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A STUDY OF PREVALENCE & ANTIBIOGRAM OF ESBL PRODUCERS AMONG GRAM NEGATIVE BACILLI FROM VARIOUS CLINICAL ISOLATES IN A TERTIARY CARE CENTRE.

Microbiology

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ABSTRACT

Antimicrobial resistance is a significant global challenge in the present era. Extended spectrum beta lactamase (ESBL) producing gram- negative bacilli have emerged as a major problem during the last two decades in many healthcare settings worldwide . The objective of the present study was to determine the prevalence and antibiotic sensitivity pattern of ESBL producing gram negative bacilli isolated from various samples from both in-patients and out-patients who attended a tertiary care hospital in western Uttar Pradesh, India. A total of 192 consecutive, non- repetitive, gram negative isolates, which were resistant to one of the third generation cephalosporins (cefotaxime, ceftriaxone or ceftazidime) were selected as "Suspicious for ESBL production". These isolates were confirmed for ESBL production by the double disc synergy test (DDST) and the phenotypic confirmatory disc diffusion test (PCDDT) as recommended by the Clinical and Laboratory Standards Institute (CLSI). Out of the total 192 gram negative bacilli isolates, 108 (56.2%) were confirmed as ESBL producing isolates. DDST detected only 101 (93.5%) ESBL producing isolates while all the 108 ESBL producing isolates were detected by PCDDT (p value <0.05). Escherichia coli was the most common ESBL producer followed by Proteus mirabilis & Klebsiella pneumoniae. The specimen wise distribution of the ESBL producers revealed maximum ESBL production in urine (63.2%), followed by pus (48.6%) and others (50%). Imipenem (91.1%), Piperacillin-Tazobactam (75%) and Amikacin (59.9%) in the decreasing order, were the most active antimicrobials for the treatment of the infections caused by the ESBL producing organisms. Reporting of ESBL production along with the routine antimicrobial susceptibility testing will help the clinicians in prescribing proper antibiotics.

KEYWORDS

Extended spectrum beta lactamase, prevalence, DDST, PCDDT

INTRODUCTION

Antimicrobial resistance is a significant global challenge in the present era. The production of β- lactamase is the predominant mechanism for resistance to the β-lactam antibiotics in gram negative bacteria responsible for the resistance to the third generation cephalosporins and aztreonam. ESBL producing gram- negative bacilli have emerged as a major problem during the last two decades in many healthcare settings worldwide [1].

The introduction of the third generation cephalosporins into clinical practice was firstly started in the early 1980s. It was heralded as a major breakthrough in the fight against bacterial resistance to antibiotics mediated by β- lactamases. The third generation cephalosporins were not only effective against most β- lactamase producing organisms but they also had the major advantage of lessened nephrotoxic effects compared to aminoglycosides and polymixins.

The first report of plasmid encoded β- lactamases capable of hydrolyzing the extended-spectrum cephalosporins was published in 1983 [2]. Other β- lactamases were soon discovered which had the ability to confer resistance to the extended-spectrum cephalosporins. Hence these new β- lactamases were coined extended-spectrum β-lactamases (ESBLs) [3,4].

There is no consensus about the precise definition of ESBLs. A commonly used working definition is that the ESBLs are β- lactamases capable of conferring bacterial resistance to the penicillins, first, second, and third- generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by β- lactamase inhibitors such as clavulanic acid [5].

The various problems associated with ESBLs include multi-drug resistance, difficulty in detection and treatment, and increased mortality. Awareness and the knowledge of proper detection methods of these enzymes is necessary for proper patient care.

Improved infection control methods along with the judicious use of antibiotics must become health care priorities in healthcare establishments.

The objective of the present study was to determine the prevalence and antibiotic sensitivity pattern of ESBL producing gram negative bacilli isolated from various samples from both in-patients and out-patients who attended a tertiary care hospital in western Uttar Pradesh, India. The current study assumes importance as no previous data is available on the prevalence of ESBL in this region.

MATERIALS AND METHODS :

A prospective study was conducted over a period of one year (March 2019 to February 2020) at K.D. Medical College Hospital & Research Centre, Mathura, Uttar Pradesh after obtaining clearance from the institutional ethical committee. A total of 192 consecutive, non-repetitive, gram negative bacterial isolates from various clinical samples (such as urine, blood, pus, sputum, tracheal aspirate, cerebrospinal fluid, ascitic fluid and pleural fluid) which were suspicious for ESBL production as per the Clinical Laboratory Standards Institute (CLSI) guidelines, were included in this study [6].

The isolates were identified on the basis of the standard bacteriological techniques and were further tested for ESBL production by using the double-disk synergy test and the phenotypic confirmatory disc diffusion test as recommended by CLSI [6-8].

Criteria For The Selection Of The ESBL Producing Strains:

The isolates were tested for their susceptibility to the third generation cephalosporins e.g. ceftazidime (30 µg), cefotaxime (30 µg) and ceftriaxone (30 µg) by using the standard disc diffusion method as recommended by the CLSI [6]. If a zone diameter of $\leq$ 22 mm for ceftazidime, $\leq$ 27 mm for cefotaxime and $\leq$ 25 mm for ceftriaxone were recorded, the strain was considered to be "suspicious for ESBL production" [6]. Only those isolates which were resistant to one or more of these third generation cephalosporins were selected for our study and were processed for confirmation of ESBL production.

The Double Disc Synergy Test (DDST):

Isolates which were presumed to be ESBL producers on the basis of the screening test results, were picked up and emulsified in saline to a 0.5 McFarland's turbidity standard. Discs of ceftazidime (30µg), cefotaxime (30µg) and amoxyclav (20µg amoxicillin/ 10µg clavulanic acid) were placed at a distance of 20 mm from center to center in a straight line, with the amoxyclav disc in the middle on a plate of Mueller Hinton Agar (MHA) inoculated with the test strain. The plates were incubated overnight at 37°C aerobically. Isolates which showed an enhancement of the zone of inhibition as greater than 5 mm on the amoxyclav side of the disc as compared to that which was seen on the side without amoxyclav, were confirmed as ESBL producers [9].

The Phenotypic Confirmatory Disc Diffusion Test (PCDDT):

All the strains which were screened out for ESBL production were also subjected to confirmation by using the PCDDT, as recommended by the CLSI [6]. The ceftazidime (30µg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 µg discs)

were applied onto a plate of Mueller Hinton Agar (MHA) which was inoculated with the test strain. An increase of ≥ 5 mm in the zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be a marker for ESBL production [6].

ANTIBIOTIC SENSITIVITY TESTING

The susceptibility of the ESBL producing bacteria to amikacin (30µg), piperacillin(100µg), piperacillin/ tazobactum(100/10µg), cefepime(30µg), cefotaxime(30µg), ceftriaxone(30µg), ceftazidime (30µg),amoxyclav(20/10µg),cotrimoxazole(25µg),ciprofloxacin(5µg), imipenem(10µg), doxycycline(30µg) and azithromycin(15µg) was determined by the Kirby-Bauer disk diffusion method according to the CLSI guidelines [6].

Quality Control:

β-lactamase negative *Escherichia coli* ATCC 25922 was used as the negative control and ESBL-producing *Klebsiella pneumoniae* ATCC 700603 was used as the positive control throughout the study [6].

Statistical Analysis:

Statistical analysis was performed by the Chi-square test and a *p* value of less than 0.05 was considered as statistically significant.

RESULTS:

Out of the total 192 gram negative bacilli isolates suspicious for ESBL production, 108 were confirmed as ESBL producing isolates. Hence, overall prevalence of ESBL strains was found to be 56.2% (108/192). Prevalence of ESBL isolates in female and male population were 57.4% (62/108) and 42.6% (46/108) respectively [Fig.-1]. DDST detected only 101 (93.5%) ESBL producing isolates while all the 108 ESBL producing isolates were detected by PCDDT (p value < 0.05) [Table-1].

Escherichia coli was the most common ESBL producer followed by *Proteus mirabilis* & *Klebsiella pneumoniae* as shown by both PCDDT & DDST [Table-1].

The specimen wise distribution of the ESBL producers revealed maximum ESBL production in urine (63.2%), followed by pus (48.6%) and others (50%) [Table-2].

The antibiotic sensitivity pattern revealed maximum sensitivity for Imipenem (91.1%) followed by Piperacillin-Tazobactam (75%), Amikacin (59.9%), Azithromycin (43.8%) and Ciprofloxacin (35.4%). High resistance rate was seen for Ceftriaxone (85.4%), Cotrimoxazole (83.8%), Doxycycline (83.3%), Amoxyclav (82.8%), Ceftazidime (82.3%) and Piperacillin (81.2%) [Table-3].

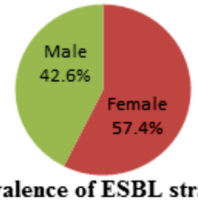

Fig.1- Prevalence of ESBL strains according to gender

Table 1: ESBL Producers Among Gram Negative Bacilli In Various Clinical Specimens

Organism	ESBL producers	
	PCDDT*	DDST#
Escherichia coli	65/98 (66.3%)	61/98 (62.2%)
Klebsiella pneumoniae	20/36 (55.5%)	18/36 (50%)
Pseudomonas aeruginosa	14/33 (42.4%)	13/33 (39.4%)
Proteus mirabilis	3/5 (60%)	3/5 (60%)
Proteus vulgaris	1/3 (33.3%)	1/3 (33.3%)
Acinetobacter spp.	3/10 (30%)	3/10 (30%)
Citrobacter spp.	2/7 (28.6%)	2/7 (28.6%)
TOTAL	108/192 (56.2%)	101/192 (52.6%)

PCDDT*- Phenotypic Confirmatory Disc Diffusion Test, DDST#- Double Disc Synergy Test

Table 2- Specimenwise Distribution Of ESBL Isolates

Samples →	Urine	Pus	Others
Distribution of ESBL isolates (%)	62/98 (63.2%)	35/72 (48.6%)	11/22 (50%)

Table 3: Antibiotic Susceptibility Pattern Of Gram Negative Bacilli In Various Clinical Isolates

Antibiotics (n =192)	Sensitive (%)	Intermediate (%)	Resistant(%)
Amikacin	115 (59.9%)	3 (1.6%)	74 (38.5%)
Ciprofloxacin	68 (35.4%)	4 (2.1%)	120 (62.5%)
Cefepime	62 (32.3%)	1 (0.5%)	129 (67.2%)
Doxycycline	30 (15.6%)	2 (1%)	160 (83.3%)
Piperacillin	36 (18.6%)	0	156 (81.2%)
Piperacillin Tazobactam (PIT)	144 (75%)	3 (1.6%)	45 (23.4%)
Imipenem	175 (91.1%)	2 (1%)	15 (7.8%)
Azithromycin	84 (43.8%)	2 (1%)	106 (55.2%)
Cotrimoxazole	28 (14.6%)	3 (1.6%)	161 (83.8%)
Amoxyclav	32 (16.6%)	1 (0.5%)	159 (82.8%)
Cefotaxime	39 (20.3%)	2 (1%)	151 (78.6%)
Ceftriaxone	27 (14%)	1 (0.5%)	164 (85.4%)
Ceftazidime	34 (17.7%)	0	158 (82.3%)

DISCUSSION

There has been a rapid worldwide spread of ESBL producing bacteria at an alarming rate. Hence, quick detection of ESBL production and effective infection control measures are required to control spread of ESBL producing bacteria. The treatment options for the infections caused by these bacteria are becoming increasingly limited due to their increasing resistance against the commonly used antimicrobials.

Previous studies from India have reported a varying prevalence of ESBL producers depending upon the geographical area of the study. In previous Indian studies, the ESBL production which was reported among gram negative bacteria by Mathur et al [10] was 68%, Singhal et al [11] was 64% and Rodrigues et al [12] was 53%. Also, Rodrigues et al [12] reported 65.8%, 61.7% and 27.9% ESBL positivity among *E.coli* , *K.pneumoniae* & *Pseudomonas aeruginosa* respectively. These findings correlated well with the findings of our study.

The prevalence of ESBL strains was more in female population. This is in accordance with the study by Sofia C.Patel et al [13]. The commonest sample received in our laboratory was urine. In the present study, higher ESBL prevalence in female population could be due to the higher prevalence of urinary tract infection in females [14,15].

In the present study, it was also observed that 66.3% *Escherichia coli* and 55.5% *Klebsiella pneumoniae* isolates were ESBL producers. Although in other studies , *K.pneumoniae* was more often reported as an ESBL producer, we found that ESBL production was more common in the *E.coli* isolates as compared to that in the *K.pneumoniae* isolates in the present study [16,17,18].

In *Pseudomonas spp*., ESBL production was observed less as compared to Enterobacteriaceae , because their resistance is mediated by various other mechanisms such as the production of metallo-beta-lactamases, lack of drug penetration due to mutations in the porins and the loss of certain outer membrane proteins and efflux pumps [11,19,20].

We observed that ESBL production among gram negative bacilli was more frequently detected by the PCDDT as compared to the DDST. The Clinical Laboratory Standards Institute (CLSI) therefore also recommended the use of PCDDT for the phenotypic confirmation of ESBL production among Enterobacteriaceae [21].

In our study, we observed that a majority of the isolates were susceptible to Imipenem (91.1%) & Piperacillin-Tazobactam (75%). This finding is in accordance with the findings of various other studies [22, 23].

ESBL producing organisms have evolved as the most common nosocomial pathogens in the present era. Hence, early detection of ESBL producing micro-organisms will help a lot in the control of nosocomial infections. Now-a-days , a majority of the ESBL producing isolates are multi-drug resistant and poses a therapeutic challenge. Among the spectrum of available antimicrobials , carbapenems are the most active and reliable treatment option for infections caused by the ESBL producing isolates.

However, carbapenem resistance is gradually increasing in gram-

negative bacteria due to its overuse. Hence, regular detection of ESBL producing bacteria should be performed by conventional methods at all tertiary care centres to combat the increasing carbapenem resistance.

Reporting of ESBL production along with the routine antimicrobial susceptibility testing will help the clinicians in prescribing proper antibiotics.

Conflict Of Interest: There are no conflicts of interest.

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