	19.2	69.2	69.2	57.7	78.8	78.8	NT	NT	92.3	NT	NT	NT	NT	53.8	38.5	30.8	53.8	73.1	100
Escherichiacoli	14	41.7	58.3	50	58.3	41.7	75	91.7	91.7	NT	NT	83.3	83.3	91.7	75	NT	NT	NT	NT	50	100
Pseudomonasaeruginosa	13	33.3	26.7	33.3	26.7	33.3	33.3	33.3	NT	53.3	40	NT	NT	NT	33.3	NT	NT	NT	NT	NT	100
Enterobacterspp	4	0	33.3	33.3	33.3	66.7	100	33.3	33.3	NT	NT	100	100	100	100	33.3	NT	NT	NT	100	100
Proteusspp	3	0	33.3	0	0	66.7	100	66.7	100	NT	NT	100	100	100	66.7	0	NT	NT	NT	100	100

The most commonest microbial pathogen was Klebsiella pneumoniae 30(19.10%)and it showed multidrug resistance pattern. The resistant pattern showed high resistance for 3[rd]generationCephalosporins (Cephazolin, Cefuroxime, Cefotaxime 90.9% and Cefepime 87%). Ciprofloxacin 87.9%, Cotrimoxazole 72.7%,Piperacillin tazobactam81.8%, Imipenem 78.8%,Meropenem 75.8 %,Gentamicin and Amikacin 66.7% ,Levofloxacin is 90.1% .But 100% sensitivity was found for Colistin .

Table no.4 Shows antibiotic resistant pattern of Gram-Positive organism

Gram-positive organism	No of isolates	CLINDAMYCIN	ERYTHROMYCIN	CEFOXITIN	PENICILLIN	DOXYCYCLINE	CLOTRIMOXAZOLE	LINEZOLID	GENTAMICIN	CIPROFLOXACIN	AMPICILLIN	VANCOMYCIN	HIGH LEVEL STREPTOMYCIN	HIGH LEVEL GENTAMICIN	TETRACYCLINE	BACITRACIN	OPTOCHIN	LEVOFLOXACIN	CEFOTAXIME
Staphylococcus aureus		62.9	18.6	25.6	7.7	76.7	34.9	100	18.6	39.5	NT	100	NT	NT	NT	NT	NT	NT	NT
Enterococcus sp.		-	33.3	NT	41.7	33.3	NT	100	NT	41.2	41.7	90.9	91.7	41.7	NT	NT	NT	NT	NT
Streptococcus.pyogens.		50	100	NT	50	NT	NT	100	NT	NT	NT	NT	NT	NT	NT	NT	100	NT	NT	50

In Gram positive bacteria Staphylococcus aureus was the mostly predominant microbial isolate and that compromised majority of methicillin- resistant Staphylococcus aureus .Screening done by Cefoxitin disc diffusion, Showed high resistance for Penicillin 93% ,Erythromycin 81.4% , Cefoxitin 37.1%C ,Ciprofloxacin 60.5% ,Gentamicin 81.4%,Co-trimoxazole 65.1%,Clindamycin 62.8% ,Doxycycline 76.7% , Linezolid and Vancomycin were 100% sensitive.

Fig. 1 Golden yellow colonies of *Staphylococcus aureus*

V. Discussion

Bloodstream infections in patients are cause of morbidity and mortality that are admitted in Medical intensive care unit & Intensive care unit .Data on the incidence of BSIs significantly vary depending upon the morbidities, Individual risk, Institutions and duration of hospital stay.

The present study was conducted in a Microbiology department of a tertiary care hospital over a period of one & half year.

In present Study there were total 508 blood samples of clinically suspected bloodstream infection received out of which,118 (23.23%) blood samples shows positive for bacterial growth .Similar with the study done by Arora U et al (2007)[14] were noted in 20.2 % of the infected patients .Study done by Ali and Kebede et al (2008)[15] , were 24.2% developed with BSI
.Similarly in other study by Jyoti P et al (2019)[16], were observed 19.95%.   This study have shown 276 (54.33%) male patients and 232 (45.67%) female patients, here male predominance was seen ,this was similarly shown in the study of, Shaikh JM et al (2008)[17], were (M-52.6% & F- 47.4%) . In other study by Prowle et al [3] (M-65.2% & F-34 .84%) .Similar Male predominance was seen in study by Ullas Bhabhor et al(2018)[7],were male was 94 (54.33%) & female were 79 (45.66%).

PATHOGENS DETECTED:

From the study we got to know that among total microorganisms causing BSI there were 72 (61.14%) isolates were Gram negative and followed by 46 (38.85%) isolates were Gram positive,causing septicaemia. Comparative studies showing Gram negative bacteria as predominant pathogen in septicaemia.

Author	Year	Percentage of Gram negative bacteria
Moghnieh et al [18]	2015	38%
Radha Rani D et al [19]	2017	54.76%
Meghana Palewar et al [20]	2022	38%
Present study	2022	61.14%

Epidemiology of microorganisms causing BSIs is changed over years ,with aconcomitant increase in antimicrobial resistance.[16]

Among the Gram negative bacteria in the present study, In majority of samples was found Klebsiella pneumonia 25(21.18%) .This finding is consistent with Jyoti P Sonawane[16]et al were *Klebsiella pneumoniae* was predominant (22.15%) followed by Staphylococcus aureus (6.59%). Among the gram positive isolates in the present study, predominant pathogen was *Staphylococcus aureus*.

In our study the other organism were Acinetobacter baumannii , Escherichia coli, Pseudomonas aeruginosa , Enterobacter ,Proteus .Enterococcus spp and Streptococcusspp.

Most of the isolated pathogens were found to be highly resistant with little treatment option against septicaemia which is dangerous clinical condition with worst outcome. Study deduce that empirical treatment for suspected cases of septicaemia should cover both Gram-negative and Gram - positive microorganisms particularly Klebsiella pneumoniae and Staphylococcus aureus . Looking at the antibiograms obtained in our set up over one and half year study Piperacillin-Tazobactam and Amikacin ,both these drugs can be added as treatment for adult septicaemia.

SomewhatsimilarpatternofresistancewerealsoseeninstudiesdonebyRadhaRanietal[19](2017)andMariyah Yousufetal[21](2020)alsoshowedthatgramnegativemicroorganismhaveresistanceto3rdgenerationCephalosporinsan dGentamicin.SamepatternofresistancewasseeninthestudydonebyJyotiP Sonawaneet al (2019)[16] ,showed *Klebsiella pneumoniae* as major Gram -negativeorganism withmultidrug resistance.

In Gram positive bacteria Staphylococcus aureus was the mostly predominant microbial isolate and that compromised majority of methicillin- resistant Staphylococcus aureus .Screening done by Cefoxitin disc diffusion, Showed high resistance for Penicillin 93% ,Erythromycin 81.4% , Cefoxitin 37.1%C ,Ciprofloxacin 60.5% ,Gentamicin 81.4%,Co-trimoxazole 65.1%,Clindamycin 62.8% ,Doxycycline 76.7% , Linezolid and Vancomycin were 100% sensitive. Vancomycin is drug of choice for MRSA . Similarly findings in resistant pattern in Staphylococcus aureus were consistence with the study by Mariyah Yousuf et al (2020) [21],were Staphylococcus aureus showed 100% sensitivity to Linezolid and Vancomycin ,the isolate was more resistant to Penicillin 87% , followed by Erythromycin 75% and Cefoxitin 62% .We found more no of MRSA ,this may be due to poor environmental cleaning & improper infection control measures in MICU.

In Jyoti P. Sonawane et al(2019) [16], Gram positive isolates were 100% sensitive to Linezolid and Vancomycin .Resistant to Penicillin, Erythromycin, Ciprofloxacin and Gentamicin.

In Cherukuri B et al (2021) [22] , among Gram positive isolates *Staphylococcus aureus* were 100% sensitive to Linezolid and tigecycline followed by Erythromycin 78.6 % , Vancomycin 71.4% , Clindamycin and Co- trimoxazole were 64.3 % .

Therefore, this study concludes that overall blood stream infection rate was found to be high among MICU patients with high occurance in the age group of 31 – 40 years age group. Klebsiella pneumonia was predominant in gram negative organisms and resistant to most of the antibiotics. Staph aureus was predominant in the gram positive group and resistant to most of the antibiotics.

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