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Research Article

PHENOTYPIC DETECTION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCING GRAM-NEGATIVE BACILLI

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Abstract

Background: Globally, antimicrobial resistance emerges as one of the major public health threats. Excessive or inappropriate use of antimicrobials in healthcare settings over the last few decades has been a contributing factor in the emergence of drug resistance in all bacterial species. The prevalence of extended-spectrum beta-lactamases (ESBL) producing Gram-negative bacteria (GNB) is on the rise in both community and health care settings.

Aim: To isolate the ESBL-producing Gram-negative organisms from clinical samples and to evaluate their frequency and antimicrobial susceptibility pattern.

Methods: A total of 100 pus samples were collected and tested for ESBL-producing Gram-negative bacilli using phenotypic screening and confirmatory methods.

Results: Out of the 100 samples, 81 had bacterial growth, with 21 (26%) and 60 (84%) of Gram-positive cocci (GPC) and Gram-negative bacilli (GNB), respectively. Of the 60 GNB, 34 (56.6%) isolates were found to be ESBL producers by both screening and confirmatory tests.

Conclusion: The prevalence of ESBL producers in the current study is 34 (56.6%) among the 60 GNB isolates. These organisms can lead to cross-infections among the hospitalized patients, therefore, it is important to have strict infection control measures in the health care settings to contain such infections and prevent their spread to the community.

Key words: Confirmatory test, *Escherichia coli*, Extended-spectrum beta-lactamases, *Klebsiella,* Screening test.

INTRODUCTION

Globally, antimicrobial resistance emerges as one of the major public health threats. Excessive or inappropriate use of antimicrobials in healthcare settings over the last few decades has been a contributing factor in the emergence of drug resistance in all bacterial species [1]. Multidrug-resistant bacterial infections narrowed the antimicrobial choice for the infection management.

Due to persistent exposure to various β-lactam antibiotics, there is a constant production and mutation of β-lactamases, which render the bacteria resistant to the novel β-lactam antibiotics. Therefore, they are called extended-spectrum beta-lactamases (ESBL) producing bacteria [2].

The prevalence of ESBL-producing Gram-negative bacteria (GNB) is on the rise in both community and health care settings. Major classes of antibiotics like penicillin, extended-spectrum cephalosporin, and aztreonam are hydrolyzed by the ESBL enzymes [3].

It is important to identify the ESBL-producing Gram-negative organisms to initiate effective antibiotics, to prevent poor clinical outcomes, to avoid prolonged hospitalizations, and to establish infection control practices promptly [4].

AIM AND OBJECTIVE

To isolate the ESBL-producing Gram-negative organisms from clinical samples and to evaluate their frequency and antimicrobial susceptibility pattern.

MATERIALS AND METHODS

PLACE OF STUDY

This cross-sectional prospective study was done at the Department of Microbiology ofa tertiary care hospital, for a period of one year after obtaining approval from the institutional research ethics committee (TIREC: 1900/Micro/2021, dated 05.03.2021).

METHODOLOGY

A total of 100 pus samples were collected from the patients admitted in the Department of General Surgery and Orthopaedics. Pus samples were collected using a disposable syringe or sterile swabs under strict aseptic precautions and were transported immediately to the laboratory for further processing. Samples were processed at the Microbiology laboratory using conventional methods. The Gram-negative bacilli isolated from the clinical specimens were subjected to antibiotic susceptibility testing using Muller Hinton agar by Kirby Bauer's disk diffusion method, and interpretations were made. The isolates were tested for ESBL production by phenotypic methods as per CLSI 2022 guidelines.

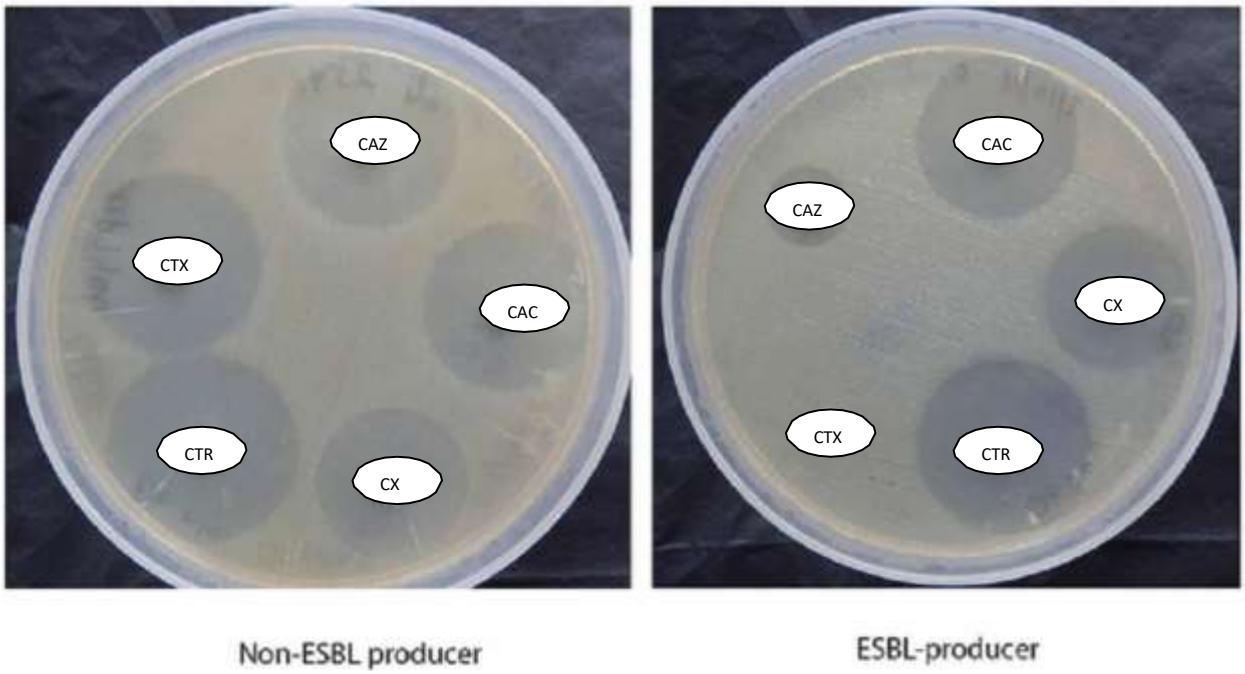
The following antibiotics were used for sensitivity testing of Gram-negative bacilli: amikacin (30µg), ciprofloxacin (5µg), ampicillin (10µg), ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg), cefepime (30µg), cefoxitin (30µg) and imipenem (10µg).

Phenotypic detection of ESBL production:

Screening test:

Screening test for ESBL production was done by disc diffusion test using ceftazidime (30µg), cefotaxime (30µg) and ceftriaxone (30µg) discs. The diameters of the zone of inhibition around the disks were measured and interpreted according to CLSI 2022 guidelines.[Figure 1].

Figure 1- Screening test for ESBL detection



Confirmatory test:

Isolates that were considered to be positive for ESBL production by the screening test were subjected to the phenotypic confirmatory test by combined disk diffusion test using ceftazidime (30µg)(CAZ) and ceftazidime + clavulanic acid (30/10µg) (CAC) discs. The diameters of the zone of inhibition around the disks were measured. Isolates showing an increase in the zone diameter by ≥5 mm around the disks containing ceftazidime + clavulanic acid over the disks containing ceftazidime alone were confirmed to be phenotypic confirmatory test positive.[Figure2]

Figure 2- Confirmatory test for ESBL detection (Combined disk diffusion):

Statistical analysis:

Data were entered in Microsoft excel data sheet and were analyzed using SPSS version 23.0 (IBM Corporation, Armonk, NY).Continuous variables are expressed as Mean and Standard deviation. Categorical and continuous variables are analysed with the Chi-square test and *t*-test, respectively.

OBSERVATION AND RESULTS

This study included pus samples and wound swabs from the patients admitted to the Department of General Surgery and Orthopaedics. A total of 86 pus samples and 14 wound swabs were collected. Of the 100 samples collected, males and females were 62 (62%) and 38 (38%), respectively. The most common age group was 31-40 years, 43 (43%), followed by the 41-50 years 31 (31%) age group. Among the 100 samples, 81 had bacterial growth, with 21 (26%), and 60 (74%) of Gram-positive cocci (GPC) and Gram-negative bacilli (GNB), respectively.

All 60 Gram-negative bacilli were subjected to ESBL screening test. Of the 60, 34 (56.6%) isolates were found to be ESBL-producing organisms by both screening and confirmatory tests. [Table 1]. These organisms were common among the age group of 31-40 years, 17 (50%) in the study, with a male preponderance, 21 (62%).

Table 1: ESBL production among Gram-negative bacilli

Gram negative bacilli	No. of isolates tested for ESBL production (n=60)		Phenotypic detection of ESBL production			
			Screening test		Confirmatory test	
	No.	%	No.	%	No.	%
E.coli	7	11.6	6	10	6	10
Klebsiella	36	60	19	31.6	19	31.6
Proteus	17	28.4	9	15	9	15
Total	**60**	**100**	**34**	**56.6**	**34**	**56.6**
ESBL: Extended spectrum beta lactamases						

In the present study, *Klebsiella* species were the most common ESBL producers with 19 (55.9%) isolates, followed by *Proteus* species *9* (26.5%) and *Escherichia coli* 6 (17.6%). [Table 1] More than 60% of ESBL-producers were sensitive to Amikacin and Imipenem, proving that these drugs continue to be effective against ESBL producers. [Table 2]

Table 2: Sensitivity pattern of the ESBL producers

Antibiotics	*Klebsiella* n=19 (%)	*Proteus* n=9 (%)	*E.coli* n=6 (%)	Total n=34 (%)
Amikacin (30µg)	10 (52.6)	5 (55.6)	5 (83.3)	20 (59)
Gentamicin (5µg)	7 (36.8)	3 (33.3)	3 (50)	13 (38)
Ciprofloxacin (5µg)	5 (26.3)	6 (66.7)	1 (16.7)	12 (35)
Ampicillin (10µg)	0 (0)	0 (0)	0 (0)	0 (0)
Imipenem (10µg)	13 (68.4)	9 (100)	5 (83.3)	27 (79.5)
Cefoxitin (30µg)	19 (100)	9 (100)	6 (100)	34 (100)
Cefepime (30µg)	0 (0)	0 (0)	0 (0)	0 (0)

DISCUSSION

Multidrug-resistant Gram-negative bacilli (MDR GNB) are globally accountable for fatal infections. Infections by ESBL-producing organisms than non-ESBL organisms are reported to have higher mortality rates [3]. Therefore, it is important to identify the resistance pattern of such MDR GNB organisms in order to aid in the appropriate usage of antibiotics and prevent the further emergence of resistant strains.

Various mechanisms, such as active removal of the antibiotic from the cell, enzymatic modification rendering the antibiotic ineffective, modification of the antibiotic's target site, and decreased uptake due to a change in membrane permeability, lead to bacterial resistance to antibiotics. The primary mechanism accounts for the enzymatic inactivation of antibiotics, and β-lactamase enzymes are the most clinically significant example [1].

Mutations in the amino acid arrangement of the genes (TEM, SHV and CTX-M) surrounding the β-lactamase enzyme are responsible for narrow-spectrum β-lactamases production. These enzymes are plasmid-encoded and are easily transferred between bacterial species [5].

The prevalence of ESBL among the Enterobacterales varies between regions, depending on the contributing factors, such as the types and duration of antibiotic usage, duration of hospitalization, and instrumentation [5].

Over the last decade, strains expressing CTX-M ESBL have emerged in many nations, despite knowing that TEM and SHV variants of ESBL are more prevalent. *Klebsiella* species and *Escherichia coli*, as well as other Enterobacterales like *Enterobacter* spp, *Proteus* spp, *Salmonella* spp., *Citrobacter* spp., *Morganella* spp., *Serratia* spp., and *Providencia* spp., are the main sources of ESBLs. GNB that produce ESBLs are responsible for longer hospitalization stays, increased use of expensive antibiotics such as carbapenems, thereby increasing the health care expenses [3].

Latin America reported a high rate of ESBL (44.9%), while other countries like Greece, Portugal, Germany and the Netherlands reported 27.4%, 15.5%, 2.6% and 2%, respectively. The Surveillance of Antimicrobial Resistance in India in 2004-2006 reported 88% of ESBL prevalence in India. This is due to the deliberate and widespread use of third-generation cephalosporins and the over-the-counter availability of antibiotics [6].

In the present study, out of the 100 pus samples collected, 60 (60%) were GNB, of which 34 (56.6%) were ESBL-producers. ESBL-producing organisms were higher in the age group of 31-40 years, 17 (50%), and were commonly observed in males, 21 (62%), compared to females, 13 (38%).

High ESBL-producing GNB in the study were *Klebsiella* spp 19 (55.9%), followed by *Proteus* spp 9 (26.5%). Few studies stated that Klebsiella spp were high ESBL-producers, with 51.6%and 63% [7, 8]. It is discordant with a study by Aiesh *et al*, where *E. coli* is more common among the ESBL-producers [2].

In this study, ESBL-producing organisms were 80%, and 59% sensitive to imipenem and amikacin, respectively. Few studies by Madhavi B*et al*[9] and Dalela*et al*[10] observed 91% and 98.5% sensitivity to Imipenem among the ESBL-producing GNB. Also 64.5% sensitivity to amikacin was reported by Dalela *et al*[10].

The limitations of the study included a single-centric study, a small sample size,

and genotypic detection of ESBLs was not performed.

CONCLUSION

The prevalence of ESBL producers in the current study is 34 (56.6%) among the 60 GNB isolates. *Klebsiella* spp 19 (55.9%) was the most common ESBL-producing organisms. More than 60% of the ESBL-producing GNB were sensitive to both amikacin and imipenem. In the hospitalized patients, these organisms may cause cross infections, therefore, it is important to have strict infection control measures in the health care settings to contain MDR GNB infections and prevent their spread to the community.

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