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PHENOTYPIC DETECTION OF EXTENDED SPECTRUM BETA LACTAMASE AND THEIR ANTIBIOTIC SENSITIVITY PATTERN IN VARIOUS CLINICAL ISOLATES OF ENTEROBACTERIACEAE

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ABSTRACT Extended-spectrum β-lactamases (ESBLs) are a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of hospitalized and community-based patients. Enterobacteriaceae group is the main cause of bacterial infection and in this family Escherichia coli and Klebsiella species are the most common causes of nosocomial infections. ESBLs represents a major threat among multidrug-resistant (MDR) bacteria isolates. These ESBL producing pathogens are now recognized globally as major causes of nosocomial and community-acquired infections. ESBL detection is important from a therapeutic point of view and for infection control purposes. Carbapenems are often used to treat infections caused by ESBL producing E. coli and Klebsiella. This study was conducted in indoor patient department of Teerthanker Mahaveer Medical College & Research Centre, Moradabad, U.P., which is a tertiary care hospital. This study was conducted during the period from April 2015 to December 2017.A total no. of 350 gram negative Enterobacteriaceae were isolated in 800 no. of samples. They were screened for the beta-lactamase production. Among the 350 isolates 210 strains were ESBL producers. The major ESBL producers were E.coli (32%) followed by Klebsiella, Enterobacter, Citrobacter, Proteus. Multidrug resistance to Fluoroquinolones and Aminoglycosides were observed in the ESBL producing organism. The most common ESBL producing organism were from ICU.

KEYWORDS : Extended-spectrum β-lactamases (ESBLs), Antibiotic sensitivity pattern.

INTRODUCTION:

Extended-spectrum β-lactamases (ESBLs) are a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of hospitalized and community-based patients. Infections due to ESBL producers range from uncomplicated urinary tract infections to life-threatening sepsis.[1]

Enterobacteriaceae group is the main cause of bacterial infection and in this family *Escherichia coli(E.coli)* and *Klebsiella* species are the most common causes of nosocomial infections. These pathogens are responsible for a broad spectrum of clinical infections in immune competent and as well as in immuno-compromised person.[2]

ESBLs represents a major threat among multidrug-resistant bacteria isolates.These ESBL producing pathogens are now recognized globally as major causes of nosocomial and community-acquired infections. ESBL detection is important from a therapeutic point of view and for infection control purposes. The first ESBL was detected in Germany in 1983, among different enterobacterial isolates recovered patients hospitalized at intensive care unit. It was recognized by the producer strains unusual resistance to cefotaxime (CTX) and ceftazidime (CAZ), which was transferable by conjugation to *E. coli*. In 1984, *Klebsiella pneumonia (K.pneumonia)* isolates were detected in different hospitals in France.[3]

Carbapenems imipenem (IPM) and meropenem (MEM), are often used to treat infections caused by ESBL producing *E. coli* and *Klebsiella*. However, carbapenemases enzymes recognize almost all hydrolysable β-lactams, and most are resistant to inhibition by all commercially viable β-lactamase inhibitors.[4]

Four classes are available in this group: Molecular classes A, C, and D include the β-lactamases with serine at their active site, whereas molecular class B β-lactamases are all metalloenzymes with zinc in active-site. *Klebsiella pneumonia* carbapenemase (KPC) enzymes are belonging to class A carbapenemases that reside on transferable plasmids and can hydrolyze all penicillins, cephalosporins, and carbapenems. The emergence of acquired metallo-β-lactamases (MBLs) has clinical and epidemiological implications and is a matter of particular concern world-wide.[4] Gram negative beta lactamase producing organisms exhibited resistance to beta lactam antibiotics (e.g. penicillin, cephalosporins, monobactams) were developed during the last 2 decades.[5]

As a result of continuous point mutations in TEM-1,TEM-2 and SHV-1 genes found among gram negative bacteria, ESBLs emerged which are enzymes first identified in 1983 and mediated resistant to 3rd generation cephalosporins (e.g. ceftazidime, ceftriaxone and cefotaxime) and monobactams (e.g. azetreonam) antibiotics and have been found in a wide range of Gram-negative bacteria, predominantly in *K.pneumonia* and *E. coli*. ESBLs are carried on plasmids and may be associated with resistance to other types of antibiotics.[6]

EXTENDED SPECTUM BETA LACTAMASES (ESBLs):

ESBLs are typically inhibitor-susceptible to β-lactamases that hydrolyze penicillins, cephalosporins, and aztreonam and are encoded by mobile genes. The most frequently encountered ESBLs belong to the CTX-M, SHV, and TEM families. ESBL producers are usually multiply drug resistant, including the 3rd generation cephalosporins (eg. cefotaxime, ceftriaxone, ceftazidime) and monobactams (eg.aztreonam) but not the cephamycins (eg.cefoxitine and cefotean) and carbapenems (eg.imipenem, meropenem, ertapenem). Organisms that produce ESBL remain an important reason for therapy failure with cephalosporins and have serious consequences for infection control.[7]

Most ESBLs can be divided into three group: CTX-M, SHV AND TEM types. *E.coli* and *K. pneumonia* remain the major ESBL

producing organisms isolated worldwide, although these enzymes have also been identified in several other organism of Enterobacteriaceae family and in certain non-fermenters. All the Beta-lactamase enzymes are commonly found in the Pseudomonas areuginosa and E.coli and also detected in K.oxytoca, Proteus mirabilis, Enterobacter, Citrobacter, Salmonella species and other members of Enterobacteri aceae.[8]

Beta-lactamase inhibitors:-

The three inhibitors of beta-lactamase has found in clinical medicine are clavulanic acid, sulbactum and tazobactum. All three inhibitors are effective against staphylococcal penicillinase and have variable effectiveness against the chromosomal enzyme of Gram-negative bacilli. Clavulanate and tazobactum are superior to salbactum in activity against plasmid mediated beta-lactamase of Gram-negative bacteria including the ESBL.[9]

MATERIALS AND METHODS:

Study Design

This study was Prospective observational study.

Study Procedure

The Sample taken for the microbiological assessment were Endotracheal suction, high vaginal swab, tip of tracheostomy tube, bronchoalveolar lavage, Foley's catheter tip, urine, pus, sputum, blood, and CSF.

The culture of the sample was done on blood agar, Mac Conkey agar and CLED media.

The Identification of the isolates was done with the help of Gram's staining, culture characteristics, motility test and standard biochemical tests which was Methyl red test, indole production test, urease production test, citrate utilization test, triple sugar iron test, Oxidative Fermentative test, oxidase test.

The Antibiotic Susceptibility Test was determined

- By the Kirby Bauer's disc diffusion method on Mueller-Hinton agar plate.
- The Choice of antibiotic disks was selected as per CLSI guidelines.

Mueller Hinton Agar is used for disk diffusion sensitivity testing of non-fastidious organisms.

Mueller Hinton Broth was recommended for preparing suspensions of microorganisms for disk diffusion sensitivity testing.

PHENOTYPIC DETECTION OF ESBLs PRODUCTION:

- ESBLs are Ambler class A penicillinases, which confer resistance to and hydrolyze the 3rd Generation cephalosporins like ceftazidime, cefotoxime, monobactam-azteronam and related oxyimino -lactams as well as older penicillins and cephalosporins.
- The Confirmatory test done by using double-disk (combined-disk) method.

The disks contained 30 μg of CAZ alone and in combination with 10 μg of clavulanic acid, respectively (Himedia Company,India).

Isolates were examined for their susceptibility to 3rd generation cephalosporins by using CAZ (30 μg) and CTX (30 μg) disks. All suspected isolates for ESBLs production were confirmed by the combination disk method on Mueller Hinton Agar plates that were inoculated with standardized inoculum (comparable to 0.5 McFarland standards) of the isolates to form a lawn culture. Separate commercial disks containing cefotaxime (30 μg) and ceftazidime (30 μg) with and without clavulanic acid (10 μg) were placed over the lawn culture. The disc was placed centre to centre on the plate and the distance of 25 - 30 mm between the cephalosporin and clavulanate containing discs were observed. An increase in zone size of more than or equal to 5 mm for cefotaxime and ceftazidime with and without clavulanic acid indicates ESBL production.[10]

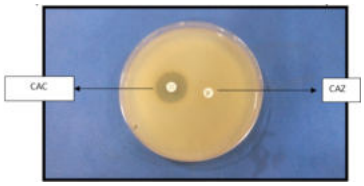

Figure 1: Showing detection of ESBL by Double Disc Method showing Zone of inhibition ≥5mm with Ceftazidime and clavulanic acid than Ceftazidime alone

RESULT :-

This study was conducted at Teerthanker Mahaveer Medical College & Research Centre, Moradabad, U.P. Total no.of 800 isolates were taken for the study. Out of 800, 350 isolates were Enterobacteriaceae group

which were taken for the study from April 2015 to December 2017. In 350 isolates 186 (53%) were isolated in females and 164 (47%) were isolated in males.

Among 350 clinical isolates majority were E.coli 175 (50%), Klebsiella spp. 80 (23%), Enterobacter spp. 54 (15%), Citrobacter spp. 36 (10%) and Proteus spp. 05 (2%) in various clinical samples like Pus followed by Urine, Blood, Sputum, High vaginal swab Endotracheal secretion Tracheal secretion, Foley's tip, CSF, Peritoneal fluid, Throat swab, BAL fluid, Thoractomy suction, Intra-uterine pack, Catheter tip , Bronchial washing and Ascitic fluid.

Among various gram negative isolated organism highest ESBL production was observed in E.coli 110(32%), followed by Klebsiella spp. 70 (20%), Enterobacter spp. 16 (04%), Citrobacter spp. 12 (03%) and Proteus spp. 2 (0.5%) were positive by Double disc diffusion test. The total no. of ESBL production was found 210 (59.5%) were positive and 140 (40.5%) were negative by Double disc diffusion test.

Table-1: Showing Gender wise distribution of total no. of samples.(n=350)

Sex	Frequency	Percent
Female	186	53.0
Male	164	47.0
Total	350	100.0

Table-2: Showing frequency of isolated organism in total no. of patients (n=350)

Isolates	Frequency	Percent
E.coli	175	50.0
Klebsiella spp.	80	23.0
Enterobacter spp.	54	15.0
Citrobacter spp.	36	10.0
Proteus spp.	05	2.0
Total	350	100.0

Table-3: Showing the presence or absence of ESBL was compared among different isolates by Combined Disc Method. A significantly higher number of samples tested positive for ESBL among E.coli (32%) and Klebsiella spp. (20%) in comparison to Enterobacter spp. (4%), Citrobacter spp. (3%) and Proteus spp. (0.5%) (n=350)

	ESBL Positive	ESBL Negative	Total
E.coli	110 (32%)	65 (18%)	175 (50%)
Klebsiella spp.	70 (20%)	10 (3%)	80 (23%)
Enterobacter spp.	16 (4%)	38 (11%)	54 (15%)

Citrobacter spp.	12 (3%)	24 (7%)	36 (5%)
Proteus spp.	2 (0.5%)	3 (1.5%)	5 (2%)
Total	210 (59.5%)	140 (40.5%)	350 (100%)

Table 4: Antibiotic sensitivity pattern of ESBL producing E.coli (n=110)

Antibiotics Name	Sensitive (S)	Resistant (R)
Ampicillin	0	110(100%)
Ampicillin/sulbactum	95(86.36%)	15(13.64%)
Piperacillin/tazobactum	97(88.2%)	13(11.8%)
Co-trimoxazole	12(10.9%)	98(89.1%)
Chloromphenicol	76(69.1%)	34(30.9%)
Gentamycin	18(16.4%)	92(83.6%)
Amikacin	91(82.7%)	19(17.3%)
Ciprofloxacin	107(97.3%)	3(2.7%)
Levofloxacin	80(72.7%)	30(27.3%)
Cefotaxime	84(76.4%)	26(23.6%)
Ceftazidime	0	110(100%)
Ceftazidime/clavulanic acid	93(84.5%)	17(15.5%)
Ceftriaxone	0	110(100%)
Cefoperazone/sulbactum	110(100%)	0
Imipenem	80(72.7%)	30(27.3%)
Meropenem	81(73.6%)	29(26.4%)
Ertapenem	81(73.6%)	29(26.4%)
Tigecycline	93(84.5%)	17(14.5%)
Polymyxin B	95(86.4%)	15(13.6%)
Nitrofurantoin	92(83.6%)	18(13.4%)

Table 5:Antibiotic sensitivity pattern of ESBL producing Klebsiella spp. (n=70)

Antibiotics Name	Sensitive (S)	Resistant (R)
Ampicillin	0	70(100%)
Ampicillin/sulbactum	64(91.4%)	6(8.6%)
Piperacillin/tazobactum	54(77.1%)	16(22.9%)
Co-trimoxazole	17(24.3%)	53(75.7%)
Chloromphenicol	26(37.1%)	44(62.9%)
Gentamycin	9(12.9%)	61(87.1%)
Amikacin	3(4.3%)	67(95.7%)
Ciprofloxacin	8(11.4%)	62(88.6%)
Levofloxacin	16(22.9%)	54(77.1%)
Cefotaxime	0	70(100%)
Ceftazidime	0	70(100%)
Ceftazidime/clavulanic acid	34(48.6%)	36(51.4%)
Ceftriaxone	0	70(100%)
Cefoperazone/sulbactum	70(100%)	0
Imipenem	67(95.7%)	3(4.3%)
Meropenem	67(95.7%)	3(4.3%)
Ertapenem	53(75.7%)	17(24.3%)
Tigecycline	58(82.9%)	12(17.1%)
Polymyxin B	45(64.3%)	25(35.7%)
Nitrofurantoin	60(85.7%)	10(14.3%)

Table 6:Antibiotic sensitivity pattern of ESBL producing Enterobacter spp. (n=16)

Antibiotics Name	Sensitive (S)	Resistant (R)
Ampicillin	0	16(100%)
Ampicillin/sulbactum	16(160%)	0
Piperacillin/tazobactum	16(100%)	0
Co-trimoxazole	14(87.5%)	2(12.5%)
Chloromphenicol	6(37.5%)	10(62.5%)
Gentamycin	1(6.3%)	15(93.7%)
Amikacin	2(12.5%)	14(87.5%)
Ciprofloxacin	11(68.8%)	5(31.2%)
Levofloxacin	11(68.8%)	5(31.2%)
Cefotaxime	0	16(100%)
Ceftazidime	0	16(100%)
Ceftazidime/clavulanic acid	6(37.5%)	10(62.5%)
Ceftriaxone	0	16(100%)
Cefoperazone/sulbactum	16(100%)	0
Imipenem	5(31.2%)	11(68.8%)
Meropenem	5(31.2%)	11(68.8%)
Ertapenem	6(37.5%)	10(62.5%)
Tigecycline	15(93.8%)	1(6.2%)
Polymyxin B	15(93.8%)	1(6.2%)
Nitofurantoin	14(87.5%)	2(12.5%)

Table 7: Antibiotic sensitivity pattern of ESBL producing Citrobacter spp. (n=12)

Antibiotics Name	Sensitive (S)	Resistant (R)
Ampicillin	0	12(100%)
Ampicillin/sulbactum	10(83.3%)	2(16.7%)
Piperacillin/tazobactum	12(100%)	0
Co-trimoxazole	2(16.7%)	10(83.3%)
Chloromphenicol	12(100%)	0
Gentamycin	1(8.3%)	11(91.7%)
Amikacin	1(8.3%)	11(91.7%)
Ciprofloxacin	12(100%)	0
Levofloxacin	12(100%)	0
Cefotaxime	12(100%)	0
Ceftazidime	0	12(100%)
Ceftazidime/clavulanic acid	10(83.3%)	2(16.7%)
Ceftriaxone	0	12(100%)
Cefoperazone/sulbactum	12(100%)	0
Imipenem	12(100%)	0
Meropenem	12(100%)	0
Ertapenem	12(100%)	0
Tigecycline	12(100%)	0
Polymyxin B	12(100%)	0
Nitrofurantoin	11(91.7%)	1(8.3%)

Table 8:Antibiotic sensitivity pattern of ESBL producing Proteus spp. (n=2)

Antibiotics Name	Sensitive (S)	Resistant (R)
Ampicillin	0	2(100%)
Ampicillin/sulbactum	2(100%)	0
Piperacillin/tazobactum	2(100%)	0
Co-trimoxazole	2(100%)	0
Chloromphenicol	2(100%)	0
Gentamycin	0	2(100%)
Amikacin	0	2(100%)
Ciprofloxacin	2(100%)	0
Levofloxacin	2(100%)	0
Cefotaxime	0	2(100%)
Ceftazidime	0	2(100%)
Ceftazidime/clavulanic acid	2(100%)	0
Ceftriaxone	0	2(100%)
Cefoperazone/sulbactum	2(100%)	0
Imipenem	2(100%)	0
Meropenem	2(100%)	0
Ertapenem	2(100%)	0
Tigecycline	2(100%)	0
Polymyxin B	0	2(100%)
Nitrofurantoin	2(100%)	0

DISCUSSION:-

The present study was carried for phenotypic detection of ESBL producer in various clinical isolates of Enterobacteriaceae and their antibiotic sensitivity pattern, which are nosocomial and community-associated bacteria commonly harbouring ESBLs among the patients attending TMMC & RC, Moradabad.

The worldwide emergence of multi-drug resistant bacterial strains is a growing concern which are usually found in those hospitals where antibiotic use is frequent and the patients are in critical condition. Broad resistance spectrum is a cause for concern and necessitates the restricted use of extended-spectrum cephalosporins, and a trial of other suitable alternatives.[14] Therapeutic options for the infections which are

caused by the ESBL producers have also become increasingly limited.[13] A study has found ciprofloxacin to be highly effective in treating multi-resistant Gram-negative infections[14] Recent studies on ESBL production among the members of Enterobacteriaceae which were isolated from clinical specimens, showed an increase in the occurrence of ESBL producers.[11]

In the present study, E. coli showed the maximum ESBL production (32%) followed by Klebsiella spp. (20%), Enterobacter spp (4%) and Citrobacter spp (3%). This was similar to the studies by Wadekar et al, in which, ESBLs were predominantly present among E. coli (50%) compared to Klebsiella spp. (37.5%), Enterobacter spp. (33.3%) and Citrobacter spp. (33.3%), Mathur et al, 62% of the E. coli and 73% of the K. pneumoniae isolates were reported to be ESBL producers.[12], Metri et al,[13] K. pneumoniae and E. coli as the major ESBL producers, Jain et al,[14] ESBL was detected in 87.2% isolates of *Klebsiella* spp ., 72.5% isolates of *Enterobacter* spp., 65.3% isolates of *E.coli*, 33.3% isolates of *Acinetobacter* spp. and in none of the isolates of *Citrobacter* or *Pseudomonas* spp.,Moyo et al,[15]a high prevalence of ESBL production by *E. coli* (39.1%) and by *Klebsiella spp* (51.5%) urinary isolates at MNH and Khanfar *et al*[16] and points to the possibility of nosocomial acquisition of UTI due to ESBL pathogens. These findings have significant implications for empirical management of patients with UTI using third generation cephalosporins.

Recent studies on ESBL production among the members of Enterobacteriaceae which were isolated from clinical specimens, showed an increase in the occurrence of ESBL producers. A study from North India on uropathogens such as Klebsiella pneumoniae, Escherichia coli, Enterobacter, Proteus and Citrobacter spp., showed that 26.6% of the isolates were ESBL producers.[17] A study from Nagpur showed that 48.3% of their cefotaxime resistant gram negative bacilli were ESBL producers.[17] A report from Coimbatore (India) showed that ESBL production was 41% in E. coli and 40% in K. pneumoniae.[28]

However, the prevalence of ESBL-producing strains of E. coli was reported to be 35.5% in the study by Anago et al.[29], the prevalence in Tanzania was reported to be 39.1%.[15]Ahoyo *et al*.[18] showed a prevalence of 22% in isolates from various nosocomial infections., Anago et al, 14.8% of *E. coli* strains isolated from urinary tract infection (UTI) were ESBL.[19], Lower ESBL prevalence was described in Morocco (1.3%) in UTI isolated strains[20], in Cameroon (16%) in strains isolated from faeces in the community.[21]

However, the contrasting results were shown in the studies by Mohsen et al,[22] the frequency of ESBL-production by *K. pneumoniae* (35.5%) was higher than that by *E. coli* (18.8%),the frequency of ESBL-production by *K. pneumoniae* was similar to our study but by E. coli was drastically lower and Vinod Kumar et al[23] from Gulbarga reported 16.8% and 48.6% of *E.coli* and *K. pneumoniae* respectively as the ESBL producers. The ESBL prevalence was different when compared to that reported in the National Surveillance of Antibiotic Resistance (NSAR) report for 2014 for Malayasia.[24]

In *Citrobacter* and *Proteus*, the ESBL production in the current study was 23.1% and 25.0% respectively which was consistent with the findings of the studies which was carried out by Metri et al, 14.3% for both *Citrobacter* and *Proteus* and Gangone et al.[25]

The prevalence of ESBL production is high in the referral centers and the intensive care units where the patients are referred from the peripheral centers and where the antibiotic use is profuse. Studies which were undertaken in Hubli by Krishna et al.[19] and in New Delhi by Wattal et al.[26] revealed a markedly higher incidence of ESBL production, which can be attributed to the subjects from the intensive care units, where the prevalence and the risk factors which are responsible for the emergence of the ESBL producers is high. Other reasons for the high prevalence of the ESBL producers were indwelling catheters, endotracheal or nasogastric tubes, gastrostomies or tracheostomies, severity of the illness, the excessive use of cephalosporins and a high rate of patient transfer from the peripheral centers.[14,27]

ESBL producing organisms, being the commonest nosocomial pathogens, it is essential to detect and treat them as early as possible. Since ESBL production is more common among the nosocomial pathogens, early detection will definitely help in controlling hospital infections which are caused by this group of organisms. Enterobacteriaceae are the common isolates in most of the laboratories. Now-a-days, a majority of these isolates are multi-drug resistant. The control of these multidrug resistant organisms is a therapeutic challenge. This difficulty is enhanced further by the co-existence of the resistance to β-lactams, aminoglycosides and fluoroquinolones, as observed in our study. Of all the available antimicrobial agents, carbapenems are the most active and reliable treatment options for infections which are caused by the ESBL producing isolates.[10]

CONCLUSION :-

- The mean age of the study population in the present study was 36.34±19.47 years with slightly more females (53%) than males (47%)..
- Majority of the isolates were E.coli (50.0%) followed by Klebseilla spp (23.0%),Enterobacter spp.(15%), Citrobacter spp (10%) and Proteus spp (2%).
- A significantly higher number of samples tested positive for ESBL among E. coli (32%) and Klebseilla spp (14.0%) in comparison to Enterobacter spp., Citrobacter spp. and Proteus spp.
- The ESBL-producing organisms are a breed of multidrug-resistant pathogens that are increasing rapidly and becoming a major problem in the area of infectious diseases. It is essential to report ESBL production along with the routine sensitivity reporting, which will help the clinicians in prescribing the proper antibiotics.
- Piperacillin-tazobactam and imipenem are the most active and reliable agents for the treatment of infections which are caused by ESBL producing organisms.
- The aim of the study to reduce the prevalence of antimicrobial resistant pathogens,ESBL producing *E.coli*, effective infection control measures like hand washing and barrier precautions are required.
- Monitoring the judicious use of extended spectrum cephalosporins, periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy would go a long way in addressing some of the problems associated with these pathogens.

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