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Original Research Paper

Aerobic Bacteriological Profile of Bloodstream Infections with Special Reference to ESBL Producers among the Gram Negative Isolates

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ABSTRACT:
Background: Bloodstream infections (BSI) refer to the presence of microorganisms in blood. The timely detection and identification of pathogens of bloodstream infection and determination of antibiotic sensitivity has great diagnostic and prognostic importance. **Aim**: To study the aerobic bacteriological profile of bloodstream infections, to determine their antibiotic susceptibility pattern and to find out the proportion of ESBL producers among the gram negative isolates. **Materials and Method**: This descriptive cross-sectional study included 950 blood samples received for culture in the Microbiology laboratory, from inpatients of Government Medical College, Ernakulam. Isolates obtained were identified and ABST done as per standard protocols. For all GNB, ESBL production was detected by phenotypic method as per CLSI guidelines. Genotyping for ESBL gene detection was also done for Enterobacteriaceae isolates. The data entered in excel spread sheet was analysed using Statistical Package for Social Sciences (SPSS) software 20.0. Fisher's exact test was used in the analysis of study variables. The level of statistical significance was taken as p value < 0.05. **Result**: The culture positivity rate was 6.5%. 74% of the isolates were gram negative bacilli. Escherichia coli was the most frequent organism isolated, followed by Staphylococcus aureus. 39.1% of the gram negative isolates were ESBL producers and majority of them were E. coli. The most prevalent beta-lactamase gene detected was bla_{CTX-M}. **Conclusion**: We could prove statistically significant association of presence of invasive device for more than 1 week with both bloodstream infections and ESBL production. We could also prove statistically significant association of ESBL production with group of antibiotic used currently.

Keywords: Bloodstream infection; ESBL

INTRODUCTION:

Bloodstream infections (BSI) constitute one of the most serious situations among infectious diseases[1]. BSIs are widely spread all over the world with direct and indirect social and economic impacts. It is estimated that BSIs affects approximately 30 million people, causing 6 million deaths each year in the world.[3,4] Prompt initiation of appropriate antimicrobial therapy is important for preventing morbidity and mortality.[5] Prevalence and susceptibility patterns of bacteria show wide geographical variations; as evidenced by a number of previous studies. It varies in the same hospital at different periods of time also.[6] Over the past several decades, there has been a shift in the nature of the infecting microbiota. The number of anaerobic isolates has decreased over that time, whereas the number of isolates of yeast and clinically significant coagulase-negative staphylococci has increased.[2] The prevalence of ESBL producers in gram negative bacilli increased from 61.6% in 2011 to 66% in 2014.[7] Hence regular survey of bloodstream infections with regards to the commonly isolated pathogens and their antibiotic profile is important as it helps the clinician to choose a proper empirical antibiotic therapy.[8]

AIMS AND OBJECTIVES:

The present study was conducted to study the aerobic bacteriological profile of bloodstream infections in samples received for culture and sensitivity in the Microbiology laboratory, Government Medical College,

Ernakulam over a period of 1 year, to determine their antibiotic susceptibility pattern and to find out the proportion of ESBL producers among the gram negative isolates.

MATERIALS AND METHODS:

Study Design: Descriptive cross-sectional study.
Study Setting: Department of Microbiology, Government Medical College, Ernakulam.
Study Period: 1 year (April 2021 to March 2022)
Sample size was calculated as 950 based on a study done by Singh AK et al in 2014 at UP on 'Bacterial and antimicrobial resistance profile of bloodstream infections: a hospital-based study'
Study Specimen: Blood samples received for culture and sensitivity over a period of 1 year in Microbiology laboratory of Government Medical College, Ernakulam.
Inclusion Criteria: Samples for blood culture received in Microbiology laboratory from adult and paediatric inpatients of Government Medical College, Ernakulam.
Exclusion Criteria: Repeat samples from same patient
Study Procedure: The study was started after obtaining approval from Institutional Research Committee and Institutional Ethics Committee (IEC- 48/2021) of Government Medical College, Ernakulam. Automated blood culture system BacT/ALERT 3D was used to process samples of blood received in the Microbiology laboratory for culture and sensitivity. When a positive culture was indicated by the automated system, it was sub-cultured onto Blood agar and MacConkey agar and a Gram stained smear was examined. The culture plates were incubated aerobically at 37^0C for 24 to 48 hours, were examined for growth and colony morphology was noted. Identification of the isolate was done by performing gram staining and appropriate biochemical reactions as per standard guidelines. Antibiotic susceptibility testing of the isolates were done by Kirby-Bauer disk diffusion method and interpreted as per CLSI guidelines.

Detection of ESBL Producing Isolates:

ESBL producers among the isolates of gram negative bacilli were detected by using both phenotypic and genotypic methods.

1. **Phenotypic Method:**

- **Screening test**: This was done for isolates of Escherichia coli and Klebsiella pneumoniae as per CLSI guidelines. Isolates with zone size of Cefotaxime ≤ 27mm and Ceftazidime ≤ 22mm were considered potential ESBL producers and were subjected to confirmatory tests.

2. **Confirmatory Tests**:

- **Double Disc Synergy Test**:

Amoxicillin clavulanate (20/10 µg) disc and 30 µg disc each of Ceftazidime and Cefotaxime were placed at a distance of 15-20 mm from center to center on Mueller Hinton agar plate on which lawn culture of the isolate had been done and incubated overnight at 37^0C. Extension of inhibition zone of either of the cephalosporin towards the amoxicillin clavulanate disc was interpreted as positive for ESBL production.

- **Combination Disc Method:**

Disc of Ceftazidime (30 µg) alone and a disc of Ceftazidime-clavulanic acid (30/10 µg) were placed at a distance of 25mm center to center on a Mueller Hinton agar plate on which lawn culture of the isolate had been done. A disc of Cefotaxime (30 µg) alone and a disc of Cefotaxime-clavulanic acid (30/10 µg) were also placed at a distance of 25 mm center to center on the same MHA plate. The plates were incubated overnight at 37^0C. Increase in inhibition zone diameter by ≥ 5 mm for either antibiotic tested in combination with clavulanate compared to the zone diameter when tested alone confirmed ESBL production.
Other gram negative bacilli were subjected to both the confirmatory tests as above for ESBL detection.

3. **Genotypic Method:**

PCR-Based Detection of ESBL Genes in the Isolates of Enterobacteriaceae*:*
This was done at Central Institute of Fisheries Technology, Wellington Island. DNA extraction was performed from overnight bacterial cultures (grown in brain heart infusion broth) by heat lysis method. All PCRs were carried out by conventional method. Details of the primers and PCR conditions used in this study are given in table below:

Primer	Primer sequence (5'-3')	Target gene	PCR conditions	Amplicon size
Multiplex primers				
OXA-23-likeF	GAT CGG ATT GGA GAA CCA	$bla_{OXA-23-like}$		501 bp
OXA-23-likeR	GA		Initial denaturation at 94 °C	
OXA-24-likeF	ATT TCT GAC CGC ATT TCC AT	$bla_{OXA-24-like}$	for 5 min; 30 cycles of 94 °C	246 bp
OXA-24-likeR	GGT TAG TTG GCC CCC TTA AA		for 25 s, 52 °C for 40 s and	

OXA-51-likeF OXA-51-likeR OXA-58-likeF OXA-58-likeR	AGT TGA GCG AAA AGG GGA TT TAA TGC TTT GAT CGG CCT TG TGG ATT GCA CTT CAT CTT GG AAG TAT TGG GGC TTG TGC TG CCC CTC TGC GCT CTA CAT AC-3'	*bla*$_{OXA-51-like}$ *bla*$_{OXA-58-like}$	72 °C for 50 s; and a final elongation at 72 °C for 6 min.	353 bp 599 bp
<u>Multiplex primers</u> MultiGES_for MultiGES_rev MultiPER_for MultiPER_rev MultiVEB_for MultiVEB_rev	 AGTCGGCTAGACCGGAAAG TTTGTCCGTGCTCAGGAT GCTCCGATAATGAAAGCGT TTCGGCTTGACTCGGCTGA CATTTCCCGATGCAAAGCGT CGAAGTTTCTTTGGACTCTG	 GES-1 to 9 and GES-11 PER-1 and PER-3 VEB-1 to VEB-6	Initial denaturation at 94 °C for 10 min; 30 cycles of 94 °C for 40 s, 60 °C for 40 s and 72 °C for 1 min; and a final elongation step at 72 °C for 7 min.	 399 bp 520 bp 648 bp
<u>Multiplex primers</u> MultiCTXMGp1_for MultiCTXMGp1_rev MultiCTXMGp2_for MultiCTXMGp2_rev MultiCTXMGp9_for MultiCTXMGp9_rev	 TTAGGAARTGTGCCGCTGYA CGATATCGTTGGTGGTRCCAT CGTTAACGGCACGATGAC CGATATCGTTGGTGGTRCCAT TCAAGCCTGCCGATCTGGT TGATTCTCGCCGCTGAAG	 variants of CTX-M group 1 including CTX-M-1, CTX-M-3 and CTX-M-15 variants of CTX-M group 2 including CTX-M-2 variants of CTX-M group 9 including CTX-M-9 and CTX-M-14	Initial denaturation at 94 °C for 10 min; 30 cycles of 94 °C for 40 s, 60 °C for 40 s and 72 °C for 1 min; and a final elongation step at 72 °C for 7 min.	 688 bp 404 bp 561 bp
<u>Multiplex primers</u> <u>MultiTSO-T_for</u> <u>MultiTSO-T_rev</u> <u>MultiTSO-S_for</u> <u>MultiTSO-S_rev</u> <u>MultiTSO-O_for</u> <u>MultiTSO-O_rev</u>	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC AGCCGCTTGAGCAAATTAAAC ATCCCGCAGATAAATCACCAC GGCACCAGATTCAACTTTCAAG GACCCCAAGTTTCCTGTAAGTG	TEM variants including TEM-1 and TEM-2 SHV variants including	Initial denaturation at 94 °C for 10 min; 30 cycles of 94 °C for 40 s, 60 °C for 40 s and 72 °C for 1 min; and a final elongation step at 72 °C for 7 min.	800 bp 713 bp 564 bp

		SHV-1 OXA-1, OXA-4 and OXA-30		

DATA COLLECTION AND ENTRY:
Clinical details of the study samples were collected from the case files and entered into the proforma. These were numerically coded and entered into Microsoft Excel spreadsheet. The growth obtained after culture, identification of the isolates, susceptibility to each antibiotic, and the results of ESBL detection were also coded and entered into the excel spreadsheet.

DATA ANALYSIS:
The data entered in the MS-Excel spreadsheet was analyzed using Statistical Package for Social Sciences (SPSS) software 20.0. Qualitative variables were summarized using frequency or percentage. Fisher's exact test was used in the analysis of study variables. The level of statistical significance was taken as p value < 0.05 in this study.

RESULTS:
Of the 950 blood samples processed, aerobic culture yielded bacterial growth in 62 samples. The culture positivity rate was 6.5%. The maximum number of isolates (14) was in the age group of 51-60 years, accounting for 22.6% (N=62) followed by 61-70 years age group, which accounted for 21% of the isolates. 54.8% of positive cultures were obtained from males. 74.2% of the positive blood culture samples were from patients admitted in the ICU.
Majority of the isolates were gram negative bacilli (74%; n=46). Out of the 46 GNB isolated, 27 were Enterobacteriaceae and 19 were non fermenters.

Table 1: Distribution of bacterial isolates in the culture positive samples

Organism	**Number (%)**
Gram negative bacilli – Enterobacteriaceae (Total no: 27)	
Escherichia coli	14 (22.6)
Klebsiella pneumoniae	9 (14.5)
Citrobacter koseri	2 (3.2)
Salmonella Typhi	1 (1.6)
Serratia marcescens	1 (1.6)
Gram negative bacilli – Non fermenters (Total no: 19)	
Pseudomonas aeruginosa	6 (9.7)
Pseudomonas species other than Pseudomonas aeruginosa	4 (6.4)
Acinetobacter species	8 (12.9)
Stenotrophomonas maltophilia	1 (1.6)
Gram positive cocci (Total no:16)	
Staphylococcus aureus	10 (16.1)
Enterococcus faecium	4 (6.4)
Streptococcus species other than beta hemolytic Streptococcus	1 (1.6)
Beta hemolytic Streptococcus species	1 (1.6)
Total	**62 (100.0)**

Escherichia coli was the most common isolate among the total as well as gram negative isolates. Among the Enterobacteriaceae, 50% were E.coli. Similarly Acinetobacter species constituted 50% of the non-fermenters. Staphylococcus aureus was the second most common among the total isolates.

Antibiotic Sensitivity of the Isolates:

Table: 2. Antibiotic Sensitivity Pattern of GNB – Enterobacteriaceae:

Organism		**Antibiotics**												
Escherichia coli		AM	PR	CX	FR	AG	ZX	PT	IM	MP	CT	CI	GM	AK
(n=14)		0	1	3	3	14	3	14	14	14	8	6	9	13

	S													
	R	14	13	11	11	0	11	0	0	0	6	8	5	1
Klebsiella pneumoniae (n=9)	**S**	0	3	3	3	9	3	8	9	9	3	5	7	9
	R	9	6	6	6	0	6	1	0	0	6	4	2	0
Citrobacter koseri (n=2)	**S**	0	0	0	0	2	2	0	2	2	1	2	1	1
	R	2	2	2	2	0	0	2	0	0	1	0	1	1
Salmonella Typhi (n=1)	**S**	0	-	-	1	-	-	-	-	-	0	0	-	-
	R	1	-	-	0	-	-	-	-	-	1	1	-	-
Serratia marcescens (n=1)	**S**	0	0	1	1	0	1	1	1	1	1	1	1	1
	R	1	1	0	0	1	0	0	0	0	0	0	0	0

S – Sensitive, R – Resistant, AM - Ampicillin (10 µg) PR - Cephalothin (30 µg) CX - Cefotaxime (30 µg) FR - Ceftriaxone (30 µg) AG - Augmentin (20/10 µg) ZX - Cefepime (30 µg) PT - Piperacillin-tazobactam (100/10 µg) IM - Imipenem (10 µg) MP - Meropenem (10 µg) CT - Cotrimoxazole (1.25/23.75 µg) CI - Ciprofloxacin (5 µg) GM - Gentamicin (10 µg) AK - Amikacin (30 µg)

All the isolates belonging to family Enterobacteriaceae were resistant to Ampicillin, but all the tested isolates were sensitive to Carbapenems.

Table 3: Antibiotic sensitivity pattern of GNB – Non fermenters

Organism		**Antibiotics**													
		FG	CX	FR	ZX	PT	IM	MP	CT	CI	LV	GM	AK	TO	MI
Pseudomonas aeruginosa (n=6)	**S**	0	-	-	-	1	5	5	-	4	-	5	5	-	-
	R	6	-	-	-	5	1	1	-	2	-	1	1	-	-
Pseudomonas species other than Pseudomonas aeruginosa (n=4)	**S**	2	-	-	-	1	2	2	0	4	-	0	0	-	-
	R	2	-	-	-	3	2	2	4	0	-	4	4	-	-
Acinetobacter species (n=8)	**S**	1	1	2	3	3	3	3	3	2	3	3	3	5	4
	R	7	7	6	5	5	5	5	5	6	5	5	5	3	4
Stenotrophomonas maltophilia (n=1)	**S**	1	-	-	-	0	0	0	1	0	-	1	0	-	-
	R	0	-	-	-	1	1	1	0	1	-	0	1	-	-

FG – Ceftazidime (30 µg) LV - Levofloxacin (5 µg) TO - Tobramycin (10 µg) MI - Minocycline (30 µg)

Table 4: Antibiotic sensitivity pattern of gram positive cocci

Organism		Antibiotics											
		PG	AM	CX_{30}	ER	CD	CT	GM_{10}	GM_{120}	CX	LZ	VA	CI
Staphylococcus aureus (n=10)	S	0	-	5	4	4	5	4	-	-	10	-	8
	R	10	-	5	6	6	5	6	-	-	0	-	2
Enterococcus faecium (n=4)	S	1	1	-	0	-	-	-	3	-	4	4	1
	R	3	3	-	4	-	-	-	1	-	0	0	3
Streptococcus species other than beta hemolytic Streptococcus (n=1)	S	0	0	-	1	1	-	-	-	0	1	1	-
	R	1	1	-	0	0	-	-	-	1	0	0	-
Beta hemolytic streptococcus species (n=1)	S	1	1	-	1	1	-	-	-	1	1	1	-
	R	0	0	-	0	0	-	-	-	0	0	0	-

PG - Penicillin (10U) CX_{30} - Cefoxitin (30 µg) ER - Erythromycin (15 µg) CD - Clindamycin (2 µg) GM_{10} - Gentamicin (10 µg) GM_{120} - Gentamicin (120 µg) LZ - Linezolid (30 µg) VA - Vancomycin (30 µg)

Table 5: Presence of invasive devices in patients with positive blood culture

Presence of invasive device	Positive blood culture (n = 62)		Total
	Number	Percent	
Nil	44	5.4	808
Yes (≤ 7 days)	11	9.7	113
Yes (> 7 days)	7	24.1	29
Total	62	6.5	950
P value < 0.001			

A statistically significant association could be shown between presence of invasive device for more than 7 days and bloodstream infections in this study.

ESBL Production:

39.1% of the gram negative isolates were ESBL producers.

ESBL Production and Location in the Hospital:

In this study there is a statistically significant association between location in hospital (ward) and ESBL producers.

Table 6: Group of antibiotic used currently and ESBL production

Antibiotic group	ESBL (n=18)		Total
	Number	Percent	
Not applicable	0	0	2
3rd generation cephalosporin	0	0	9
Beta lactam-beta lactamase inhibitor combination	3	50	6
Carbapenem	3	60	5

>1 class of antibiotic and at least one of these is a beta lactam	11	47.8	23
Others *	1	100	1
Total	18	39.1	46
P value = 0.004			

*****Fluoroquinolones

There is a statistically significant association between group of antibiotic used currently and ESBL production in our study.

Table 7: Presence of invasive device and ESBL production

Presence of invasive device	ESBL (n=18)		Total
	Number	Percent	
Nil	12	38.7	31
Yes, ≤ 7 days	2	25	8
Yes, > 7 days	4	57.1	7
Total	18	39.1	46
P value = 0.009			

Our study showed a statistically significant association between presence of invasive device for more than 1 week and ESBL production.

Table 8: Distribution of beta-lactamase genes among the ESBL positive Enterobacteriaceae isolates

Gene	Escherichia coli (n=11)	Klebsiella pneumoniae (n=6)	Citrobacter koseri (n=1)
Only bla_{CTX-M}	2	0	0
Only $bla_{OXA-1-like}$	0	0	1
Only bla_{TEM}	0	0	0
Only bla_{SHV}	0	0	0
Both bla_{CTX-M} and $bla_{OXA-1-like}$	5	0	0
Both bla_{CTX-M} and bla_{TEM}	4	0	0
All four genes present ($bla_{CTX-M,}$ $bla_{TEM,}$ $bla_{SHV,}$ and $bla_{OXA-1-like)}$	0	6	0

DISCUSSION:

A total of 950 blood samples received from patients admitted in Government medical college, Ernakulam were included in the study. The aim of the study was to find out the aerobic bacteriological profile of bloodstream infections, to determine their antibiotic susceptibility pattern and also to find out the proportion of ESBL producers among the gram negative isolates.

In our study, aerobic culture yielded bacterial growth in 62 out of the 950 samples (6.5%). This was similar to the culture positivity rate in a study carried out in 2017 at Kathmandu, Nepal, which was 7.48%.[9]

Our study had more samples received from ICU (60%) compared to wards (40%). Majority of the culture positive samples were also from ICU (74.2%) and the culture positivity rate was also higher in samples received from ICU (8% for ICU and 4.2% for wards). This finding was in concordance with the finding in the studies conducted at Kathmandu and Jaipur.[9, 10]

In our study, gram negative bacilli accounted for 74% (46 isolates) of the culture positive samples. Similar finding was seen in studies conducted at New Delhi[11], Manipur[12], Kanpur[13], Bhopal[14], North Karnataka[15] and North Kerala.[16]

Our study had E. coli as the most frequent isolate (22.6%), followed by Staphylococcus aureus (16.1%). Similar finding was seen in studies conducted at Pune[17] and Mathura[18].

All the Enterobacteriaceae isolates obtained in our study were resistant to Ampicillin. This was consistent with study done by Vasudeva N et al,[10] and the study from northwest Ethiopia.[19] All the isolates tested were sensitive to Imipenem and Meropenem in our study. This was similar to the study conducted by Kotgire et al in Maharashtra[17].

Our study showed a statistically significant association between presence of invasive device for more than 1 week and positive blood cultures. This was in concordance with the studies done at Ethiopia[22], Israel[23] and Iran[24] which also noted higher BSI rate in patients on invasive device.

39.1% of the gram negative isolates from our study were ESBL producers. This was similar to the study from Gujarat, where the prevalence of ESBL producers among blood culture isolates was 39.6%.[20]

E. coli (61.1%) constituted majority of the ESBL producing bacteria in our study, followed by K. pneumoniae (33.3%) and Citrobacter koseri (5.6%). This was similar to the study from China which also observed E. coli as the most common ESBL producer followed by K. pneumoniae.[21]

In our study, 66.7% of the gram negative isolates from wards were ESBL producers, whereas 29.4% of the gram negative isolates from ICU were ESBL positive. We got statistically significant association between location in hospital (ward) and ESBL producers. However, in the study conducted at Pune, ESBL prevalence was higher in isolates from ICU.[25] The higher prevalence of ESBL producers in wards in our study may be attributed to the use of multiple antibiotics with at least one Beta lactam and use of Fluoroquinolones in wards. All the patients infected with ESBL positive bacteria in our study had history of current antibiotic intake. There was a statistically significant association between the group of currently used antibiotic and ESBL positivity in our study. ESBL production was found to be more associated with use of Fluoroquinolones, Carbapenems, Beta lactam-beta lactamase inhibitor combination and more than one class of antibiotic with at least one Beta lactam. In the study done in Israel, similar finding was seen, with exposure to Beta lactam agents and to Fluoroquinolones being significant predictors for BSI with ESBL positive Enterobacteriaceae.[26]

In our study, 57.1% of the patients with gram negative bacteremia who had invasive device for > 1 week were infected with ESBL producing bacteria. We could find a statistically significant association between presence of invasive device for > 1 week and ESBL positive isolates. Studies from Israel also observed presence of invasive device or invasive procedures to be associated with bacteremia with ESBL producing Enterobacteriaceae.[26,27]

Our study found bla_{CTX-M} as the most prevalent beta-lactamase gene, that was detected in all the ESBL positive isolates of E. coli and K. pneumoniae, either singly or in combination with other beta-lactamase genes. The ESBL positive Citrobacter koseri had only $bla_{OXA-1-like}$ gene. This was consistent with the studies conducted at Kathmandu[9], and Puducherry[28], which detected bla_{CTX-M} as the most prevalent ESBL gene.

CONCLUSION:

A total of 950 blood samples received in the Microbiology lab from patients admitted in Government Medical College, Ernakulam were included in the study from 1st April 2021 to 31st March 2022. The culture positivity rate was 6.5%. We could prove statistically significant association of bloodstream infection with presence of invasive device for more than 1 week. Majority of the aerobic bacteria isolated were gram negative bacilli, constituting 74% and the remaining 26% were gram positive cocci. Most frequent organism isolated was Escherichia coli, which accounted for 22.6% of the isolates. It was followed by Staphylococcus aureus (16.1%). 39.1% of the gram negative isolates were ESBL producers. 61.1% of the ESBL producers in our study were E. coli, followed by K. pneumoniae (33.3%) and Citrobacter koseri (5.6%). We could prove statistically significant association of ESBL positive isolates with group of antibiotic used currently, and presence of invasive device for more than 1 week. The most prevalent beta-lactamase gene detected in ESBL positive isolates in this study was bla_{CTX-M}.

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