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Original Research Article

Characteristics of blood infections and phenotypic detection of extended-spectrum β-lactamases in ICU patients in Central India posing a challenge

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ABSTRACT

Background: Bloodstream infection (BSI) is defined by positive blood cultures in a patient with systemic signs of infection and may be either secondary to a documented source or primary, that is, without identified origin. The aim of this study was to improvise blood culture systems for a quicker, optimum diagnosis and prompt treatment.
Methods: A prospective study was conducted with total of 309 samples for determining the bacteriological profile and prevalence of ESBL in BSI's in patients admitted in the ICU's (surgical/medical/gynaecological) with the suspicion of sepsis. Samples received in department of microbiology were processed as per standard protocol and identification of bacteria was carried out with the help of relevant biochemical tests. AST for both the ICUs was done together by Kirby-Bauer disk diffusion method according to CLSI guidelines.
Results: Of the total, 149 (48.22%) samples were positive for growth with the major isolates out of these being gram negative bacilli, 104 (69.79%) and 45 (30.21%) were gram positive cocci. Among the gram negative bacilli, *Klebsiella pneumoniae* 35 (33.65%) was the most common bacteria while the least frequent organism isolated was *Acinetobacter baumanii* 10 (9.62%). Imipenem, piperacillin-tazobactam and levofloxacin were the most sensitive antibiotics whereas cefepime, cefuroxime were the most resistant antibiotics.
Conclusions: This study highlights the incidence of gram negative bacilli in ICU's and the emergence of multi-drug resistant organism. Infections with MDR organisms can lead to inadequate or delayed treatment which is associated with adverse patient outcomes.

Keywords: Gram negative bacilli, Sepsis, Extended spectrum beta lactamase, Multidrug resistant bacteria

INTRODUCTION

Bloodstream infection (BSI) is defined by positive blood cultures in a patient with systemic signs of infection and may be either secondary to a documented source or primary, that is, without identified origin.[1] BSI's usually occur after the patient undergoes intravascular catheterization. Thus, the microbiological profile of bacteria causing these infections can be both gram negative as well as gram positive bacteria. Despite the recent advances in treatment and supportive care, BSI infections continue to be a major cause of morbidity and mortality in such patients.[2] The laboratory diagnosis of these infections is routinely done with blood cultures. Since blood is a sterile fluid, the positive predictive value of blood culture is high.[3] The case fatality rate associated with BSIs in intensive care unit (ICU) patients is between 35-50%.[4] The epidemiology of the pathogens causing BSIs has drastically changed over the years with a significant rise in antimicrobial resistance. Risk factors for BSIs include intubation, arterial catheter, tracheostomy, duration of intubation, duration of catheter use, duration of nasogastric catheter, underlying comorbidities like diabetes mellitus/hypertension/Chronic

renal failure, immunocompromised status.[5] *Staphylococcus spp.*, coagulase negative *Staphylococcus* and *Streptococcus spp.* are the predominant gram positive bacteria while *Escherichia coli*, *Enterobacter spp.*, *Klebsiella spp.*, *Pseudomonas spp.*, *Acinetobacter spp.*, *Salmonella typhi*, *Citrobacter spp.*, are the predominant gram negative bacteria isolated in blood cultures of sepsis patients.[6] Development of resistance among the bacteria causing these infections is a major concern. Infections with multi drug resistant (MDR) organisms can lead to inadequate or delayed treatment which is associated with adverse patient outcomes.

The introduction of third generation cephalosporins was heralded as a major breakthrough in the fight against β lactamase mediated bacterial resistance to antibiotics.[7] However resistance to these extended spectrum cephalosporins was also discovered soon and hence these new β lactamases were coined extended-spectrum β-lactamases (ESBLs). They are mostly plasmid mediated β lactamases that efficiently hydrolyse oxyimino cephalosporins and monobactams, yet are inhibited by β-lactamase inhibitors.[7]

The aim of this study was to improvise the blood culture systems for a quicker, optimum diagnosis and treatment of these infections with a lookout on the judicious antimicrobial therapy. This study was carried out in a tertiary care hospital with a view to study the bacteriological profile and determine the antimicrobial sensitivity patterns.

METHODS

A prospective study was conducted for determining the bacteriological profile and prevalence of ESBL in BSI's in patients admitted in the ICU's (surgical/medical/ gynaecological) with the suspicion of sepsis, from February 2021 to August 2022. The study was conducted from the department of microbiology, Netaji Subhash Chandra Bose (N. S. C. B.) Medical College and Hospital, Jabalpur (MP). Institutional approval for the study was taken. Ethical approval for this study wasn't required. The study was conducted on the blood culture samples received in the department of microbiology. Inclusion criteria for the samples included patients admitted to the ICU's and those with a clinical suspicion of sepsis. Patients less than 15 years old were excluded from the study. In the study, 309 blood samples for blood culture were collected under all aseptic precautions and transferred to previously prepared blood culture bottles containing BHI broth and transported to the Bacteriology section of the Department of Microbiology with minimal delay. After overnight incubation at 37 °C samples were sub-cultured on to nutrient agar, blood agar, chocolate agar and MacConkey agar to look for any growth. Any growth that was observed after overnight incubation at 37°C was identified with the help of colony morphology, gram staining and relevant standard biochemical test such as catalase test, coagulase test, triple sugar iron, oxidase, citrate utilization test, urease production test, methyl red test, indole production, Vogues Proskauer test.[9] Antibiotic susceptibility tests were done in Muller Hinton agar by the Kirby Bauer disc diffusion method as per clinical and laboratory standards institute (CLSI) guidelines.[8] Blood culture bottles showing no signs of any growth after 5 days of incubation, either growth on MacConkey/blood agar or haemolysis/turbidity were reported as negative after a final confirmatory subculture.

Statistical analysis

All statistical analyses were conducted for ESBL production using SPSS method. Phenotypically detected ESBL producing organisms were compared with ESBL-negative study isolates by using the Chi-square test. P values <0.05 were considered significant.

RESULTS

A total of 309 samples that were received from ICU during the study done from a period of February 2021 to August 2022 at the department of microbiology, Netaji Subhash Chandra Bose Medical College, Jabalpur. All the samples were cultured in brain heart infusion broth.

Table 1: Distribution according to positive cultures.

Total no. of samples	No growth	Growth	Gram positive cocci	Gram negative bacilli
	N (%)	N (%)	N (%)	N (%)
309	160 (51.77)	149 (48.22)	45 (30.21)	104 (69.79)

Table 2: Age distribution of the cases.

Age (years)	No. of cases
	N (%)
18-28	77 (24.91)
29-38	84 (27.19)
39-48	53 (17.15)
49-58	42 (13.59)
59-68	36 (11.66)
69-78	12 (3.88)

Continued.

Age (years)	No. of cases
79-88	4 (1.29)
89-98	1 (0.33)
Total	309 (100)

Table 3: Antibiotic susceptibility pattern of GNB.

Isolated organisms	*K. pneumoniae* (n=35)		*E. coli* (n=34)		P. aeruginosa (n=25)		A. baumanii (n=10)	
Antibiotics	No.	%	No.	%	No.	%	No.	%
GEN	22	62.85	16	47.05	17	68	5	50
CZ	4	11.42	2	5.88	1	4	0	0
CEC	23	65.71	19	55.88	17	68	NT	NT
AMC	5	14.28	3	8.82	NT	NT	2	20
PTZ	25	71.42	27	79.41	20	80	5	50
CXM	2	5.71	0	0	0	0	1	10
CTX	2	5.71	2	5.88	1	4	2	20
DOX	11	31.42	10	29.41	8	32	2	20
IMP	33	94.28	30	88.23	20	80	10	100
CPM	4	11.42	3	8.82	2	8	1	10
CAZ	5	14.28	3	8.82	6	24	1	10
LE	27	77.14	28	82.35	17	68	5	50
COT	10	28.57	7	20.58	7	28	3	30

Table 4: Antibiotic susceptibility pattern of GPC.

Isolated organisms	*S. aureus* (n=25)		Coagulase negative *Staphylococcus species* (n=19)		*Streptococcus spp.* (n=1)	
ANTIBIOTICS	No.	%	No.	%	No.	%
GEN	15	60	16	84.21	1	100
VA	25	100	19	100	1	100
AMC	14	56	8	42.1	0	0
E	9	36	4	21.05	0	0
CD	9	36	4	21.05	1	100
DOX	23	92	15	78.94	1	100
P	0	0	3	15.78	0	0
LE	20	80	18	94.73	1	100
COT	11	44	4	21.05	0	0

Table 5: Organism showing ESBL production (n=52).

S. No	Name of organism isolated	ESBL (52)	Non ESBL (42)
		N (%)	N (%)
1.	*K. pnuemoniae*	21 (40.38)	10 (23.81)
2.	*E. coli*	17 (32.69)	12 (28.57)
3.	*P. aeruginosa*	14 (26.93)	20 (47.62)
Total		52 (100)	42 (100)
P value		0.023	

Of the total, 149 (48.22%) samples were positive for growth with the major isolates out of these being gram negative bacilli (GNB), 104 (69.79%) and 45 (30.21%) were gram positive cocci (GPC). In this study, females outnumbered males in this study by a very small margin (1%). 50.16% of cases were females and 49.84% were males.

Majority of cases belonged to the age group of 29-38 years (27.19%) followed by the 18-28 age group (24.91%).

Among the gram negative bacilli, *K. pneumoniae* 35 (33.65%) was the most common bacteria, followed by *E. coli* 34 (32.69%) and *P. aeruginosa* 25(24.04%) while

the least frequent organism isolated in gram negative bacteria was *A. baumanii* 10 (9.62%) (Figure 1).

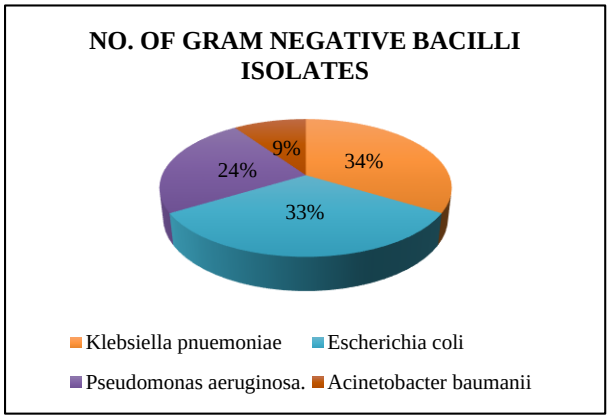

Figure 1: Distribution among GNB.

Most common amongst the GPC were *S. aureus* 25 (55.55%) followed by coagulase negative *Staphylococcus* species 19 (42.22%) and *Streptococcus spp.* 1 (2.23%).

Amongst the GNB, *K. pneumoniae* showed maximum sensitivity to imipenem 33 (94.28%), levofloxacin 22 (77.14%) and piperacillin-tazobactam 25 (71.42%). It was least sensitive to cephalosporin group of drugs i.e., cefuroxime 02 (5.71%), cefotaxime 02 (5.71%) and cefazolin 04 (11.42%) followed by cotrimoxazole 10 (28.57%).

E. coli was most sensitive to imipenem 30 (88.23%), levofloxacin 28 (82.35%) and piperacillin tazobactam 27 (79.41%) while it was least sensitive to cefuroxime 0 (0%), cefotaxime 2 (5.88%) and cefazolin 2 (5.88%).

In the study, *P. aeruginosa* was found to be most sensitive to imipenem 20 (80%) and least sensitive to cefuroxime 0 (0%). A. baumanii was similarly most sensitive to imipenem 10 (100%) and least sensitive to cefazolin 0 (0%).

Amongst the GPC, *S. aureus* showed maximum sensitivity to vancomycin 25 (100%), followed by doxycycline 23 (92%) and levofloxacin 20 (80%). It was least sensitive to penicillin 0 (0%), erythromycin 09 (26%) and clindamycin 9 (36%) (Table 4).

Coagulase negative *Staphylococcus* species was most sensitive to vancomycin 19 (100%), levofloxacin 18 (34.73%) and gentamicin 16 (84.21%) while it was least sensitive to penicillin 3 (15.78%), erythromycin 4 (21.05%) and clindamycin 4 (21.05%) (Table 4).

In the present study, *Streptococcus spp.* was found to be 01 (100%) sensitive to gentamicin, vancomycin, clindamycin, doxycycline and levofloxacin while it was 00 (0%) sensitive to penicillin, erythromycin, amoxycillin-clavulanic acid and cotrimoxazole (Table 4).

Of the 94 GNB tested for ESBL production, 52 (55.31%) were positive for ESBL production which was compared with non-ESBL producers 42 (44.69%). The difference was statistically significant ($p<0.05$).

Out of the ESBL producing bacilli, *K. pneumoniae* 21 (40.38%) was the most frequent ESBL producer followed by *E. coli* 17 (32.69%) and *P. aeruginosa* 14 (26.93%) (Table 5).

DISCUSSION

In this study, blood stream infections were found to be positive in 149 (48.22%) of 309 cases (Table 1). This was comparable to the findings of Komori et al where among the included patients 54.5% had bacteraemia.[10] This correlated with the study of Banerjee et al in which the prevalence rate of blood stream infection was 51.55% of cases.[11]

In the present study, etiological agents were more commonly gram negative bacteraemia (69.79%) which was greater than gram positive bacteraemia (30.21%). This was in comparison with the study by Manyahi et al where they found that the majority (74%) were GNB.[12] This was found to be similar to the study of Agrawal et al who concluded that in positive samples GNB and GPB were 68.35% and 31.65% respectively.[6]

There was a female preponderance accounting for 50.16% in this study (Table 3). Similar to this, another study done by Komori et al showed that bacteraemia was less prevalent in males compared with females 359 (56.4%) vs. 350 (65.9%).[13]

From 149 BSI positive cases, in our study, *K. pnuemoniae* was the most common pathogen isolated in 35 (23.48%) followed by *E. coli* 34 (22.81%), *P. aeruginosa* in 25 (16.77%). Similar pattern was observed by Bhadauria et al that frequent pathogen identified among gram negative bacteria were *Klebsiella* 24 (42.8%), followed by *E. coli* 18 (32.14%), *Acinetobacter* 10 (17.85%), *Pseudomonas* 2 (3.57%).[14]

Among gram negative organisms most organisms showed sensitivity to imipenem (90.62%), levofloxacin (69.37%) and piperacillin-tazobactam (69%). Most GNB showed resistance to cephalosporins. Bhadauria et al showed that after polymyxin B, isolated GNB showed high sensitivity for levofloxacin (60.71%), cefixime (57.78%), gentamicin, meropenem, piperacillin/tazobactam (50%).[14] In a study done by Mehta et al they concluded that in gram negative isolates, amikacin showed more activity (76.61%) against *Enterobacteriaceae*; whereas for non fermenters, including *Pseudomonas spp.* and *Acinetobacter spp.*, ciprofloxacin showed higher activity (65.17%), followed by amikacin (62.50%).[15]

In the present study most GPC were resistant to penicillin G. Similar results were observed in the study by

Vasudeva et al 16 that most GPC were most sensitive to vancomycin (100%) and least sensitive to penicillin G (0%).[16]

The commonest bacteria isolated in this study, *K. pneumoniae* showed 94.28% sensitivity to imipenem, 77.14% sensitivity to levofloxacin, 71.42% sensitivity to piperacillin-tazobactam and least sensitive to cefepime (11.42%). This was comparable to the study by Vasudeva et al where *Klebsiella spp.* was 100% sensitive to imipenem, 83.33% sensitive to piperacillin-tazobactam and 16.66% sensitive to cefepime.[16]

In the present study *E. coli* showed maximum sensitivity to imipenem (88.23%), levofloxacin (82.35%) and piperacillin-tazobactam (79.41%) and most resistant to cefuroxime 34 (100%) followed by cefepime 31 (91.18%). Rani et al concluded in their study that *E. coli* was 65.57% sensitive to imipenem, 54.09% sensitive to piperacillin-tazobactam and only 13.11% sensitive to levofloxacin which was in contrast to the present study; while the study showed similar resistance to cefepime (81.97%).[17]

P. aeruginosa in our study was found to be most sensitive to imipenem (80%) and piperacillin-tazobactam (80%). It was least sensitive to cefepime (8%). Rani et al showed similar results with imipenem (77.20%) and piperacillin-tazobactam (86.30%) sensitive.[17] Resistance was comparable to the study by Agrawal et al where it showed only (16.67%) sensitivity to ceftazidime.[6]

In the current study *A. baumanii* showed maximum sensitivity to imipenem (100%) and least to ceftazidime (10%). Easow et al and Banerjee et al showed similar pattern where *Acinetobacter spp.* was 100% sensitive to imipenem.[11,18] In their study, Agrawal et al concluded that *Acinetobacter spp.* was 25% sensitive to ceftazidime.[6]

Amongst GPC, *S. aureus* showed maximum sensitivity to vancomycin (100%), doxycycline (92%) and levofloxacin (80%); whereas it was most resistant to penicillin (100%) and erythromycin (64%). This was comparable to the study by Agrawal et al where *S. aureus* was 89.47% sensitive to vancomycin and 63.16% resistant to erythromycin.[6] The resistance to penicillin was comparable to a study by Gill et al which showed that 85% isolates of *S. aureus* were resistant to penicillin.[3]

The second common isolate among GPC was CONS which showed 100% sensitivity to vancomycin, 94.73%, to levofloxacin. It showed resistance of 78.95%, to erythromycin. This was similar to the study by Pal et al where CONS was most sensitive to vancomycin (84%), 80% to levofloxacin and 70% resistance to erythromycin.[28]

In the present study *Streptococcus spp* was most sensitive to vancomycin (100%), gentamicin (100%). It was most resistant to amoxycillin-clavulanic acid (100%). This was in concordance to Sonawane et al who concluded that *Streptococcus spp* was most sensitive to vancomycin (100%), and had a lesser sensitivity for gentamicin (83.33%).[27]

In the present study, out of the total 104 GNR isolates, ESBL production was seen in 52 (50%) of the isolates. This was comparable to the study by Kateregga et al who concluded in their study that that 62% of *Enterobacteriaceae* isolates were of the ESBL phenotype.[19]

ESBL production was maximum amongst *K. pneumoniae* 21 (40.38%), followed by *E. coli* 17 (32.69%) and *P. aeruginosa* 14 (26.93%). This was comparable to the study by Taneja et al who concluded that ESBL was detected in 10 (38.5%) isolates of *E. coli*.[20] Kazemian et al in their study demonstrated similar results where phenotypic ESBL detection tests indicated that 36 (40%) *K. pneumoniae* isolates and 23 (35.4%) *E. coli* isolates were ESBL producers.[21] Similar results were seen in the study by Zandi et al where among the 152 samples of *E. coli*, 45 strains (30%) were producers of ESBLs and among the 118 samples of *K. pneumoniae*, 44 strains (37.3%) were producers of ESBLs.[22]

In contrast, ESBL was produced by 23% and 40% of *K. pneumoniae* and *E. coli* respectively as demonstrated by Easow et al.[18] Similarly, a study by Laudy et al concluded that ESBL-type enzyme production was detected in 110 out of 720 isolates (15%) in at least one of the phenotypic assays.[23]

In the present study, *P. aeruginosa* showed over 14 (56%) ESBL producing strains. Comparable results were seen in a study by Kothari where out of total 100 samples studied 42% were seen as ESBL positive *Pseudomonas spp*.[24] A lower incidence of ESBL production was seen in the study by Begum et al where of 82 strains of *Pseudomonas spp.* tested for ESBL, 31 (37.8%) were found as ESBL-positive.[25] Similarly a lower positive rate was seen in the study by Agrawal et al where out of out of 148 *P. aeruginosa* isolates, 30 (20.27%) were found to be positive for ESBL production.[26] This difference in ESBL production in the present study and other studies maybe attributed to increased use of antibiotics among the included patient population and possible higher incidence of hospital acquired MDR *P. aeruginosa* infections.

CONCLUSION

This study highlights the incidence of gram negative bacilli in medical and surgical ICU's and the emergence of multi-drug resistant organism. The administration of the antimicrobials among ICU patients is highly based on a combination of three or more agents covering a broad spectrum of pathogens. Infections with MDR organisms

can lead to inadequate or delayed treatment which is associated with adverse patient outcomes.

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