Original Article

DOI: 10.7860/JCDR/2021/51550.15608

Microbiology Section

Prevalence of Extended Spectrum Beta-lactamase and Carbapenemase Producers in Gram Negative Bacteria causing Blood Stream Infection in Intensive Care Unit Patients

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ABSTRACT

Introduction: Blood Stream Infections (BSI) in Intensive Care Unit (ICU) patients at initial stages are acute infections which might even become life threatening. In developing countries, increasing antimicrobial resistance and emergence of Extended Spectrum Beta-Lactamases (ESBL) and carbapenemase has also added an extra burden on physicians.

Aim: To study the prevalence of emergence of ESBL and carbapenemase producing Gram Negative Bacteria (GNB) causing BSI in ICU patients.

Materials and Methods: The present cross-sectional study was conducted on 1537 blood samples which were received in duration of two years from 2018 to 2020 in the Department of Microbiology, Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, Haryana, India from various ICUs. A 5-7 mL of blood was aseptically added to BACTEC bottles and bottles after proper labeling were inserted into the machine and incubated upto five days. 0.1 mL of broth from positively flagged bottles was cultured on Blood and MacConkey Agar. These plates were incubated at 37°C for 24 hours and processed as per standard microbiological procedures. Data was entered locally and calculated on the Microsoft Excel database.

Results: Among 1537 samples, 263 (17.11%) samples were flagged positive by BACTEC system. On culture out of 263 samples, 51 (19.40%) were Gram Positive Cocci (GPC), 21 (07.98%) were *Candida* spp. and 191 (72.62%) were GNB. Among 191 Gram negative isolates, *Escherichia coli* 64 (33.51%) was the predominant organism followed by *Klebsiella spp.* 60 (31.41%). For all gram negative isolates, meropenem was the most sensitive drug followed by imipenem. Tigecycline (81.25%) was the second most effective drug against *Acinetobacter baumannii.* ESBL detection was done by Combine Disc Test on 124 samples (*Escherichia coli* and *Klebsiella* spp.) which showed *Klebsiella* spp. 25 (20.16%) as the highest ESBL producing organism The rate of carbapenemase producer was 20 (10.45%) among all the gram negative isolates.

Conclusion: For BSI in ICU patients, culture and sensitivity along with screening for prevalence of ESBL and carbapenemase producers should be done prior to starting antibiotics.

Keywords: Combined disc test, *Escherichia coli*, Modified carbapenem inactivation method

INTRODUCTION

The Blood Stream Infections (BSI) in its initial stages are acute events which are often misdiagnosed with other conditions and with late diagnosis it leads to life threatening multiorgan failure [1,2]. World Health Organisation (WHO) in 2017 listed BSI as global health priority due to increase in cases of sepsis leading to high mortality and morbidity [3]. The incidence rates of BSI infection in developing countries varies due to lack in implementation of guidelines on antimicrobial regime and unavailability of antimicrobial susceptibility testing which has led to the evolution of novel drug resistant bacteria resulting in poor therapeutic outcomes in BSI [4,5].

Patients admitted in ICU are critically ill and use of central venous catheters, invasive ventilation, urinary catheters, other invasive devices and equipment disrupts anatomical barrier which further increases the risk of developing BSI [6].

The aetiology of BSI has seen a dramatic shift in these years being dominated by GNB [7]. In Asia-Pacific region, Multidrug-Resistant Gram-Negative Bacteria (MDR-GNB) infections dominate in the Asia-Pacific region. MDR-GNB, ESBL organisms, carbapenemase producing Enterobacteriaceae, carbapenem-resistant *Acinetobacter* species, MDR *Pseudomonas aeruginosa* are the major culprits [8].

The increasing prevalence of ESBL and carbapenemase producing gram negative isolates causing BSI are major concern in developing countries as it leads to 50% mortality [9]. Keeping in mind the high fatality rate of BSI and increasing antimicrobial resistance this study was conducted to know the prevalence of ESBL and producing GNB causing BSI in ICU patients.

MATERIALS AND METHODS

The present cross-sectional study was conducted for duration of two years i.e., January 2018 to December 2020 on blood samples received from Medicine, Surgery, Obstetrics and Gynaecology and Neonatal ICUs in the Department of Microbiology, MMIMSR, Mullana, Haryana, India for determining the prevalence of ESBL and carbapenemase producing GNB in BSI. Ethical clearance for the study was taken from Institutional Ethical Committee vide letter no. MMIMSR/IEC/2017/1154. Informed consent was obtained from all the patients involved in this study.

Inclusion criteria: All blood samples coming from various ICUs were included in the study.

Exclusion criteria: Blood samples from general wards of various departments were excluded from the study.

Processing of Samples

A total of 1537 blood samples were received from patients admitted in various ICUs of the institute. A 5-7 mL of blood was aseptically added to BACTEC bottles and bottles after proper labeling were inserted into the machine and incubated upto five days. In between the positively flagged bottles were taken out and with the help of syringe 0.1 mL of broth were cultured on Blood and MacConkey Agar. These plates were incubated at 37°C for 24 hours and after given time processed as per standard operating procedures [10,11].

Antibiotic Susceptibility Tests

A 0.5 McFarland suspension of GNB was lawn cultured on Mueller Hinton Agar (MHA, Hi-Media, Mumbai) and antimicrobial sensitivity testing was performed by using Kirby-Bauer disk diffusion method and interpreted according to guidelines of Clinical and Laboratory Standards Institute (CLSI), 2018 [12]. Following antibiotics were applied for gram negative isolates:

(a) For *Escherichia coli, Klebsiella* spp., *Citrobacter* spp. *and Enterobacter* spp.: Ciprofloxacin (5 µg), piperacillin-tazobactam (100/10 µg), amikacin (30 µg), ceftrixone (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), cefepime (30 µg), trimethoprim-sulphomethoxazole (1.25/23.75 µg). Additionally ceftazidime-clavulanic acid (30/10 µg) was used for *Escherichia coli, Klebsiella* spp.

(b) For *Pseudomonas* spp.: Ciprofloxacin (5 µg), levofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), cefepime (30 µg), ceftazidime (30 µg), netilimycin (30 µg), tobramycin (10 µg), ticarcillin-clavulanic acid (75/10 µg). Additionally, amikacin (30 µg) was also tested against *Pseudomonas* spp.

(c) For *Acinetobacter baumannii,* Ceftriaxone (30 µg), trimethoprim-sulphomethoxazole (1.25/23.75 µg), piperacillin-tazobactum (100/10 µg), minocycline (30 µg). Tigecycline (15 µg) was also additionally tested as per European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [13].

Phenotypic Tests

(i) Extended Spectrum β-Lactamases (ESBL) Detection:

A phenotypic confirmatory test recommended by the CLSI was done for *Escherichia coli and Klebsiella* spp. by combined disc diffusion method. Ceftazidime (30 µg) and a disc of ceftazidime-clavulanic acid (30/10 µg) was placed at a distance of 20 mm clinical isolate on MHA along with other antibiotics [12]. ESBL production was concluded if the inhibition zone was increased by 5 mm towards the ceftazidime-clavulanic acid disc in comparison to ceftazidime disc alone.

(ii) Modified Carbapenem Inactivation Method (mCIM) for detection of Carbapenamase producing isolates:

Meropenem resistant organisms were taken for this method. A loopful of testing isolate was mixed in Tryptic Soy Broth (TSB) aliquot (2 mL) and meropenem (10 µg) disc was added to TSB aliquot and was incubated at 37°C for four hours. Just before finishing of TSB-meropenem disc suspension incubation, 0.5 McFarland suspension of *Escherichia coli* (ATCC 25922) was prepared and lawn culture from this suspension was done on MHA. After 3-5 minutes, new meropenem disc was applied to MHA. Also, meropenem inactivated disc was also taken out from TSB aliquot and was applied to same MHA plate and plate was incubated for 18-24 hours at 37°C [12].

Interpretation of Carbapenemase producing isolates:

- Carbapenemase Positive: Zone diameter of 6-15 mm or presence of pin-point colonies.
- Carbapenemase Negative: Zone diameter of ≥19 mm.

STATISTICAL ANALYSIS

Data was captured manually and entered into MS-Excel. All the calculations were made by using formula in MS-Excel.

RESULTS

A total of 1537 samples from various ICUs were received and out of which, 263 (17.11%) were flagged positive by BACTEC 9050. A 1274 (82.89%) were incubated for up to five days and were declared sterile. Out of 1537 samples, majority was received from Medicine ICU 903 in which 181 samples were flagged positive by BACTEC 9050 showing positivity rate of 20.04% followed by Surgery ICU from which 362 samples were received in which 64 samples were flagged positive and positivity rate was 17.67% [Table/Fig-1].

Out of 263 (17.11%) samples, 51 (19.40%) were Gram Positive Cooci (GPC), 21 (07.98%) were *Candida* spp. and 191 (72.62%) were gram negative bacilli. GPC and *Candida* spp. were excluded from the study. Out of 191 samples, *Escherichia coli* was predominant followed by *Klebsiella* and *Pseudomonas* spp. [Table/Fig-2]. Overall, meropenem was the most sensitive drug for all the gram negative isolates followed by imipenem. Tigecycline (81.25%) was the second most effective drug against *Acinetobacter baumannii* [Table/Fig-3]. ESBL detection

Intensive Care Unit (ICU)	Total no. of sample received (S)	No. of sample flagged positive by BACTEC (P)	Positivity rate (P/S) %
Medicine	903	181	20.04
Surgery	362	64	17.67
Obstetrics and Gynaecology	190	11	05.78
Neonatal	82	07	8.53

[Table/Fig-1]: Sample wise distribution and positivity rate in various ICUs.

No. of culture positive	GPC* (%)	Gram negative bacilli (N=191) (72.62%)						*Candida* spp. (%)
		Escherichia coli (%)	*Klebsiella* spp. (%)	*Citrobacter* spp. (%)	*Enterobacter* spp. (%)	*Pseudomonas* spp. (%)	*Acinetobacter baumannii* (%)	
263	51 (19.39)	64 (33.50)	60 (31.41)	15 (7.85)	08 (4.19)	28 (14.66)	16 (8.38)	21 (7.99)

[Table/Fig-2]: Percentages of organisms causing Blood Stream Infection (BSI).
*Gram positive cocci

	Escherichia coli (%) N=64	*Klebsiella* spp. (%) N=60	*Citrobacter* spp. (%) N=15	*Enterobacter* spp. (%) N=08	*Pseudomonas* spp. (%) N=28	*Acinetobacter baumannii* (%) N=16
Ciprofloxacin	35 (54.68)	27 (45)	9 (60)	5 (62.5)	16 (57.14)	7 (43.75)
Levofloxacin	NT+	NT+	NT+	NT+	14 (50)	8 (50)
Piperacillin-Tazobactam	45 (70.31)	31 (51.67)	11 (73.33)	6 (75)	NT+	10 (62.5)
Ticarcillin-Clavulanic acid	NT+	NT+	NT+	NT+	19 (67.85)	10 (62.5)
Ceftriaxone	33 (51.56)	27 (45)	8 (53.33)	4 (50)	NT+	8 (50)
Cefuroxime	33 (51.56)	25 (41.67)	7 (46.66)	5 (62.5)	NT+	NT+
Cefotaxime	32 (50)	28 (46.66)	8 (53.33)	4 (50)	NT+	NT+
Cefepime	40 (62.5)	35 (58.33)	10 (66.66)	5 (62.5)	19 (67.85)	10 (62.5)

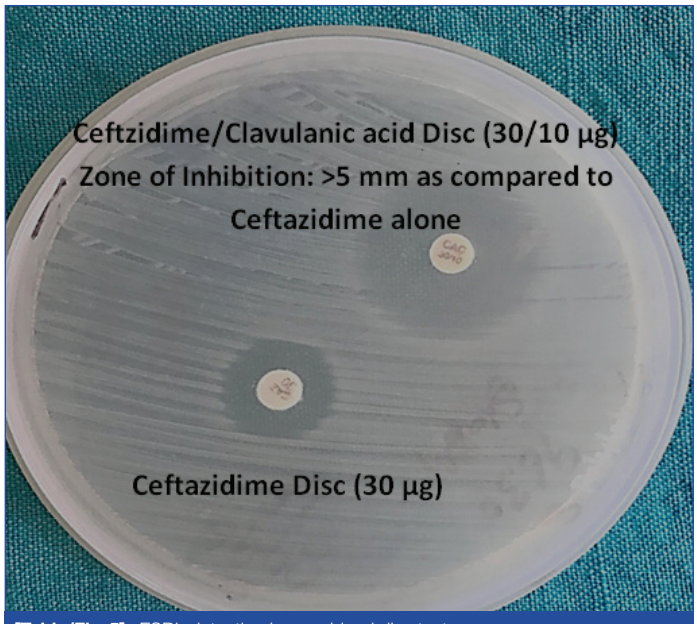
Ceftazidime	38 (59.37%)	33 (55%)	8 (53.33%)	6 (75%)	18 (64.28%)	8 (50%)
Ceftazidime-Clavulanic acid	50 (78.12%)	43 (71.66%)	NT+	NT+	NT+	NT+
Gentamicin	54 (84.37%)	49 (81.66%)	12 (80%)	6 (75%)	22 (78.57%)	12 (75%)
Amikacin	51 (79.68%)	42 (70%)	12 (80%)	6 (75%)	24 (85.71%)	NT+
Imipenem	56 (87.5%)	52 (86.66%)	13 (86.67%)	7 (87.5%)	23 (82.14%)	12 (75%)
Meropenem	58 (90.62%)	51 (85%)	14 (93.33%)	7 (87.5%)	25 (89.28%)	14 (87.5%)
Trimethoprim-Sulphmethoxazole	26 (40.62%)	21 (35%)	8 (53.33%)	4 (50%)	NT+	6 (37.5%)
Netilimycin	NT+	NT+	NT+	NT+	22 (78.57%)	12 (75%)
Tobramycin	NT+	NT+	NT+	NT+	19 (67.85%)	10 (62.5%)
Minocycline	NT+	NT+	NT+	NT+	NT+	8 (50%)
Tigecycline	NT+	NT+	NT+	NT+	NT+	13 (81.25%)

[Table/Fig-3]: Sensitivity profile of gram negative isolates causing BSI.
NT+: Not Tested

was done by combined disc test on 124 samples (*Escherichia coli* and *Klebsiella* spp.) which showed *Klebsiella* spp. 25 (20.16%) as the highest ESBL producing organism than *Escherichia coli* 18 (14.51%). A 191 samples were processed for carbapenemase detection by mCIM which showed highest carbapenemase production in *Klebsiella* spp. 6 (03.14%) followed by *Escherichia coli* 4 (02.09%) and *Acinetobacter baumannii* 4 (2.09%) [Table/Fig-4-6].

Total no. of isolates	Carbapenamase producers N=20 (10.47%)					
191	*Escherichia coli* (%)	*Klebsiella* spp. (%)	*Citrobacter* spp. (%)	*Enterobacter* spp. (%)	*Pseudomonas* spp. (%)	*Acinetobacter baumannii* (%)
	4 (2.09)	6 (3.14)	2 (1.05)	1 (0.52)	3 (1.58)	4 (2.09)

[Table/Fig-4]: Carbapenemase detection by Modified Carbapenem Inactivation Method (mCIM).



[Table/Fig-5]: ESBL detection by combined disc test.

[Table/Fig-6]: Carbapenemase detection by mCIM method.

DISCUSSION

BSI's are life threatening infections and the increasing rate of ESBL and carbapenemase producers has set an alarm in the medical community. Hence, the proper identification of ESBL and carbapenemase producers should be done so as to start the antimicrobial therapy.

The present study was conducted on 1537 blood samples received from various ICU's of our tertiary care hospital. Overall positivity rate was 17.11% which was supported by study conducted by Nannan Panday RS et al., in which positivity rate was (13.9%) [14]. The invasive and intubation procedures in ICU patients leads to increased risk of bacteremia [6].

Out of 1537 samples, majority of samples were received from Medicine ICU 903/1537 (58.75%) followed by Surgery ICU 362/1537 (23.55%) and Obstetrics and Gynaecology ICU 190/1537 (12.36%). The highest positivity was noted in blood samples from Medicine ICU 181/903 (20.04%) followed by Surgery 64/362 (17.67%). Similar studies conducted by Parajuli NP et al., which also showed higher prevalence in Medicine and Surgery ICU (adult group) [7].

Among 263 positively flagged bottles, when culture was done it showed highest prevalence of GNB followed by GPC i.e., 72.62% and 19.40%. Among 191(72.62%) isolates of GNB, *Escherichia coli* (33.51%) were the predominant organism followed by *Klebsiella* spp. (31.41%) and *Pseudomonas aeruginosa* (14.66%). The similar study conducted by Bajaj A et al., which also showed high prevalence of GNB in BSI and showed *Klebsiella* spp. (31.01%) as predominant organism followed by *Pseudomonas aeruginosa* (17.72%) [15]. GNB have built-in abilities to find new ways to be resistant and can pass along genetic materials that allow other bacteria to become drug-resistant as well [16].

The antibiotic sensitivity testing was done by Kirby-bauer disc diffusion method. In the present study, Meropenem was the most sensitive drug for all the gram negative isolates followed by Imipenem. Tigecycline (81.25%) was the second most effective drug against *Acinetobacter baumannii*. These results were in accordance with the study conducted by Siwakoti S et al., in which they also reported high percentage of MDR bacteria [8]. Prior hospitalisation and antibiotic exposure have been identified as risk factors for infections caused by resistant bacteria in different studies [16].

The occurrence of ESBL producers in BSI leads to high mortality and morbidity. The ESBL detection was done for *Escherichia coli* and *Klebsiella* spp. as per CLSI, 2018 guidelines by Combined disc Method (Ceftazidime-Clavulanic acid). Among 124 isolates, 43 (34.67%) isolates were ESBL producers. The *Klebsiella* spp. producers were 20.16% followed by *Escherichia coli* (14.51%). High ESBL producers (61.8%) were reported in studies conducted by Sangare SA et al., [17]. Another study conducted by Adeyemo AT et al., showed 26.2% as ESBL producers [18]. The antibiotic sensitivity pattern of ESBL producers showed high resistance to beta-lactam antibiotics along with moderate resistance to aminoglycosides.

Carbapenemase production is plasmid mediated so resistance is easily acquired by GNB and can also lead to 50% mortality in BSI. Therefore in present study, among 191 isolates, carbapenemase detection was done by mCIM which showed 10.47% carbapenemase producers. In present study carbapenemase production was higher in *Klebsiella* spp. (3.14%) followed by *Escherichia coli* (2.09%). Higher prevalence of carbapenemase producers has also been reported in study conducted by Okoche D et al., in which the prevalence was 22.4% [19]. Similar study conducted by Subhedar V and Jain SK showed 16.8% carbapenemase producers [20]. The increasing incidences of ESBL and carbapenemase producing GNB force us to screen these producers.

Limitation(s)

The study excludes gram positive bacteria and *Candida* spp. isolates which are also a major cause of BSI. Also, genotypic screening for resistance genes could not be performed due to the limited resources.

CONCLUSION(S)

BSI among ICU patients itself are life threatening infections and emergence of ESBL and carbapenemase producer increases mortality by 50%. Moreover, as present study suggested that, for ESBL production, combined disc test can be used for screening of every sample as it does not require extra labour and expertise. Also, carbapenemase production was confirmed by mCIM method and gave good results and CLSI guidelines shows this test as confirmatory and hence, recommended to be used in MDR infections.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 27, 2021
- Manual Googling: Sep 20, 2021
- iThenticate Software: Sep 28, 2021 (11%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 24, 2021**
Date of Peer Review: **Sep 10, 2021**
Date of Acceptance: **Sep 29, 2021**
Date of Publishing: **Nov 01, 2021**