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Phenotypic Detection of Extended Spectrum β-lactamases Producing Clinical Isolates in Blood Stream Infections in a Tertiary Care Hospital in Northern India

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A B S T R A C T

BSI is caused by viable infectious microorganisms in the bloodstream (BS). Causing shock, disseminated intravascular coagulation, multiple organ failure, and demise. Extended-spectrum β-lactamases (ESBLs) are plasmid-mediated enzymes that pose a major therapeutic struggle in hospitalized and community-based patients these days. Aim of the study is to detect ESBL isolates in multidrug resistant clinical isolates from blood stream infections. The multidrug resistant organisms were isolated using the standard protocol followed in the laboratory followed by using double disc synergy method for ESBL detection. Staphylococci were the most common among the gram-positive organisms (thirty-seven percent) followed by methicillin-resistant *Staphylococcus aureus* (twenty percent) and *Staphylococcus epidermidis* (5%). *Escherichia coli* (12%), *Klebseilla pneumoniae* (10%) and *Pseudomonas aeruginosa* (7%) were the widely known gram-negative organisms. *Escherichia coli* and *Klebseilla pneumoniae* were the main multi drug resistant organisms out of that 3 (80%) of the tested *Escherichia coli* (5) and 2 (50%) of the tested *Klebseilla pneumoniae* (4) were ESBL-producers. For effective management and prevention of drug resistance, reasonable antibiotic use, establishment of antibiotic policy, and timely BSI therapy are necessary. Finally, any delay in initiating appropriate antibiotic therapy has the potential to be fatal.

Introduction

BSI are caused by viable infectious microorganisms in the bloodstream (BS). BSI increase illness and death, especially in ICU patients. They are severe because they often cause "shock, disseminated intravascular coagulation, multiple organ failure, and demise."Hospital stays and expenses have risen the most (Viscoli, 2016). "Extended-spectrum ß-lactamases (ESBLs)" are "plasmid-mediated enzymes" that pose a major therapeutic struggle in hospitalized and community-based patients these days. ESBL-producing infections include UTIs and sepsis" (Anderson *et al.,* 2006). The older TEM was isolated from a Greek patient named Temoniera. Clavulanic acid inhibits enzymes that hydrolyze cephalosporins and aztreonam. ESBL-producing organisms are resilient to multiple antibiotic classes, limiting treatment options. "Clinical and Laboratory Standards Institute" offers guidelines for detecting "ESBLs in *Klebseilla pneumoniae*, *E. coli*, and *Proteus mirabilis* (Laupland,

2013)." All ESBL-detection methods share the general principle that clavulanic acid enhances extended-spectrum cephalosporins activity against ESBL-producing organisms.

ESBLs show how gram-negative bacteria can adapt to new antimicrobials. To prevent further selection and spread of these increasingly resistant disease-causing organisms, effective infection-control practices and intervention strategies, such as antibiotic rotation, are needed.

BSIs are more common in critically ill patients, with approximately seven percent of them developing within the initial month of hospitalization in an ICU. The mainstay of treatment for infections caused by multidrug-resistant ESBL-producing organisms are Carbapenems but recent reports of acquired carbapenem resistance in these organisms are a cause for serious concern (Woodford *et al.,* 2004). Timely investigation will increase our understanding of these serious infections and allow us to elaborate more effective policies and practice guidelines for reducing their frequency and the morbidity and mortality that they cause (Mario Tumbarello *et al.,* 2006).

Materials and Methods

Research Design

The research was carried out at Government Medical College and Hospital's Department of Microbiology. The study lasted for one year (Dec 2021- Nov 2022). Samples of blood from patients over the 18 years who were admitted to "ICUs, medicine/surgery/gynaecology wards, or OPDs" by a medical doubt of bacteraemia were directed to a microbiology lab. The "double disc synergy test, combined disc method, and E-test" are the most commonly used approaches. For finding of the "ESBL phenomenon, several automated systems have been developed, and some laboratories use molecular methods" (Laupland, 2013).

Sample Collection

Blood samples were taken under strict aseptic and antiseptic conditions. "Phlebotomy" was used to collect the samples. "Subcultures on a blood agar plate and MacConkey's agar were performed after incubation at 37^{o}C aerobically for 24 hours." After seven days of incubation and a final subculture, blood culture broth that showed no signs of growth of bacteria ("hemolysis or turbidity") was reported negative. Microbiological blood culture was used to confirm BSI.

The "positive blood culture plates were gram stained" and testified to the appropriate wards right away. Standard guidelines were followed for identification and antibiotic susceptibility testing (Thaden *et al.,* 2017).

Sample Processing

Blood for culture was collected from 181 clinically suspected bacteremia cases under strict aseptic precautions. A volume of 5–10 ml from adults were obtained for culture. The same was inoculated into conventional blood culture bottles containing Brain Heart Infusion broth (1:10 dilution). These were then incubated aerobically at 37°C for 24h. After incubation, a blind subculture was done to appropriate solid culture media irrespective of the turbidity status.

The bottles were taken out and visually observed for turbidity every morning and then manually agitated for aeration. The bottles showing turbidity were sub-cultured on MacConkey agar and Blood agar. All the negative bottles were incubated for 7 days and another blind subculture was done at the end of 7 days of incubation before reporting them as negative (CLSI, 2017) (*CLSI Document M47-A*).

Any growth obtained was subjected to standard protocol of processing the samples followed in the department that is Gram-staining, colony morphology, and standard biochemical tests. Antibiotic susceptibility testing was performed according to Kirby–Bauer's disc diffusion method and interpreted according to CLSI guidelines using HiMedia (Mumbai) antibiotic discs (Clinical and Laboratory Standards Institute, 2017) (*CLSI Document M100-S27*).

Microbiology studies for detection of ESBL.

In our study, all of the *E. coli* and *K.pneumoniae* isolates which were resistant to cefotaxime (30 µg) or ceftazidime (30 µg) were subjected to ESBL screening according to the CLSI criteria. Both ceftazidime and cefotaxime with and without clavulanic acid (10 µg) disks were placed 25 mm apart from each other on Muller-Hinton agar inoculated with 0.5 McFarland suspension of the test isolate. A difference of ≥5mm between the inhibition zone diameters of cefotaxime or

ceftazidime disk in combination with clavulanic acid was considered as a positive ESBL phenotype.

Results and Discussion

Total 181 patients were considered in this work. Most common "age group was >60 years (44%) followed by 41-60 years (38%) and 19-40 years (19%)." "Male patients (60%) were more than female (40%)." "We noted positive blood culture report in "23% patients."

"Respiratory infection (29%), genitourinary infection (22%) & metabolic disorders (12%)" were widely known initial clinical diagnosis for source of bacteremia.

Staphylococci were the most common among the gram-positive organisms (thirty-seven percent) followed by "methicillin-resistant *Staphylococcus aureus*" (twenty percent) and "*Staphylococcus epidermidis*" (5%). "*Escherichia coli*" (12%), "*Klebseilla pneumoniae*" (10%) and "*Pseudomonas aeruginosa*" (7%) were the widely known "gram-negative organisms".

Imipenem, Ertapenem, Piperacillin–tazobactam, Gentamicin, Levofloxacin, Amikacin, Ceftazidime, and Aztreonam were found to be sensitive drugs against gram negative bacteria in this study.

Antibiotics like Vancomycin, Linezolid,Gentamicin, Clindamycin, and Ciprofloxacin were found to be more effective against *Staphylococcus aureus* and MRSA in this study.

All *Escherichia coli* and *K.pneumoniae* isolates were subjected to Double disc diffusion testExtension of zone of inhibition towards amoxicillin-clavulanic acid was interpreted as ESBL producer [Figure 1]. Results showed that 3(80%) of the tested *Escherichia coli* (5) and 2(50%) of the tested *Klebseilla pneumoniae* (4) were ESBL-producers.

Imipenem, meropenem, and amikacin resistance was found in gram negative isolates. Fifty-five percent were resistant to 3 of the 4 drugs or drug classes: fluoroquinolones, aminogycosides, Carbapenems.

Antimicrobinial vulnerability results were reported to doctors 48 to 120 hours (mean SD, 72 hours and 24 hours) after the "index blood culture" was collected. The "nosocomial isolates" had a pointedly higher rate of resilient to the "β-lactam–-lactamase inhibitor combinations (P = 0.001)" bacteria when compared to isolates that caused infections associated with health care and community-acquired (ESBL detection method).

In terms of clinical manifestations, bacteremia can range from "self-limiting infections to life-threatening septicemia" that necessitates quick and balanced therapy for disinfectant. Culture of blood remains the topmost standard for diagnosis, despite vast improvements in diagnostic techniques (Cohen *et al.,* 2015).

Ammerlaan (2013) stated that "local patterns of bacterial infection" as well as vulnerability to several antibiotics shall be assessed on a regular basis. When dealing with potentially life-threatening infections like BSI, it's critical to understand the regional epidemiological and microbiological data, because accuracy in predicting bacteria and resistance profiles is critical for successful therapy (Ammerlaan, 2013).

Ammerlaan (2013) The most common types of BSIs are "catheter-related BSIs (defined as the growth of the same pathogen from the catheter tip and peripheral blood culture), which account for up to 30% of cases, and primary BSIs, which account for around 35% of cases., 10 VAP, a common complication when mechanical ventilation is needed, is bacteremia in about 15% of cases and is the most common source of secondary bacteremia in critically ill patients" (Ammerlaan, 2013).

Early discovery and handling of BSI improves patient outcomes. Patients in the study had 23% BSIs. Other Indian studies using routine microbiological blood culture had varying bacterial isolation rates.

Our BSI findings are similar to Prabhu *et al.,* (2010), (20.9%), Thaden *et al.,* (2017) reported (20.02 %). Cohen *et al.,* (2015) reported (10.16%) and Carey *et al.,* (2018) (16.5%) found fewer BSIs. Difference in BSI rates between studies may be due to "blood culture volume, culture system, medium formulation, and patient type."

Thaden *et al.,* (2017) discussed about patients are given broad-spectrum antibiotics before coming to "tertiary care hospitals in India," the rate of isolation of BSI pathogens varies such as "Acinetobacter species, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *S. aureus*, MRSA, Enterococci, Coagulase negative staphylococci (CoNS), and alpha-hemolytic Streptococci" (Thaden *et al.,* 2017).

Table.1 Clinical and demographic characteristics of patients with suspected BSI

Characteristics	Patients' No.	(%)
Age gps (yrs.)		
19 to 40	35	19.00%
41 to 60	68	38.00%
More than 60	80	44.00%
Gender-Male (M) & Female (F)		
M	109	60.00%
F	72	40.00%
Blood culture report		
+ve	42	23.00%
-ve	139	77.00%

Table.2 Primary medical diagnosis for bacteremia source

Primary diagnosis Cases In%	Patients' No.	%
Infection in Respiratory System	53	29.00%
Genitourinary infection	39	22.00%
Metabolic disorders	22	12.00%
Infection in Cardiovascular system	16	9.00%
Gastrointestinal infection	13	7.00%
Central nervous system infections	12	7.00%
Skin and soft tissue infections	10	6.00%
Immunosuppressive conditions	7	4.00%
Autoimmune disorders	5	3.00%
Nonspecific	3	2.00%

Table.3 Organisms distribution in positive samples

Organism	Patients' No.	%
Gram positive		
Staphylococcus aureus	15	36.00%
Methicillin-resistant *Staphylococcus aureus*	8	19.00%
Staphylococcus epidermidis	2	5.00%
Staphylococcus hominis	1	2.00%
Enterococcus faecalis	2	5.00%
Gram Negative		
Escherichia coli	5	12.00%
Klebsiella pneumoniae	4	10.00%
Pseudomonas aeruginosa	3	7.00%
Acinetobacter baumannii	2	5.00%
Acinetobacter lwoffii	1	2.00%
Enterobacter cloacae	1	2.00%
Fungi		
Candida glabrata	1	2.00%
Candida tropicalis	1	2.00%

Table.4 Gram-positive bacteria's antibiotic susceptibility pattern

Antibiotics	MRSA (n=8)	*Staphylococcus aureus* (n=15)	Enterococcus (n=2)
Cefoxitin	8 (100 %) R	15 (100%)	2 (100%)
Linezolid	8 (100 %)	15 (100%)	2 (100%)
Vancomycin	8 (100%)	15 (100%)	2 (100%)
Gentamicin	7 (88 %)	11 (73 %)	1 (50%)
Ciprofloxacin	3 (38 %)	4 (27 %)	
Clindamycin	3 (38 %)	4 (27 %)	
Erythromycin	5 (63 %)	4 (27 %)	1 (50%)
Ampicillin			1 (50%)
Doxycycline	6 (75 %)	4 (27 %)	1 (50%)
Tetracycline	3 (38 %)	4 (27 %)	2 (100%)

Table.5 Gram-negative bacteria antibiotic susceptibility pattern

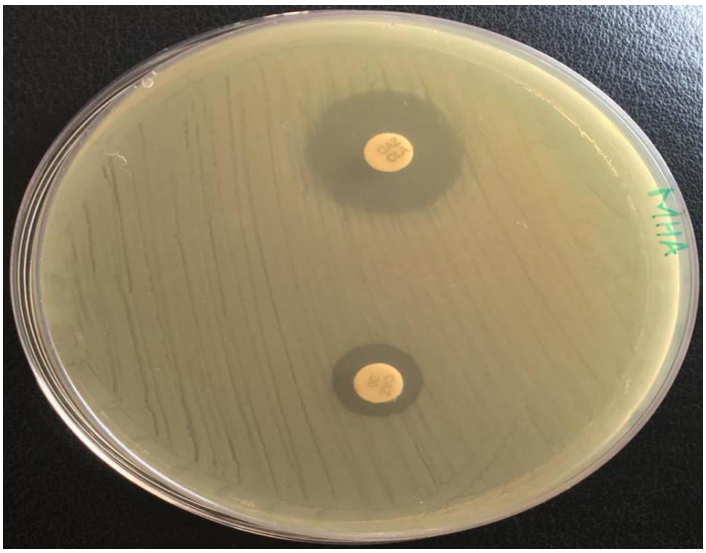
Antibiotics	Sensitivity			
	Escherichia coli (n=5)	Pseudomonas (n=3)	Klebsiella (n=4)	Acinetobacter (n=1)
Imipenem	5 (100%)	3 (100%)	4(100%)	1(100%)
Ertapenem	5(100%)	3 (100%)	4(100%)	1(100%)
Piperacillin/Tazobactum	5(100%)	3 (100%)	4(100%)	1 (100%)
Gentamicin	5(100%)	3 (100%)	4 (100%)	1(100%)
Levofloxacin	*	2 (67%)	2 (50%)	1(100%)
Cefuxorime sodium	*	2 (67%)	3 (75%)	*
Ciprofloxacin	*	1 (33%)	1 (25%)	*
Cefotaxime	*	1 (33%)	1 (25%)	*
Ampicillin	*	1 (33%)	1 (25%)	*
Erythromycin	5(100%)	1(33%)	1 (25%)	*
Cotrimoxazole	*	1 (33%)	1 (25%)	*
Meropenem	5(100%)	2(67%)	4(100%)	*
Amikacin	5(100%)	2(67%)	3(75%)	1(100%)
Azithromycin	*	2(67%)	1 (25%)	*
Cefepime	*	2(67%)	1 (25%)	*
Doxycycline	*	1 (33%)	1 (25%)	1(100%)
Amoxicillin/Clavulanic acid	*	2(67%)	1(25%)	*
Ceftazidime	1(20%)	2(67%)	3(75%)	1(100%)
Netilimicin	*	2(67%)	1(25%)	*
Aztreonam	1(20%)	1 (33%)	1 (25%)	*

(# Resistant-*)

Table.6 Analysis of risk factors associated with ESBL-producers

BSI's Epidemiological category (no. of isolates)					No. In (%) of isolates displaying resilient to the below-mentioned antimicrobial				
	AMC	TZP	IPM	MEM	AMK	GM	CIP	LEV	Less than 3 DRUGS
Nosocomial	36 (33.0)	20 (18.3)				37 (33.9)	100 (91.7)	100 (91.7)	48 (44.0)
Health Care Associated	3 (16.7)						5 (27.8)	17 (94.4)	7 (38.9)
Community acquired							one	2	one

Figure.1 Phenotypic confirmation by double disc diffusion test — showing an increase in zone size of >5 mm for ceftazidime-clavulanic acid



The isolation rate was 32.2 %in a study by Prabhu *et al.,* (2010). "*S. aureus* was the most common (42.23%), followed by *E. coli* (16.77%), CONS (14.90%), Klebsiella spp (11.20%), Pseudomonas (6.83%), Proteus spp (4.97%), and Citrobacter spp (4.97%). (3.10 %)" (Prabhu *et al.,* 2010).

According to Berenholtz (2004), Candida BSI are a global health problem. Candidemia is common in hospitalized patients exposed to antibiotics, immunosuppressive therapy, parenteral nutrition, and invasive medical procedures (Berenholtz, 2004).

Ammerlaan (2013) "Overall 12% of study participants had BSI due to MDR pathogens. Male sex, age 60, previous antimicrobial therapy, liver disease, and *K. pneumonia* bacteremia were independent MDR infection risk factors (Ammerlaan, 2013)". This study analysis confirms that initial treatment for "ESBL-producing *E. coli* and *Klebseilla pneumoniae* BSI" is often inadequate. In all of our cases, observed treatment had begun instantly after the "index culture," but 43.4% of patients received ineffective drugs.

The CLSI recommends a two-step method, the first step being a screening test for reduced susceptibility to more than one of the indicator cephalosporins (cefotaxime, ceftriaxone, ceftazidime, cefpodoxime and aztreonam). Reduced susceptibility indicates a positive result. A subsequent confirmation of ESBL production is then given by the demonstration of synergy between ceftazidime or cefotaxime and clavulanate. The presence of an ESBL is confirmed in *E. coli*, *Proteus mirabilis, K. pneumoniae* or *K. oxytoca* if: (a) the MIC values in the

presence of clavulanate are reduced by at least three two-fold dilutions; or (b) the diameter of the inhibition zone is increased by at least 5 mm when the tested cephalosporin is combined with clavulanate (CLSI, 2005).

Due to limited resources available we were able to screen ESBL by the two step method with results showing (80%) of the tested *Escherichia coli* (5) and 2 (50%) of the tested *Klebseilla pneumoniae* (4) were ESBL-producers which was consistent with Shakya *et al.,* (2017).

Other studies Gilbert *et al.,* (2013), BSI caused by "ESBL-producing organisms" found even higher rates (66%). In our country, illogical use of antibiotics, OTC accessibility of higher disinfectant agents, higher infection prevalence, and poor antibiotic vulnerability surveillance in medical centers may be the main causes of antimicrobial resistance.

BSIs increase patient suffering, costs, and sepsis. Rise of "Gram-negative organisms" that is resilient to multiple drugs is alarming; additional research is needed to develop treatment and preventive strategies.

BSI are a main reason of sickness and demise in critically ill patients. For effective management and prevention of drug resistance, reasonable antibiotic use, establishment of antibiotic policy, and timely BSI therapy are necessary. Finally, any delay in initiating appropriate antibiotic therapy has the potential to be fatal.

Even when the "MICs fall within the susceptible range (8 g/ml)," microbiology laboratories play a significant part in finding "ESBL producers," as bad results have been seen when serious infections with "ESBL-producing organisms" are given with "oxyimino cephalosporins" and "aztreonam." Over expression of a "chromosomally encoded AmpC enzyme" or acquisition of "plasmid-encoded AmpC enzymes" in "ESBL-producing organisms" is also a concern, as the "CLSI ESBL confirmatory tests" may miss "ESBLs" in these isolates. This could lead to cefepime therapy that isn't needed.

Author Contribution

Rajni Bharti: Investigation, formal analysis, writing—original draft. Harman Multani: Validation, methodology, writing—reviewing. Deep Shikha:—Formal analysis, writing—review and editing. Shashi Sudhan Sharma: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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