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Research Article

DETECTION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ESBL PRODUCING GRAM NEGATIVE BACTERIA

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ABSTRACT

Objective: The ESBL producing organisms has been steadily increasing over the past years. The detection and treatment of these ESBL organisms are extremely limited. In the present study, to determine the Extended Spectrum Beta Lactamases producing organism which were isolated from various samples of Multispeciality hospital in Salem, India. Methods: A total of 278 gram negative isolates were received from various samples were used to detect the ESBL production by using double disk approximation test and Phenotypic confirmatory test with combination disc. The susceptibility of ESBL producers were analysed by antibiotic susceptibility test by using 4 commercially available beta-lactamase inhibitors combinations (Piperacillin/ tazobactam, Ampicillin/Sulbactam, Ticarcillin/Clavulanic acid and Amoxycillin/Clavulanic acid). Results: A total of 351 samples were received, from that 53 *Escherichia coli*, 73 *Klebsiella* spp, 37 *Pseudomonas aeruginosa*, 58 *Salmonella* spp, 31 *Enterobacter* spp and 26 *Proteus* spp were isolated and tested; from the 278 isolates 151 were found to be ESBL producers. From the 151 ESBL producers, P/T exhibited best activity against 134(88.74%) followed by A/C 128(84.76%) and A/S 126(83.44%). T/C 108(71.52%) shown the poor activity against all the organisms compare to other combination antibiotics. P/T exhibited significantly greater inhibitory activity against *Klebsiella* spp (96.15%), *E. coli* (92.68%), *P. aeruginosa* (90.47%), whereas, A/C exhibited considerable antimicrobial activity against *Klebsiella* spp (92.30%) and *P. aeruginosa* (90.47%). The A/S exhibited greater inhibitory activity against *P. aeruginosa* (90.38%) and *Klebsiella* spp (90.38%). P/T, A/C and A/S exhibited better activity against rest of the organisms. All the four agents show good activity against *Proteus* spp. Conclusion: These data suggest that Amoxycillin/Clavulanic acid and Ampicillin/Sulbactam combination antibiotics showed maximum inhibitory activity against ESBL producers. So, it can be used for the treatment of ESBL infection.

Keywords: Extended Spectrum Beta Lactamases (ESBL); beta lactam inhibitors- Piperacillin/ tazobactam, Ampicillin/Sulbactam, Ticarcillin/Clavulanic acid, Amoxycillin/Clavulanic acid.

INTRODUCTION

Antimicrobial drug resistance among the pathogens represents an ongoing worldwide therapeutic challenge. The growing bacterial resistant to antibiotics may lead to an increase in appropriate empirical antimicrobial treatment of infections with a delay in the correct therapy [1-3]. The widespread use of broad spectrum antibiotics has led to the emergence of infections caused by drug resistant microbes [4]. The gram negative bacilli are rapidly acquiring resistance to multiple antibiotics [5], making treatment selection problematic. The β-lactam antibiotics are the most widely used antimicrobial agents which are destructed by the bacterial enzymes called β-lactamases. Extended spectrum β-lactamases (ESBL) represents a major group of β-lactamases, currently being worldwide identified by large numbers [6]. Beta lactamases are widely distributed in members of Enterobacteriaceae [7]. Gram negative pathogens harboring ESBL are becoming increasing therapeutic problems [8].

ESBL are plasmid mediated enzymes that lead to multidrug resistance in organisms. Most of this plasmid not only contain DNA encoding ESBL enzymes, but also carry a gene which confers resistance to several non-β lactams antibiotics. The most frequently coresistant found in ESBL producing organisms are aminoglycosides, fluoroquinolones, tetracyclines, Chloramphenicol and sulfamethoxazole-trimethoprim [9, 10]. ESBL have the ability to hydrolyses and cause resistant to various type of newer β-lactam antibiotics including the extended–spectrum (or third–generation) cephalosporin (e.g. cefotaxime, ceftazidime) and monobactams (aztreonam) but not the cephamycins (cefoxitin and cefotetan) and carbapenems (imipenem, meropenem and extrapenem) [11]. A number of studies have confirmed that extrapenem is resistant to hydrolysis mediated by many β-lactamases (both plasmidic and chromosomal) including ESBL [12-15]. And they are also inhibited by clavulanate (CA), sulbactam and tazobactam with the combination of amoxicillin/clavulanate, Ticarcillin/clavulanate, Ampicillin/sulbactam and Piperacillin/tazobactam. Originally ESBL production is observed in *Escherichia coli*, and *Klebsiella* spp., now it was documented in other gram negative bacilli including *Proteus* spp., *Pseudomonas aeruginosa*, *Enterobacter* and *Salmonella* spp., [16-24]. The incidence of ESBL producing strains among clinical *Klebsiella* spp., and *E. coli* isolates has been steadily increasing over past years [25]. The highest risk of infection with ESBL producing were in prolonged hospitalization, high score of severe illness, recent surgery, instrumentations, admission to an intensive care unit and catheterization [26]. Laboratory detection of ESBL production can be problematic [16-24]. Detection of ESBL producing strains is important because its spread within the hospital may lead to endemic occurrence and repeated outbreaks from time to time. Another important reason of it detection is failure to treat ESBL producing organisms because of limited therapeutic choices [8]. Several molecular methods' are available for research and epidemiological studies, but they are not appropriate for routine detection of ESBL production in clinical setting [27, 28]. The present study was designed to evaluate the detection of ESBL producing gram negative bacilli isolated from the patients of Multispeciality hospital and also determine the susceptibility pattern of ESBL producers against commercially available beta-lactamases inhibitors combinations.

MATERIALS AND METHODS

Different types of organism were isolated from various specimens such as pus, urine, blood and faecal. The isolates were screened for

ESBL production by double disk approximation test which was described by [29] and the phenotypic confirmation of ESBL producers was determined by Phenotypic confirmatory test with combination disc (CLSI) [30] guidelines. Then the ESBL producers were tested against commercially available beta-lactamase inhibitor combination antibiotics as per Clinical Laboratory Standards Institute (CLSI) guidelines [30].

Double disk approximation method (Primary Isolation Method)

The double disk approximation test is used as primary isolation method to identify the ESBL producing organisms. An overnight culture suspension of the test isolates which was adjusted to 0.5 McFarland's standard was inoculated by using a sterile cotton swab on the surface of a Muller Hinton Agar plates. Antibiotic discs of Amoxicillin / Clavulanic acid (20/10µg) and cefotaxime (30µg) were placed at a distance of 15 mm apart and incubated. After incubating overnight at 37°C that showed a clear extension of cefotaxime inhibition zone towards the disc containing clavulanic acid were considered as ESBL producer. The organisms which were screened and found positive for ESBL production were subjected to confirmatory test.

Phenotypic confirmatory test with combination disc

Phenotypic confirmation of ESBL producers was carried out by as per CLSI guidelines [30]. This test requires the use of a third-generation cephalosporin antibiotic disc alone and in combination with clavulanic acid. In this study, a disc of Ceftazidime (30µg) alone and a disc of Ceftazidime + Clavulanic acid (30µg/10µg) were used. Both the discs were placed at least 25 mm apart, center to center, on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate and incubated overnight at 37°C. Difference in zone diameters with and without clavulanic acid was measured. A ≥ 5mm increase in the zone diameter of of inhibition for any of the antimicrobial agents tested in combination with clavulanic acid compared to the zone diameter of inhibition when tested alone was considered as confirmatory for ESBL production.

Antibiotic sensitivity testing

The susceptibility of ESBL producers to Amoxicillin/clavulanate, Ticarcillin/clavulanate, Ampicillin/sulbactam and Piperacillin/tazobactam was determined by the Kirby-Bauer disk diffusion method according to Clinical Laboratory Standard Institute Guidelines [30].

RESULTS

A total of 351 samples were received from Multispeciality hospital there were 132 urine sample, 63 pus, 81 blood and 75 feacal samples were collected (Table 1).

Table 1: A total number and the percentage of collected samples

Samples	No. of samples	%
Urine	132	38
Pus	63	18
Blood	81	23
Faecal	75	21
Total	351	100

There were different types of Gram negative bacterial isolates were obtained such as *Escherichia coli* 53(19.06%), *Klebsiella* spp., 73(26.25%), *Pseudomonas aeruginosa* 37(13.30%), *Salmonella* spp., 58(20.86%), *Enterobacter* 31(11.15%) and *Proteus* spp., 26(9.35%). The majority of isolates were obtained from faecal sample followed by pus, urine and blood samples (Table 2).

Table 2: Distribution of organisms from different specimens

Bacterial types	Total no. of isolates (%)	No. of isolates (%) Urine	Pus	Blood	Faecal
Escherichia coli	53(19.06)	21	0	1	31
Klebsiella spp.,	73(26.25)	22	34	13	4
Pseudomonas aeruginosa	37(13.30)	0	37	0	0
Salmonella spp.,	58(20.86)	20	0	10	28
Enterobacter spp.,	31(11.15)	0	0	0	31
Proteus spp.,	26(9.35)	15	11	0	0
Total	**278**	**78(28.05)**	**82(29.49)**	**24(8.63)**	**94(33.81)**

Totally 278 isolates were tested for ESBL production by using double disk approximation test and Phenotypic confirmatory test with combination disc. From the primary screening we found *Escherichia coli* (49), *Klebsiella* spp., (61), *Pseudomonas aeruginosa* (27), *Salmonella* spp., (21), *Enterobacter* (19) and *Proteus* spp., (14) totally 191(68.70%) isolates were resistant to third generation cephalosporins. Among that *Escherichia coli* 41(77.35%), *Klebsiella* spp., 52(71.23%), *Pseudomonas aeruginosa* 21(56.75%), *Salmonella* spp., 17(29.31%), *Enterobacter* 11(35.48%) and *Proteus* spp., 9(34.61%) totally 151 (54.31%) were confirmed as ESBL producers by Phenotypic confirmatory test with combination disc (Table 3).

Table 3: Rate of ESBL producers by primary and secondary isolation methods

Name of the organisms	No. of isolates	Double disk approximation (1° screening) %	Phenotypic confirmatory test with combination disc test (2° screening) %
Escherichia coli	53	49 (92.45)	41(77.35)
Klebsiella spp.,	73	61(83.56)	52(71.23)
Pseudomonas aeruginosa	37	27(72.97)	21(56.75)
Salmonella spp.,	58	21(36.20)	17(29.31)
Enterobacter spp.,	31	19(61.29)	11(35.48)
Proteus spp.,	26	14(53.84)	9(34.61)
Total	**278**	**191 (68.70)**	**151 (54.31)**

A total of 278 gram negative bacteria were isolated of which 151(54.31%) were found to be ESBL producers. Then the ESBL producers were used to detect their susceptibility by using Amoxicillin/clavulanate (A/C), Ticarcillin/clavulanate (T/C), Ampicillin/sulbactam (A/S) and Piperacillin/tazobactam (P/T). Overall, P/T exhibited best activity (88.74% susceptible organisms) followed by A/C (84.76% susceptible organisms) and A/S (83.44% susceptible organisms). T/C exhibited poor activity against all the organisms compare to other antibiotics. When P/T, A/C and A/S were compared, P/T exhibited the greater antibacterial activity against *Escherichia coli, Klebsiella* spp., and *Pseudomonas aeruginosa* whereas, A/C exhibited greater antibacterial activity against *Klebsiella* spp., *Pseudomonas aeruginosa* and *Proteus* spp. For A/S also exhibited greater inhibitory activity against *Klebsiella* spp, *Pseudomonas aeruginosa* and *Salmonella* spp. P/T, A/C and A/S were marginally better activity against rest of the organisms. All the four antimicrobial agents reveal best activity against *Proteus* spp. Among the four antibiotics Piperacillin/Tazobactam (P/T) revealed high susceptible pattern than other three antibiotics (Table-4).

Table 4: Resistance and susceptible pattern of ESBL producers against beta-lactamase inhibitor combination antibiotics

Total no. of isolates		Piperacillin/ Tazobactam (P/T)	Ampicillin/ sulbactam (A/S)	Ticarcillin/ Clavulanic acid (T/C)	Amoxycillin/ Clavulanic acid (A/C)
Escherichia coli (53)	41	38(92.68)	33(80.48)	30(73.17)	34(82.92)
Klebsiella spp., (73)	52	50(96.15)	48(92.30)	41(78.84)	47(90.38)
Pseudomonas aeruginosa (37)	21	19(90.47)	19(90.47)	12(57.14)	19(90.47)
Salmonella spp., (58)	17	12(70.58)	14(82.35)	09(52.94)	11(64.70)
Enterobacter spp., (31)	11	8(72.72)	5(45.45)	10(90.90)	9(81.81)
Proteus spp., (26)	9	7(77.77)	7(77.77)	6(66.66)	8(88.88)
Total	**151**	**134(88.74)**	**126(83.44)**	**108(71.52)**	**128(84.76)**

DISCUSSION

Infections caused by resistant pathogens result in significant morbidity and mortality, and contribute to rising healthcare cost worldwide. Inspite of the availability of new antibiotics, emerging antimicrobial resistance has become an increasing problem in many pathogens throughout the world [31] and rapid detection in clinical laboratories is essential for the prompt recognition of antimicrobial resistant organisms [32]. Nosocomial infections are mainly caused by gram negative bacteria, due to intrinsic and acquired capabilities to develop resistance to anti-microbial agents, they are difficult to treat. One of the important mechanisms of antimicrobial resistance is the production of extended spectrum β-lactamases [33].

ESBL producing organisms pose a major problem in clinical therapeutics. The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past years, resulting in limitations of therapeutic options [34]. ESBL occurs worldwide with varying prevalence and rapidly changing overtime, unfortunately these organisms often possess resistant determinant to other important antibiotic groups, such as fluoroquinolenes and aminoglycosides. Thus, antibiotic options in the treatment of these organisms are extremely limited [35]. A study shown the prevalence and antibiogram of ESBL producers, they use the combination disk method and double disk approximation method to detect the ESBL producers [36]. Likewise in our study, we also detect the ESBL producers by double disk approximation test for screening the potential ESBL producers and phenotypic confirmatory test with combination disc method was used to confirm the ESBL producers.

A study from North India on uropathogens such as *Klebsiella pneumoniae*, *E. coli*, *Enterobacter* spp, *Proteus* spp and *Citrobacter* spp showed that 26.6% of the isolates were ESBL producers [37]. Likewise, in our study, we observed that *E. coli* 41(77.35%), *Klebsiella* spp 52(71.23%), *Pseudomonas* spp 21(56.75%), *Salmonella* spp 17(29.31%), *Enterobacter* spp 11(35.48%), and *Proteus* spp 9(34.61%). Totally 151(54.31%) isolates were ESBL producers. The another study explain the incidence of Multidrug resistance and ESBL producing *Klebsiella* spp from 6-17% of nosocomial isolates involved in Urinary tract infections [38]. Similarly a study show vey high incidence of ESBL production in *E. coli* (89.5%) [33]. In Canada, a study revealed that the rate of ESBL producing *E. coli* is 3.5% and for *Klebsiella pneumoniae* 1.8% [39]. In Winnipeg, 1-3% of *E. coli* and 0.4% of *Klebsiella* Spp. were ESBL producers [39].

A study was recorded that, *E. coli* was the predominant ESBL producer followed by *K. pneumoniae*. Though *K. pneumonia* is more often reported as the major ESBL producer, we observed that *E. coli* was the most common ESBL producer as compared to *K. pneumoniae* [37]. Comparably another study also observed that 81% of the *E. coli* and that 74 % of the *K. pneumoniae* isolates were ESBL producers. So we also observed that ESBL production was more common among the *E. coli* isolates as compared to the *K. pneumoniae* isolates [29, 37, 40]. The prevalence of 36.4% and 37.8% for *K. pneumoniae* and *E. coli* in this study also agreed with the findings of [40], who reported 40 and 41% ESBL positivity among *K. pneumoniae* and *E. coli*, respectively [41]. The current study revealed that, the prevalence of ESBL producers like *E. coli* (77.35%), *Klebsiella* spp (71.23%) also agreed with the findings of Mathur et al, 62% of the *E. coli* and 73% of the *K. pneumoniae* isolates were reported to be ESBL producers [42].

A study observed that majority of the isolates were susceptible to P/T was the best combination followed by C/S [43]. In another study, however P/T exhibited greater in vitro activity only against *E. coli, P. vulgaris* as compared to P/S [44]. Similarly the majority of the isolates were susceptible to imipenem and P/T [45, 36]. In our study, we demonstrate that ESBL producers are susceptible to P/T was the best combination antibiotic as followed by A/C and A/S.

CONCLUSION

Major outbreaks involving ESBL strains have been reported from all over the word thus making them emergence pathogen [46] and number of nosocomial outbreaks caused by ESBL producing organisms [47]. The prevalence of ESBL among clinical isolates varies greatly worldwide and the pattern are changing overtime [48]. The spread of ESBL positive strains in hospitals, there is a need to formulate a policy of empirical therapy in high risk unit were infection due to resistant organism is much higher [49]. Equally important is the information on an isolate from a patient to avoid the misuse of extended spectrum Cephalosporins, which still remain as an important component of antimicrobial therapy in high risk wards [50]. Carbapenems are the most active and reliable treatment options for infections which are caused by the ESBL producing isolates [20]. The knowledge in the awareness and the detection of the resistant pattern of the microorganisms is necessary for the judicious antibiotic use. But there is a need to emphasize on the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. In addition, regular antimicrobial susceptibility surveillance is essential.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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