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Original Research Paper

Microbiology

PROPORTION OF ESBL AND CARBAPENEMASE PRODUCING ENTEROBACTERIACEAE CAUSING BLOODSTREAM INFECTIONS AND SHOWING MULTI DRUG RESISTANCE IN INFECTED PATIENTS IN TERTIARY CARE HOSPITAL AT AGARTALA, TRIPURA

Lija Ghosh	Department of Microbiology, Agartala govt. Medical college, Agartala, Tripura, India.
Arun B	Department of Microbiology, Kannur University Kerala,India.
Puja Ghosh	Department of Microbiology, Agartala govt. Medical college, Agartala, Tripura, India.
Tapan Majumdar	Department of Microbiology, Agartala govt. Medical college, Agartala, Tripura, India.

ABSTRACT

BACKGROUND: Enrerobacteriaceae species are the major leading cause of bloodstream infections in many developing countries. Moreover, ESBL and Carbapenemase-producing Enterobacteriaceae species are often associated with high resistance to a wide class of antibiotics. There are few studies regarding the bloodstream infections causing by Enterobacteriaceae with the production of Extended-spectrum β-lactamases and Carbapenemase enzymes in Tripura, North-East India. This study aimed to determine the " proportion of ESBL and carbapenemase-producing Enterobacteriaceae causing bloodstream infection and showing Multi-drug resistance (MDR) in infected patients" in Tertiary care Hospital at Agartala, Tripura.

METHOD: Blood samples were collected from all indoor and outdoor patients suspected of Bloodstream infection. Then specimens were inoculated in various culture media after that with this culture isolates we performed gram staining and many biochemical tests (as per CLSI guidelines) to identifies the Enterobacteriaceae species. And the production of β-lactamases and Carbapenemase was confirmed by the combined disk test and Modified Hodge method.

RESULTS: In this study out of 760 samples processed in the laboratory 77 (10.13%) was tested positive for bacteremia from which 42 (54.54%) blood specimens were infected by Enterobacteriaceae. The members of the Enterobacteriaceae family isolated in patient samples are *E.coli* (22/42, 52.38%), *K. pneumoniae* (11/42, 52.38%) others are *Enterobacter* spp. (8/42, 52.38%) and *S.typhi* (1/42, 2.38%).

In all 42 Enterobacteriaceae species, 17 (40.47%) isolates were found ESBL positive on antibiotic screening which is confirmed by Combined disc diffusion test, and out of 17 Beta-lactamase producers 8 (47.05%) were *E. coli*, 5 (29.41%) were *K. pneumoniae* and 4 (23.52%) were *Enterobacter* spp.

And among 42 isolates of Enterobacteriaceae 16 (38.09%) isolates showed Carbapenemase producers, in that 8 (50%) were *E.coli*, 5 (31.25%) were *K. pneumoniae*, and 3 (18.75%) were *Enterobacter* spp.

CONCLUSION: This study aims to provide an early, rapid, and effective phenotypic method for identifying Multi-drug resistant (MDR) Bloodstream infections (BSIs) causing by the species of the Enterobacteriaceae family.

KEYWORDS : Enterobacteriaceae, ESBL, Carbapenemase, MDR, CLSI,SME, KPC, IMI.

INTRODUCTION –

The incidence of bloodstream infections is increasing day by day and becoming a high rate cause of the occurrence of infectious diseases in about 30 million people and 6 million deaths worldwide [1]. Bloodstream infections with multidrug-resistance bacterial strains are 41.7% mainly due to inappropriate antibiotic therapy [2]. Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs) have emerging a serious pathogen in hospitals as well as in communities and represent a major worldwide threat and contribute to morbidity and mortality among patients [4,5].in India ESBL producing *E.coli* increased from 40% in2002 to 61% in 2009 [3]. The ability of pathogenic bacteria to become resistant to the broad spectrum of antibiotics is a growing public health problem, and causing the initiation of major health concerns nowadays, which is continuously affecting the treatment outcome of Bloodstream infections (BSI) in hospitals.

Most of ESBLs are derived from broad-spectrum Beta-lactamases enzymes are TEM-1, TEM-2, and SHV-1 cause enzymatic hydrolysis of Beta-lactam drugs and shows resistance to oxyimino-cephalosporins and aztreonam [7]. On the other hand, Carbapenemase-producing Enterobacteriaceae can hydrolyze penicillins, Cephalosporins, monobactams, and carbapenems. These carbapenemase producers cause chronic bloodstream infections by inactivating the bactericidal ability of Beta-lactam drugs. Carbapenemase is a member of molecular class A, B, and D Beta-lactamases. Among these class, A & D enzymes have a serine-based hydrolytic mechanism, and class B enzymes are Metallo-Beta-lactamases that contain zinc in the active site. The class A carbapenemase group contains SME, IMI, NMC, GES & KPC enzymes, KPC-producing by *K. pneumonia*-causing BSIs very frequently [15].

METHODS –

This is a hospital-based cross-sectional study, was carried out in the Department of Microbiology of Agartala Government Medical College, Agartala, Tripura for a period of 3 months from Oct 2020 to Dec 2020. A total of 760 blood samples were processed during this study period, 77 samples were culture positive, out of which 42 belonged to the Enterobacteriaceae family which were included in this study.

SAMPLE PROCESSING -

- A continuous sampling method was used in this study.
- The blood samples obtained from patients were subjected to culture on blood agar, McConkey agar plates by quadrant streaking methods, the plates were then incubated at 37◦C for 18-24 h and observed for growth.
- Microscopic identification of bacterial isolates by gram staining.
- Biochemical identification.
- Antimicrobial susceptibility test for the detection of ESBL and Carbapenemase enzymes.

ANTIBIOTIC SENSITIVITY TEST (AST) BY KIRBY-BAUER DISK DIFFUSION METHOD

Isolated pure colonies of the test organism were inoculated in

a suitable liquid medium (peptone water broth) and incubated at 35-37◦C for 4-6 h. The density of the organism in broth is adjusted to approximately 1.5×108CFU/ml by comparing its turbidity with that 0.5 MacFarland opacity standard tube. Then inoculated on Mueller-Hinton agar by a sterile cotton swab and antibiotic disks were placed on plates. The plates were incubated at 37◦C for 18-24 h. after incubation the results were interpreted according to the CLSI guidelines [8].

DETERMINATION OF THE PRODUCTION OF EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) AND CARBAPENEMASE BY TEST ORGANISMS

Detection of ESBL was done by two major techniques –

- **Initial screening test** – Initial screening for ESBL was done based on diameters of zone of inhibition produced by test organisms. This test was performed with Ceftazidime, cefepime, and cefoperazone/sulbactam to observe the zone of inhibition diameters produced by test organisms according to CLSI standard[8].
- **Phenotypic ESBL confirmation test** – After the screening, ESBL enzyme production was confirmed by the combined disc test (CDT).

  Detection of Carbapenemase was done by using a Modified Hodge test (MHT).
- **Initial screening test** – This test was done based on the zone of inhibition size in diameters by test organisms against the Carbapenem drugs (Imipenem (10μg), Meropenem (10μg), Doripenem (10μg) according to the CLSI guidelines[8].
- **Phenotypic Carbapenemase production test** – Detection of Carbapenemase was done with the help of the Modified Hodge method or Cloverleaf test.

OBSERVATION & RESULTS

In this study total, 760 samples were processed in the laboratory, out of these 77(10.13%) were tested positive for bacteremia, and from these 42(54.54%) blood samples were infected by Enterobacteriaceae which were included in this study.

Among Enterobacteriaceae isolated, the most frequent isolates were *E.coli* (22/42, 52.38%), *K.pneumoniae* (11/42, 26.19%) others are *Enterobacter* spp. (8/42,19.04%) and *S.Typhi* (1/42,2.38%).

Out of 42 patients, the most common age group affected with bacteremia was 1-20 yrs. of age. A total of 13 (30.95%) patients were present in this group.

Out of 42 Enterobacteriaceae isolates, 16(38.09%) were isolated from males and 26 (61.90%) were isolated from females.

Antibiotic sensitivity test by Kirby-Bauer disk diffusion method

Among 42 Enterobacteriaceae were included in this study, 17(40.47%) isolates were found ESBL positive on screening, which was confirmed by combined disk diffusion test and 25(59.52%) strains were non-ESBL producers. And out of 17 ESBL producers, 8(47.05%) were *E.coli*, 5(29.41%) were *K.pneumoniae*, and 4 (23.52%) were *Enterobacter* spp.

Among 42 isolates of Enterobacteriaceae 16 (38.09%) isolates showed Carbapenemase producers, in that 8(50%) were E.coli, 5(31.25%) were *K.pneumoniae*, and 3(18.75%) were *Enterobacter*.

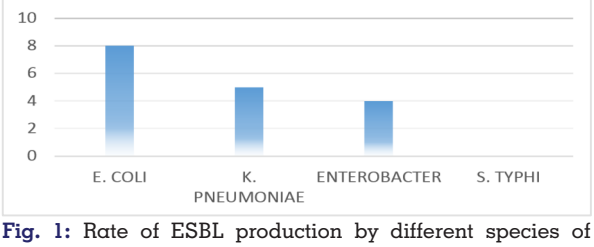

Fig. 1: Rate of ESBL production by different species of Enterobacteriaceae.

Table no.1

Age group	ESBL Producers n = 17		Non ESBL Producers n = 25		Total no. of patients
	Male	Female	Male	Female	42
0 – 20 Y	1	2	4	3	10
21 – 40 Y	1	2	2	5	10
41 – 60 Y	3	4	3	2	12
61 – 80 Y	2	2	2	4	10

Here the percentage of ESBL producers among all Enterobacteriaceae isolates are 40.47%, and highest rate of ESBL production seen in *E.coli*. Most of ESBL producing strains were isolated from Females (58.8%) as compare to Males (41.17%) and the age group 41 to 60 years were mostly affected by ESBL producing strains.

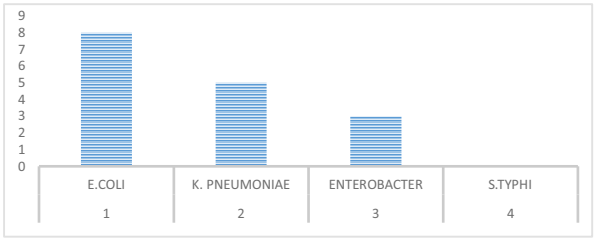

Fig. 7: Rate of Carbapenemase production by different species of Enterobacteriaceae.

Table no. 2

AGE GROUP	Carbapenemase producers n = 16		Non Carbapenemase producers n = 26		Total no. of patients
	Male	Female	Male	Female	42
0 – 20 Y	2	1	3	6	12
21 – 40 Y	2	2	6	2	12
41 – 60 Y	2	3	3	2	10
61 – 80 Y	1	3	3	1	8

In this observation, the rate of Carbapenemase production was 38.57% and carbapenemase production was high in *E.coli*. Females(56.25%) were more infected by these Carbapenemase producers in contrast to males (43.75%).

DISCUSSION:

Bloodstream infections are implicated as one of the severe public health problems that impose high morbidity and mortality among patients [9].

In our research, we analyzed the rate of bloodstream infection in this specific geographical area is 10.13%, among them a percentage of Enterobacteriaceae associated Bloodstream infections are 54.54% which is correlated with the study of Surbhi Khurana and Nidhi Bhardwaj et al., who found that Gram-negative bacterial infection was high(82%) than Gram-positive bacteria(18%)and Enterobacteriaceae(52-57%) were the most commonly isolated organism and also showed very high levels of antimicrobial resistance during their study period(2013-2016) in Northern India [10].

Daniel Z Uslan et al. have studied that, a total of 1051 patients with positive blood cultures results were identified, among the most common organism identified were *E.coli* and the incidence rate of Bloodstream infections is higher in females than males [11]. which is also seen in this present study to a certain extent, here we got among all Enterobacteriaceae species *E.coli* is accounting highest rate (52.38%) causing BSIs after that *K.pneumoniae* (26.19%) is the 2nd most important organism causing BSIs. And females (61.90%) are getting more infected than males (38.09%).

According to our study 0 – 20 years, age group is getting more infected with BSIs as compared to other age groups, here out

of 42 Enterobacteriaceae isolates a total of 13 (30.95%) isolates were belong to this age group. Maybe it's due to the lowered immune response in newborns, children, and young adults because of their lifestyle in rural areas (community-acquired infection), early pregnancy in females, or prolonged hospital stay (nosocomial infection). And increased production of ESBL and carbapenemase was seen in the 41-60 years of age group. There was another research conducted in Northern Vietnam by Vu Quoc Dat et al. BMC Infect Dis. 2017 concludes that BSIs in ESBL by Enterobacteriaceae were high in the 36-60 years of age group which resembles my research paper [16].

In this study, we've analyzed that *E.coli* topped in ESBL (47.05%) and carbapenemase(28.57%)production among all members of Enterobacteriaceae which is similar to the study of Aishwarya Govindaswamy et al., they found that ESBLs, Amp C, and carbapenemase production was high among the *E.coli* isolates from a tertiary care hospital India [12].

Pravin K Nair et al. have stated that the resistance of carbapenemase-producing Enterobacteriaceae is becoming serious public health due to high mortality and mostly it is seen in hospitalized patients in India [13]. In his study, the prevalence rate of Carbapenem-resistant Enterobacteriaceae (CRE) was 12.26% but in our study, we got 28.57% of carbapenemase producers among all tests isolates which are comparatively high.

During the study of antibiotic sensitivity pattern of all test isolates, we conclude that Tigecycline drug is very efficacious against most MDR test strains especially *E.coli* and *K.pneumoniae* .W. R. Heizmann, P.-A. Loschmann et al., and Subhasree Roy et al., in 2013 May also conclude that Tigecycline alone or in combination achieved high clinical success rates in patients infected with MDR strains mainly in *E.coli* and *K.pneumoniae* [14].

The knowledge of the proportion of Enterobacteriaceae in Bloodstream infections and antibacterial sensitivity pattern of the bacterial strains in this geographical area will help in guiding an appropriate and judicious antibiotics use.

SUMMARY & CONCLUSION–

E.coli and *K. pneumonia* were the commonest agents responsible for bloodstream infection. The age group of 1 to 20 years was the most commonly affected with bloodstream infection. And the proportion of ESBL, carbapenemase-associated BSIs was higher in older patients. Females are most frequently affected with Bloodstream infection as compared to males. Tigecycline seems to be a responsible alternative to Cephalosporins for the treatment of Bloodstream infection. A higher rate of ESBL and Carbapenemase production was seen in *E.coli* followed by *K.pneumoniae* in this present study.

Our reports highlight that Bloodstream infection by the species of Enterobacteriaceae needs to strictly monitor in communities as well as in hospitals to prevent Nosocomial and Community-acquired Bloodstream infections in our societies.

ETHICAL APPROVAL

This study was approved by the institutional Ethics Committee.

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