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Detection of extended spectrum beta-lactamase producing organisms and its antibiotic resistance pattern among Enterobacteriaceae isolates in a teaching hospital of Eastern India

Soma Mondal [1, *], Avijit Mondal [2], Abhijit Basak [3]

[1] *Department of Microbiology, College of Medicine and JNM Hospital, Kalyani, Nadia, West Bengal, India*
[2] *Department of Physiology, College of Medicine and JNM Hospital, Kalyani, Nadia, West Bengal, India.*
[3] *Department of Immunology and Immunotoxicology, Labcorp Drug development, Bengaluru, India.*

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Abstract

Introduction: ESBL-producing Enterobacteriaceae have been responsible for innumerable outbreaks of infection throughout the world and are becoming a real challenge to control the spread of infection.

Aim To determine the prevalence of ESBL producing organisms among Enterobacteriaceae isolated from different clinical samples and to detect their antibiotic susceptibility patterns.

Material and Methods The study was conducted in the Department of Microbiology, College of Medicine and JNM hospital. A total of 241 samples showing pure growth of Enterobacteriaceae were included in the study. Identification followed by antibiotic susceptibility tests were performed according to CLSI guidelines. Organisms showing reduced susceptibility to third generation cephalosporins were further tested by double disc synergy test to detect ESBL production and those ESBL screening positive isolates were further tested by Phenotypic confirmatory test according to CLSI 2019 guidelines.

Results: Prevalence of ESBL producing Enterobacteriaceae was found to be 31.04% with the highest rate among E.*coli* (34.5%) followed by *K. pneumoniae* (27.6%). and *P. mirabilis* (24.2%) .All the ESBL isolates were sensitive to meropenem and imipenem. Nitrofurantoin (84.2%) was found to be most effective drug for ESBL isolates from urine samples. Among β-Lactam/ β-Lactam inhibitor drugs, amoxicillin/clavulanic acid was found to be less sensitive compared to cefoperazone/sulbactam (67%) . Most of the ESBL isolates were found to be multidrug resistant.

Conclusion: Screening of all the gram-negative organisms for ESBL detection and judicial use of antibiotics as per sensitivity report is utmost important to prevent the spread of resistance strains in the community as well as in hospital. Formulation of infection control committee in every hospital may help to combat the present situation.

Keywords: Enterobacteriaceae; ESBL; Antimicrobial susceptibility; Phenotypic confirmatory disc diffusion test.

1 Introduction

The Gram-negative bacilli belonging to the Enterobacteriaceae are the most frequently encountered bacterial isolates recovered from different clinical samples. During last few decades different resistant bacterial strains are emerging and it poses a great threat to mankind. Production of extended-spectrum β-lactamases (ESBLs) is one of the significant resistance mechanism that hinders the antimicrobial treatment of infections caused by Enterobacteriaceae and is a

* Corresponding author: Soma Mondal

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serious threat to the mankind[1]. Extended spectrum B- lactamases (ESBLs) are plasmid mediated, TEM and SHV derived enzymes, first isolated in Western Europe in mid 1980s, most commonly found in Klebsiella *spp*., followed by Escherichia *coli*. These enzymes are capable of hydrolyzing broad spectrum cephalosporins, penicillin and monobactams such as aztreonam but inactive against cephamycins and imipenem and are usually inhibited by beta-lactamase inhibitors such as clavulanic acid. It has primarily been detected in either *Klebsiella pneumoniae* or *Escherichia coli,* although ESBLs occur in other Enterobacteriaceae such as Enterobacter *spp*. and Citrobacter *spp.* but detection is more difficult because other types of β-lactamases, especially Amp C enzymes are commonly present in these genera[2]. Resistance to antibiotics can be developed either by mutations[3] or by the acquisition through horizontal gene transfer of resistance genes[4,5]. Inappropriate and widespread use of antibiotics has led to the emergence of drug resistance mechanisms in common pathogens. It is also seen that spread and the burden of ESBL-producing bacteria is becoming greater in the developing countries and are prevalent in low-income groups. Crowded hospitals, more extensive self-treatment and poorer hygiene in general and particularly in hospitals and less effective infection control measures all lead to development and spread of resistant bacterial strains[6,7] . Most of the ESBL producers remain undetected by routine antibiotic susceptibility tests. The Clinical and Laboratory Standards Institute (CLSI) has created guidelines for the minimum inhibitory concentration (MIC) and disk diffusion breakpoints for aztreonam, cefotaxime, cefpodoxime, ceftazidime, and ceftriaxone for *E. coli,* Proteus, and Klebsiella *spp*., as well as for cefpodoxime, ceftazidime, and cefotaxime for *P. mirabilis*[8] for detection of ESBL. The sensitivity of the screening test increases with the use of more than a single drug for detection of resistance. The present study was carried out with an objective to find out the prevalence rate of ESBL producing Enterobacteriaceae in clinical isolates and to find out the antibiotic resistant pattens of them. The knowledge would be helpful to frame the hospital antibiotic policy and thus plan for hospital infection control could be formulated to prevent the spread of infection to the mankind.

Aim and objective

To detect the prevalence of ESBL producing bacteria isolated from different clinical samples and to determine antibiotic susceptibility pattern of them.

2 Material and methods

The present study was conducted from September 2018 to April 2019 in the Department of Microbiology, College of Medicine and JNM Hospital, WBUHS, Kalyani, Nadia, after receiving the ethical clearance from the Institutional Ethical committee. During the study period different samples such as urine, pus and blood samples of patients from both outpatient and inpatient departments were tested for the growth of any organisms. Cultures showing pure growth of microorganisms were identified by Gram stain, motility test and other conventional biochemical tests. Samples showing pure growth of Enterobacteriaceae were included in the study and were tested for their antimicrobial susceptibility pattern on Muller- Hinton agar media using appropriate antibiotics according to CLSI guidelines and the data was meticulously noted and analyzed.

2.1 Phenotypic screening test for ESBL

The Enterobacteriaceae isolates were screened for ESBL production by Kirby-Bauer's disc diffusion method on Mueller-Hinton agar. On it bacterial isolates showing reduced susceptibility to cefpodoxime (10 μg), cefotaxime (30μg), ceftazidime (30 μg), ceftriaxone (30 μg) and aztreonam (30 μg) are considered to be screening positive for ESBLs[8].Cut-off zone sizes of used antibiotic discs on Mueller-Hinton Media as an indicator of ESBL producer were ≤ 27 mm for cefotaxime, ≤ 22 mm for ceftazidime, ≤ 25 mm for ceftriaxone, ≤ 27 mm for aztreonam and ≤ 17 mm for cefpodoxime[8]. The strains which showed a diameter of less than 25 mm for ceftriaxone and less than 27mm for cefotaxime were selected as screening positive. The screening positive isolates were further tested by phenotypic confirmatory disc diffusion test for ESBL detection.

2.2 Phenotypic confirmatory disc diffusion test (PCDDT) for ESBL detection: (fig 1)

The test was done by placing a disk of ceftazidime (30 μg) or cefotaxime (30 ug) alone and ceftazidime + clavulanic acid (30/10 μg) or cefotaxime + clavulanic acid (30/10 μg) on Mueller-Hinton agar plate at least 20 mm apart from the previously placed discs . After overnight incubation plates were examined. The zone diameter around ceftazidime + clavulanic acid or cefotaxime + clavulanic acid disc ≥ 5mm larger than that around ceftazidime or cefotaxime disk respectively was indicated as ESBL production. Antimicrobial susceptibility pattern of *E. coli,* Klebsiella, and Proteus was determined for various samples namely urine, blood and pus was analyzed accordingly. After collecting and completion of the data, it was put in MS excel sheet and was analyzed. Percentage and mean were calculated and for calculation of p value, Epi info 7 statistical calculator was used.

3 Results

A total of 306 clinical samples showing pure growth of Enterobacteriaceae were included in the present study .From them E.*coli* (54.9%) was found to be the most commonly isolated spp., followed by *K. pneumoniae* (34.3%) and *P. mirabilis* (10.7%) [Table 1]. From 306 clinical isolates 95 was detected as ESBL producers (31.04%), among them most commonly ESBL was detected from *E.coli* (34.5%), followed by *Klebsiella pneumoniae (*27.6%) and Proteus *mirabilis* (24.2%). [Table 2].

Table 1 Distribution of Enterobacteriaceae from different clinical samples

Samples	***E. coli***	***K. pneumoniae***	***P. mirabilis***
Urine (170)	124	34	12
Pus (102)	38	46	18
Blood (34)	6	25	3
Total (306)	168 (54.9%)	105 (34.3%)	33 (10.7%)

Table 2 Prevalence of ESBL producers among different Enterobacteriaceae

Organisms	**Total number of Isolates**	**ESBL producers**	**% of ESBL producers**
E. coli	168	58	34.5%
K. pneumoniae	105	29	27.6%
P. mirabilis	33	8	24.2%
Total	306	95	31.04%

Table 3 Antimicrobial susceptibility pattern of Urine isolates (n =170)

Name of Antibiotic disks	**Escherichia coli (n=124)**	***Klebsiella pneumonia*e (n=34)**	***P. mirabilis* (n=12)**
Amoxicillin/Clavulanic acid	46%	50.3%	66.7%
Piperacillin/Tazobactam	68%	79.2%	66.7%
Cefoperazone/Sulbactam	67%	75%	100%
Cefixime	31%	46.7%	33.3%
Cefuroxime	28%	39.7%	33.3%
Ceftazidime	33%	66.7%	66.7%
Cefotaxime	33%	66.7%	66.7%
Ceftriaxone	33%	66.7%	66.7%
Cefepime	38%	70.8%	100%
Imipenem	82%	83.3%	100%
Meropenem	82%	83.3%	100%
Ciprofloxacin	30%	79.2%	66.7%
Levofloxacin	28%	75%	66.7%
Gentamicin	41%	58.3%	66.7%

Amikacin	65%	66.7%	100%
Netilmicin	79%	75%	100%
Norfloxacin	47%	62.5%	66.7%
Nitrofurantoin	90%	62.5%	6.7%
Co-trimoxazole	46%	70.8%	26.5%

*Antibiotic sensitivity rate is expressed in percentage

In the present study ESBL was detected maximum in number from blood sample (34.7%), followed by urine (33.6%) and pus (31.5%**)**. ESBL positivity rate was slightly higher among indoor (36.2%) patients as compared to outdoor (26.7%) patients as seen in the present study. ESBL producing organisms were more commonly isolated from male patients (33.6 %) than that of female patients (29.7%). Antimicrobial susceptibility results of urine and pus isolates were summarized in Table 3 and Table 4 respectively.

The present study depicts that in the cases of UTI caused by *E. coli*, nitrofurantoin (90%) was most effective antibiotic followed by imipenem (82%), meropenem (82%), whereas in case of *K. pneumoniae*, imipenem and meropenem both were sensitive to 83.3% of the isolates. Ciprofloxacin (79.2%), levofloxacin (75%) and netilmicin (75%), third generation cephalosporins (66.7%), cefepime (70.8%), co-trimoxazole (70.8%) and nitrofurantoin (62.5%) were also found to have good sensitivity. All the isolates of *Proteus mirabilis* were sensitive to most of the above-mentioned drugs including piperacillin -tazobactum, cefoperazone -sulbactum and netilmicin. Imipenem, Meropenem.

Table 4 Antimicrobial susceptibility pattern of Pus isolates (n= 102)

Name of Antibiotic disks	***Escherichia coli* (n=38)**	***Klebsiella pneumonia* (n=46)**	***P. mirabilis* (n=18)**
Amoxicillin/Clavulanic acid	33.3%	28.9%	61.5%
Piperacillin/Tazobactam	35.9%	34.2%	69.2%
Cefoperazone/Sulbactam	48.7%	36.8%	69.2%
Cefixime	20.5%	20.5%	53.8%
Cefuroxime	17.9%	23.7%	53.8%
Ceftazidime	20.5%	26.3%	53.8%
Cefotaxime	20.5%	26.3%	46.2%
Ceftriaxone	20.5%	26.3%	53.8%
Cefepime	23.1%	31.6%	61.5%
Imipenem	69.2%	65.8%	92.3%
Meropenem	69.2%	65.8%	92.3%
Ciprofloxacin	17.9%	39.5%	61.5%
Levofloxacin	15.4%	36.8%	46.2%
Gentamicin	46.1%	39.5%	53.8%
Amikacin	56.4%	42.1%	61.5%
Netilmicin	66.7%	44.7%	69.2%
Co-trimoxazole	51.3%	31.6%	30.8%

*Antibiotic sensitivity rate is expressed in percentage

In the present study meropenem (69.2%), imipenem (69.2%), netilmicin (66.7%) had good sensitivity against *E.coli* isolated from pus samples but strains of *K. pneumoniae* were found to be multidrug resistant which showed good

sensitivity only to meropenem and imipenem (65.8%) Meropenem and imipenem as well as amikacin, netilmicin, piperacillin- tazobactum were also found to have good sensitivity rates for Proteus spp.

Meropenem (100%) and imipenem (100%) were found to be most useful whereas cefoperazone-sulbactum, levofloxacin, amikacin, netilmicin all showed approximately more than 70% sensitivity rate for E.coli isolates of blood. Similarly, *K. pneumoniae* isolated from blood showed good sensitivity to meropenem (94.4%) and imipenem (94.4%) again. In the present study only three *P. mirabilis* were isolated from blood and they were found to be resistant to most of the antimicrobial drugs except for meropenem, imipenem, and netilmicin.

Table 5 Comparison of sensitivity pattern in ESBL and Non ESBL isolates

Antibiotics	ESBL (n= 95)	Non ESBL(n = 211)
Amoxicillin/Clavulanic acid	37.7	43.47
Piperacillin/Tazobactam	59.4	62.8
Cefoperazone/Sulbactam	66.7	68.6
Ciprofloxacin	31.9	41
Levofloxacin	29	38.2
Gentamicin	34.8	46.8
Amikacin	59.4	60.2
Netilmicin	73.9	75.5
Co-trimoxazole	44.2	51.3
Norfloxacin*	36.8	55.1
Nitrofurantoin	84.2	85.3

*Norfloxacin p value - 0.030 using Epi info 7

4 Discussion

One of the major challenges to our health care system in recent times is rapidly emergence of antibiotic resistance to commonly used antibiotics. In this scenario the present study assessed the antibiotic susceptibility patterns of Enterobacteriaceae and the prevalence rate of ESBL among them. In India the prevalence of ESBL production varies greatly in different studies[9,10,11,12]. The present study depicts that the prevalence of ESBL producing Enterobacteriaceae is 31.04%. However high prevalence rate was seen in few other Indian studies held at different geographical areas like, Pune (78.8%), Jaipur (52.49%), and Bhopal (48.27%)[13,9,10]. One possible reason for such variation might be varying number of samples in different studies. No countrywide study has been conducted so far for detection of the prevalence of ESBL production in India. Individual studies were done in different parts of the country, which showed various prevalence rates. The probable reasons of gradual increase in ESBLs detection in various Indian studies might be the use of random and inappropriate use of third generation cephalosporins which contribute to the evolution of ESBLs[14] .

E.coli (54.9%) was the most common Enterobacteriaceae isolated from different clinical samples, the finding is quite parallel to few other studies[10,11,12,13]. ESBL production was seen highest among *E.coli* (34.5%) compared to *K. pneumoniae* (27.6%) and *P. mirabilis* (24.2%). However, the most common species presenting ESBL activity was *K. pneumoniae* (92%), followed by *E. coli* (87%), *K. oxytoca* (87%), *P. mirabilis* (80%) as seen in a study by Baguma et. al[15]. Another study from Serbia showed the frequency of ESBL production was highest in *Serratia spp*. (85.2%), followed by *Klebsiella spp.* (81.8%), *Proteus mirabilis* (70.6%), *Morganella morganii* (71.4%), and lowest in *Escherichia coli* (33.9%)[16].

In the present study, most frequent ESBL producers were isolated from blood samples (34.7%), followed by urine (33.6.%) and pus (31.5%). In some other studies, urine was found to be the major source of ESBL production[17,18], whereas it was found in a study by Vemula et al that blood isolates were the commonest source of ESBL producers [19] similar to the current study.

The present study most of the ESBL isolates was sensitive to meropenem and imipenem. Similar results were also obtained by some other studies[15,17,18,20]. For urine isolates, nitrofurantoin (90%) was most effective antibiotic against *E. coli* followed by carbapenems (82%). We also found that 75% sensitivity to nitrofurantoin among urinary ESBL isolates in a different study[13].

Here it was found that meropenem, imipenem, netilmicin were effective against most of the pus isolates. Antibiogram results from a study showed that *E. coli* was more resistant to amoxicillin-clavulanic acid and cephalosporins similar to present study, while being least resistant to amikacin, imipenem, gentamicin, and meropenem[21].High drug resistance was observed for amoxicillin–clavulanate (100%) and co-trimoxazole (100%), while amikacin (0%) was found to be very effective drug according to Kassam et al[22].

The present study revealed that for blood isolates meropenem and imipenem were most effective whereas among remaining beta-lactam/beta-lactam inhibitor drugs have shown 50% sensitivity rates and all the third generation cephalosporins were resistant. Among β-lactam/ β-lactam inhibitor drugs highest sensitivity was observed with cefoperazone/sulbactum in case of all isolates followed by piperacillin-tazobactum and high resistance was observed with amoxicillin/clavulanic acid. This accords with a study by Sharma. et al[9] but discords with their finding that piperacillin/ tazobactum in case of Klebsiella spp. which showed higher sensitivity than cefoperazone/sulbactum[9]. In most of the studies it was found that the choice of oral medication is quite limited for ESBL producers as many of them are multidrug resistant.

The prevalence of ESBL producers were higher in male (33.6%) compared to female (29.7%) patients. The findings is in agreement with the other studies where the relation of ESBL-producing Enterobacteriaceae with respect to age and gender was estimated and more ESBL-positive isolates in males [10,23] was found. This can be explained by the fact that self-medication practice is common in men however contrary results were also found in other studies[24,25] Here the frequency of ESBL producers were slightly higher in inpatients (36.2%) compared to outpatients (26.7%). Higher prevalence of ESBL-producing isolates from outpatients was also seen in a different study[26]. ESBL prevalence was significantly higher in isolates from inpatient as reported by Vemula et al [19] , where ESBL detection rate from hospital was very high (28%) compared to the detection from community (6%). While comparing the sensitivity pattern of different drugs for ESBL and non ESBL isolates it was seen that norfloxacin had better sensitivity rate for non ESBL isolates than ESBLs with a p value of 0.030, similar result was also found in a different study where we found norfloxacin having sensitivity of 50% for non ESBL isolates[13]

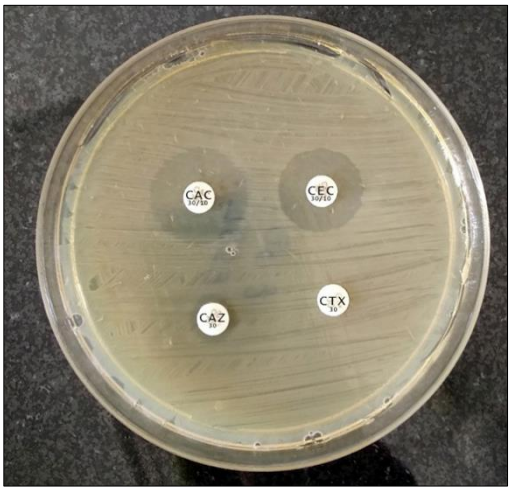

Figure 1 Phenotypic confirmatory disc diffusion test (PCDDT) for ESBL detection

CAZ- Ceftazidime, CAC- Ceftazidime-clavulanate, CTX- Cefotaxime, CEC- Cefotaxime -clavulanate

5 Conclusion

Prevalence of ESBL was found to be moderately high (31.04%) in the study population, Most of the isolates are sensitive to imipenem, meropenem, cefoperazone- sulbactum and piperacillin- tazobactum .Nitrofurantoin showed good sensitivity for the urinary pathogens and it was also noticed that norfloxacin had better role for non ESBL isolates . Most of the ESBL isolates showed reduced susceptibility to commonly available drugs so antibiotic susceptibility test as well

as ESBL screening must be done in every laboratory. A proper infection control measures should be undertaken to prevent the spread of resistant strain.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have no conflict of interest.

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