Bacteriological Profile, Antibiogram and Phenotypic Resistance Flagging of Blood Culture Isolates by Automated Methods in a Tertiary Care Hospital

Manish Kumar Rout[1], Bibhudutta Rautaraya[2]

ABSTRACT

Introduction: Microorganism present in blood whether continuously or intermittently are threat to every organ in the body. The surveillance of etiological agents in these infections is essential for their prevention and treatment. Awareness of the baseline microbial resistance specific to a hospital prevents irrational use of antibiotics in that hospital. Thus helps progress a step forward in the prevention of spread of antibiotic resistance.

Material and methods: A retrospective study was conducted in the department of Microbiology. During the study period, blood samples collected from all age group OPD, IPD and ICU patients suspected of bacteremia and septicemia were analyzed. All Gram-negative bacilli, Gram-positive cocci and Yeast were investigated while anaerobic bacteria and cultures with mixed growth were excluded.

Results: During the study period of Jan 2017-Dec2017, 1885 blood cultures were analyzed. 305 (16.1%) were found to be positive, out of which 236 were from ICU, 58 were from IPD and 11 from OPD. Among Gram positive cocci, CoNS is commonest followed by *Staphylococcus aureus* whereas in Gram negative bacilli, *Klebsiella pneumoniae* was commonest organism followed by *Pseudomonas aeruginosa*. In our study, 90% of Enterobacteraceae were ESBL producers. MRSA were isolated in 50% and MRCoNS in 71%.

Gram-positive isolate were least sensitive to penicillin (10%) while it was most sensitive to tigecycline in 100% followed by vancomycin in 95%. Gram negative isolates of Enterobacteriaceae were least sensitive to Aztreonam (11%) and while it was most sensitive to Colistin in 87% of cases. Candida species were isolated in 54 (18%) of which NICU accounted for majority of cases. *Candida albicans* was least sensitive to Fluconazole (82%) and Non albicans candida to Amphotericin B (70%) while they were sensitive to all other antifungals.

Conclusion: The retrospective study conducted showed both gram positive and gram negative bacteria were responsible for blood stream infections. Most of the strains were multi drug resistant. Rapid isolation and identification of pathogens by automated blood culture system and antibiogram with minimum inhibitory concentration (MIC) value provides early and appropriate treatment to the seriously ill patients leading to reduce mortality and reduce duration of hospitals.

Resistance flagging of the bacterial isolates guides us to perform barrier nursing and isolate the patient to prevent spread of infection. The daily analysis of resistance flagging and MIC values give important information for choosing selective antibiotics leading to good antibiotic stewardship which in turn reduces patient morbidity and mortality.

Keywords: Blood Stream Infections (BSIs), Antibiotics, Resistance Flagging, Septicemia, Klebsiella

INTRODUCTION

Microorganism present in circulating blood whether continuously or intermittently are threat to every organ in the body. Approximately 200,000 cases of bacteremia and fungemia occur annually with mortality rates ranging from 20-50%.[2]

Blood cultures of patients have isolated a wide range of organisms in BSIs. These include *Acinetobacter spp., Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* among Gram-negative bacteria and *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), enterococci and alpha-hemolytic streptococci among Gram-positive bacteria.[3]

Candida species are the fourth most common cause of nosocomial bloodstream infections worldwide.[4]

The surveillance of etiological agents in these infections is essential for their prevention and treatment. Microbial invasion of the bloodstream can have serious immediate consequences i.e., shock, multiple organ failure, disseminated intravascular coagulation (DIC) and death.[6]

Sensitive bacterial strains are now being replaced by multi-drug resistant (MDR) strains of *Klebsiella*, *Pseudomonas*, *Acinetobacter*, and *Citrobacter* species. Increase in incidence is also seen among Gram-positive isolates such as methicillin resistance in *Staphylococcus aureus* (MRSA) and vancomycin resistance in Enterococci. This increasing antimicrobial resistance is a worldwide concern and is subjected to regional variation.[7]

Awareness of the baseline microbial resistance specific to a hospital prevents irrational use of antibiotics in that hospital. Thus helps progress a step forward in the prevention of spread of antibiotic resistance and antibiotic stewardship. Therefore early diagnosis and appropriate treatment of these infections can make the difference between life and death.

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MATERIAL AND METHODS

A retrospective study was conducted in the department of Microbiology in our hospital from January 2017 to December 2017.

During the study period, blood samples collected from all age group OPD, IPD and ICU patients suspected of bacteremia and septicemia were analyzed. All samples were collected in BacT/ALERT FA plus and BacT/ ALERT PF plus bottle and BD Bactec Media plus Aerobic/F bottle irrespective of antibiotics administration. Quantity of blood sample from adult and children was 5-10 ml and 1-5 ml respectively collected with all aseptic precautions.

Samples were incubated in the automated BacT/ALERT 3D system (BIOMERIEUX, USA) and BACTEC 9050 (BD Bactec™ 9050 Blood Culture System) for 7 days. The negative results were followed up to 7 days and final report was issued.

The preliminary signal of bacterial growth was detected and displayed on the 3D monitor of BacT/ALERT system and BD Bactec™ 9050 Blood Culture System mentioning the detection time. Further identification of all blood culture positive samples was accomplished by sub-culture on Blood agar, Chocolate agar and MacConkey agar media and direct Gram's staining from positive blood culture.

The VITEK®2 system (bioMérieux) was used for identification and antimicrobial susceptibility testing of bacteria grown in standard aerobic blood culture bottles. All Gram-negative bacilli, Gram-positive cocci and Yeast were investigated while anaerobic bacteria and cultures with mixed growth were excluded.

For identification and susceptibility testing of bacteria by the standard method, approximately 0.1 mL of culture fluid from a blood culture bottle was inoculated on Blood agar, MacConkeys agar and Chocolate agar plates and incubated aerobically at 37°C and examined after 18-24 hours.

Bacteria were then suspended in 0.45% saline to a McFarland unit value of 0.5-0.63 and then loaded into appropriate VITEK identification and antimicrobial susceptibility testing cards, as described above. Approximately 3 mL of this suspension was automatically loaded into the VITEK 2 ID-GNB (identification-Gram-negative bacilli) and AST (antimicrobial susceptibility testing)-N281 cards, N364 cards (for Gram-negative bacilli), VITEK 2 ID-GPC (identification-Gram-positive cocci) and AST-P628, ST03 cards (for Gram-positive cocci and Streptococcus respectively), and VITEK 2 ID-Yeast (identification-Yeasts) and AST-YS08, YS07 (for Yeasts), using the VITEK 2 system with the 2.01 release software.

Reference strains including Escherichia coli American Type Culture Collection (ATCC) 25922, Pseudomonas aeruginosa ATCC 27853, and Staphylococcus aureus ATCC 29213 were used as controls.

The study was approved by Institutional Ethics Committee, Bhubaneswar with Ref. no. IEC/AMRI/BBSR/2019/0020

RESULTS

During the study period of Jan 2017-Dec2017, 1885 blood cultures were analyzed. 305 (16.1%) were found to be positive, out of which 236 were from ICU, 58 were from IPD and 11 from OPD. (Figure 1)

Out of positive cases, 198 (65%) were male and 107 (35%) were female. Among Gram positive cocci, *Coagulase negative Staphylococcus* is commonest in 74 (24%) followed by *Staphylococcus aureus* in 12 (4%) whereas in Gram negative bacilli, *Klebsiella pneumoniae* was commonest

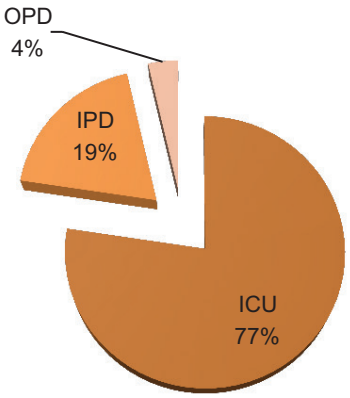

Figure-1: Department-wise distribution of culture positive cases.

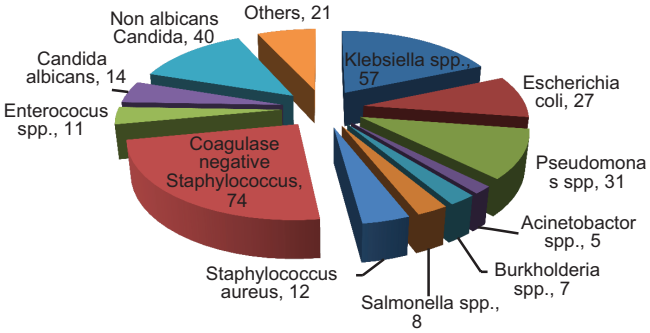

Figure-2: Frequency distribution of bacterial blood isolates.

Antibiotic Organism	CIP	CD	COT	DAP	E	GEN	LE	LZ	OXA	P	RIF	TEI	TE	TIG	VA
CoNS	32	39	48	87	19	44	28	72	13	11	58	94	89	100	96
Staphylococcus aureus	38	13	75	88	13	75	38	63	50	0	75	100	50	100	88

Table-1: Antibiotic sensitivity profile of Gram positive cocci isolated in the ICU. (in percentage)

Antibiotic Organism	AK	AT	CPM	CFS	CAZ	CIP	CL	COT	DOR	GE	IPM	LE	MRP	MIN	PIT
Klebsiella pneumoniae	53	4.3	13	4.3	4.3	24	83	48	22	41	46	26	24	52	65
Escherichiae coli	80	19	48	75	21	24	95	24	71	70	71	24	71	76	57
Pseudomonas aeruginosa	33	38	33	52	38	52	45	35	24	29	38	62	29	24	33

Table-2: Antibiotic sensitivity profile of Gram Negative Bacilli isolated in the ICU. (in percentage)

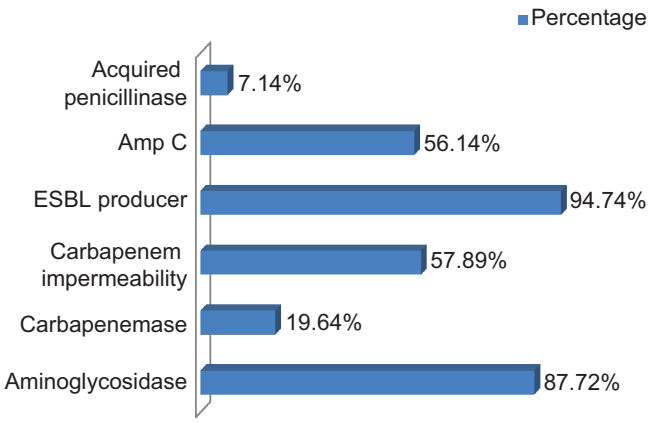

Figure-3: Resistance flagging of Klebsiella isolates.

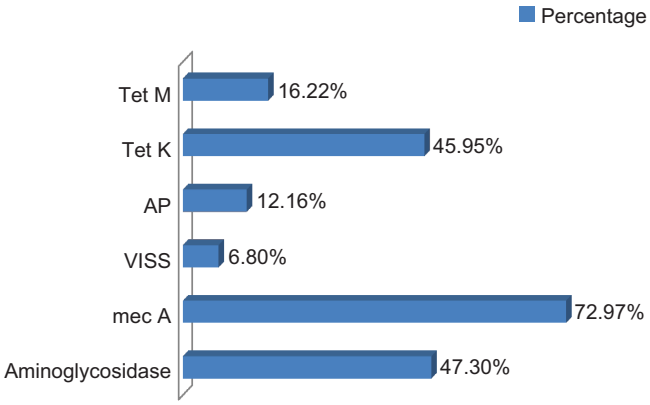

Figure-4: Resistance flagging of Staphylococcus species.

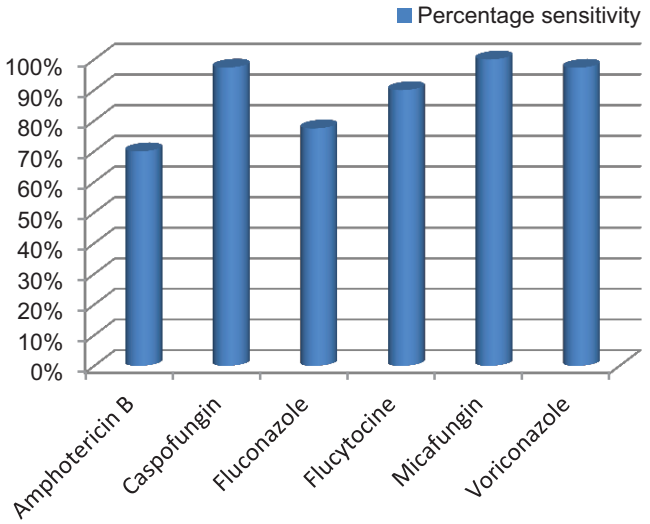

Figure-5: Antibiotic sensitive pattern of Candida species.

organism isolated in 57 (19%) followed by *Pseudomonas aeruginosa* in 31 (10%) from positive blood culture. (Figure 2)

In our study, 94% of Enterobacteraceae were ESBL producers. Methicillin resistant *Staphylococcus aureus* were isolated in 50% and Methicillin resistant *Coagulase negative Staphylococcus* in 71%. (Figure 3,4)

Gram-positive isolate were least sensitive to penicillin (10%) while it was most sensitive to tigecycline in 100% followed by vancomycin in 95%. If we translate the same in MIC values, Penicillin was MIC 0.5 (90%) cases followed by Erythromycin at MIC 8 (80%). Tigecycline had MIC 0.12 (50%), 0.25 (22%), 0.5(22%) and 1 (6%) followed by vancomycin with MIC 0.5 (11%), 1 (48%), 2 (30%), 4 (5%), 8 (2%), 32 (4%). (Table 1)

Gram negative isolates of Enterobacteriaceae were least sensitive to aztreonam (11%) and while it was most sensitive to colistin in 87% of cases. If we translate the same in MIC values, aztreonam had MIC 64 (7%), 32 (1%), 16 (3%). Colistin showed 87% sensitivity in Enterobacteriaceae with MIC 0.5 (81%), 1 (1%), 2 (5%) 4 (1%), 8 (3%), 16 (9%). (Table 2)

Candida species were isolated in 54 (18%) of which NICU accounted for majority of cases. *Candida albicans* was least sensitive to Fluconazole (82%) and *Non albicans candida* to Amphotericin B (70%) while they were sensitive to all other antifungals. (Figure 5)

DISCUSSION

In our study, Blood culture positivity was seen in 16.1% which was similar to Gupta et al[7] (16.5%) and Dash et al[12] (17%) while lower than that of Parihar et al[4] (29%) and Manjunath et al[6] (20%).

CoNS was the most common organism isolated (24%) of case followed by *Klebsiella* species (19%) which is in accordance to Vatkar et al[13] and Dash et al.[12] *CoNS* being the most common contaminant, 2 sets of blood culture from different sites were taken or clinically correlated positive samples were considered clinically significant.

Klebsiella was the most common Gram negative bacilli isolated which was in accordance to Parihar et al[4] and Dash et al[12] while in discordance to Vasudev et al[11] and Gupta et al[7] where *E. coli* was the most common organism isolated.

Candida species were isolated in 18% of cases, mostly in neonatal ICU setting which is in higher frequency than other studies and is probably due to sporadic surge of cases and their spread to others. The isolation of patients and proper barrier nursing were done to prevent the same in the future.

More than 90% of Enterobacteriaceae were ESBL producers which are higher than Arora et al[2] (34%) and Manjunath et al[6] (66%). MRSA was isolated in 50% of cases which was in accordance to Vasudeva et al[11] (50%) while higher than Gupta et al[7] (26.5%) and Vatkar et al[13] (22%). The higher resistance flagging is probably due to catering of more intensive care patients in our hospitals which have recieved treatment from other healthcare providers.

More than 80% of organisms were multi drug resistance which is similar to Arora et al[2] (71%).

Gram-positive isolate showed least sensitivity to erythromycin (10%) while it was sensitive for tigecycline in 100% followed by vancomycin and teichoplanin in 95%.

Gram negative isolates in Enterobacteriaceae were least sensitive to aztreonam (11%) while it was most sensitive to colistin in 95% of cases. It was in accordance to Arora et al[2] and Dash et al[12] while in discordance to Manjunath et al[6] where carbapenems were most sensitive for Gram negative bacilli and linezolid for Gram positive bacilli.

Our studies showed an increase in MIC value, with many

antibiotics being on the higher side of MIC chart. With more than 90% of Enterobactereaceae were ESBLs, we had most cephalosporins with MIC more than 64. Carbapenemase production and impermeability to carbapenem were responsible for organisms resistant to imipenem and meropenem, which were one of the last line antibiotics. The similar trends were also seen with colistin for *Pseudomonas aeruginos* and *Klebsiella pneumoniae* due to development of resistance to polypeptides.

It shows a worrying trend with MIC of more number of antibiotics would fall within resistance range in the future. The increase in MIC makes it difficult for the clinicians to use the drug within safe range and had to increase the concentration of drug for its action.

CONCLUSION

The retrospective study conducted showed both gram positive and gram negative bacteria were responsible for blood stream infections. Coagulase negative Staphylococcus and Klebsiella were among the most common Gram-positive and Gram-negative organisms identified causing adult sepsis respectively. Most of the strains were multi drug resistant.

The report on the current knowledge of bacterial resistance profile of the patient, which is provided by microbiology laboratory from time to time, is necessary for early diagnosis and treatment.

Rapid isolation and identification of pathogens by automated blood culture system and antibiogram with minimum inhibitory concentration (MIC) value provides early and appropriate treatment to the seriously ill patients leading to reduce mortality and reduce duration of hospitals.

MIC value also helps in determining the therapeutic index for the antibiotics which further helps the clinicians.

Resistance flagging of the bacterial isolates guides us to perform barrier nursing and isolate the patient to prevent spread of infection. The daily analysis of resistance flagging and MIC values give important information for choosing selective antibiotics leading to good antibiotic stewardship which in turn reduces patient morbidity and mortality.

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