Extended Spectrum beta lactamase producing gram negative bacteria causing nosocomial Urinary tract infection and its susceptibility pattern

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Abstract

The antibiotic resistance is a problem of deep concern both in hospital and community setting. Production of extended spectrum beta lactamase is a significant resistance mechanism that impedes the antimicrobial treatment of infection caused by *Enterobacteriacae*. ESBL are rapidly evolving group of beta lactamase which share the ability to hydrolyze third generation cephalosporin but are inhibited by clavulanic acid. In this present work about 25 urine samples was collected from Medical College. About seven different strains were isolated and identified. Detection of ESBL producers were carried out by many methods with included screening for ESBL producers and phenotypic confirmatory test. It was observed that *E.coli, Klebsiella pneumonia* and *Pseudomonas sp* are the common ESBL producers. Identifying ESBL producing organism is a major challenge for the clinical microbiology. Further the isolated ESBL producing gram negative bacteria underwent antimicrobial susceptibility test using Kirby Bauer method. *E.coli* showed more sensitivity to nitrofuratoine, gentamicin. *Pseudomonas sp* showed sensitivity towards gentamicin, meropenem, ciprofloxacin. *K. pneumoniae* showed towards imipenem, gentamicin, piperacillin and meropenem. There is no doubt that ESBL producing infections are a grave concern to medical world. The aim of this study was to determine the rate of ESBL producing Gram-negative bacteria causing nosocomial Urinary Tract Infection as well as their susceptibility pattern to the most commonly used antimicrobials to identify the most appropriate antibiotic treatments for these infections.

Key words: *Antibiotic resistance, Enterobacteriacae, Urinary Tract Infection, Gentamicin*

I.INTRODUCTION

Nosocomial infection is a significant complication of hospitalization. Urinary tract infections (UTIs) are the most common type of nosocomial infections (Sharif *et al*., 2013). Gram-negative bacilli are the most important cause of these infections (Gaynes *et al.*, 2005). These bacteria are showing rising rates of resistance to current therapies. The production of extended-spectrum β-lactamase (ESBL) enzymes is a common mechanism of resistance. ESBLs are enzymes that confer resistance to most beta-lactam antibiotics including penicillins, cephalosporins, and the monobactamaztreonam (Pitout *et al*., 2008). These enzymes have been found exclusively in Gram-negative organisms (Jain and Roy *et al.*, 2003). Although the prevalence of ESBL-producing *Escherichia coli* can vary from country to country, resistance rates to many commonly used therapies have increased throughout the world (Hawser *et al.*, 2011). *E. coli* is the most common cause of UTI (Sievert *et al*., 2013). Cases of UTI caused by ESBL-producing *E. coli* and *Klebsiella pneumoniae* as well as *Pseudomonas aeruginosa*, including multidrug-resistant (MDR) strains, are increasing (Zilberberg *et al*.,2013).

Antibiotic resistance may also be either mutational or acquired. This implies changes in the bacteria that prevent the antibiotic from exerting its effect on the bacterial target, which may have resulted from either (1) mutation of existing genetic material within the bacteria or (2) acquisition of new genetic material from other bacteria. For example, *Escherichia coli in* its natural state may be susceptible to both Ampicillin and Ciprofloxacin. However, the mutation of existing genetic material may lead to Ciprofloxacin resistance, and the acquisition of genes that encode for beta-lactamase production may lead to resistance of E. coli to Ampicillin. The problems of antibiotic resistance are typically magnified in a hospital setting. Exposure to antibiotics while a patient is in the hospital may lead to genetic mutations that contribute to antibiotic resistance. Patients may be inadvertently exposed to the bacterial flora of other patients. As a result, antibiotic-resistant bacteria may colonize multiple patients. Exposure of these patients to antibiotics may eliminate all but the most resistant bacteria. These resistant organisms may transfer antibiotic resistance genes to other bacteria, there by multiplying the problem (David *et al*., 2007).

2. MATERIALS AND METHODS

2.1 Sample Collection

The urine sample was collected from Medical College, Trivandrum. About 25 samples were collected from patient suffering from nosocomial urinary tract infection. The samples were incubated at 37◦C for 24 -48 hours. The culture were inoculated to plates containing Cetrimide agar, Mac Conkey agar, Eosin Methylene Blue agar,XLD agar,SS agar and CLED agar and incubate (Dubey, 2007).

2.2 Characterization of bacterial isolates

Bacterial isolates were characterized using Bergey's manual of Determinative Bacteriology (Holt *et al*., 1993) based on morphology, microscopic, macroscopic, biochemical and physiological characters.

2.3 Disk-Diffusion methods

The Clinical and Laboratory Standards Institute (CLSI) has proposed disk-diffusion methods for screening for ESBL production. Muller Hinton agar was prepared and swabbed with 24 hours old culture of isolated sample. Antibiotic discs such as Cefpodoxime, ceftazidime, aztreonam, cefotaxime or ceftriaxone disks are placed on the plates. The plates are incubated at 37◦C for 24 hours. After incubation note the zone of inhibition was measured (Wayne, 2009).

2.4 Screening by dilution antimicrobial susceptibility tests

Ceftazidime, aztreonam, cefotaxime or ceftriaxone were used at a screening concentration of 1 µg/mL or cefpodoxime at a concentration of 1 µg/mL for *Proteus mirabilis*; or 4 µg/mL, for the others. Muller Hinton broth was prepared and the above antibiotics were added. Then the test organism is inoculated. After incubation growth was noted (Silva, 2000).

2.5 Phenotypic confirmatory tests for ESBL production

The CLSI advocated the use of cefotaxime (30 µg) or ceftazidime (30 µg) disks with or without clavulanate (10 µg) for phenotypic confirmation of the presence of ESBLs in *Klebsiella* and *Escherichia coli, P. mirabilis and Salmonella species*. The disk test was performed with confluent growth on Mueller-Hinton agar. After incubation the zone diameter is measured in both the plates (Wayne, 2009).

Antibiotic disks containing ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg) and aztreonam (30 µg) was placed on the clavulanate- acid containing agar plates and regular clavulanate-free Mueller-Hinton agar plates inoculated with test orgamism. Then the plates are at 37^0C for 24 hours and zone of inhibition was noted.

2.6 Disk approximation test

Muller Hinton agar plates were prepared and swabbed with the test organism. Cefoxitin disk at a distance of 2.5 cm from cephalosporin disk. Then the plates were incubate at 37^0C for 24 hours and result was observed (Revathi,1997).

2.7 Double Disk Diffusion Test

Muller Hinton agar plates were prepared and disk of amoxicillin and disk of cefotaxime were placed 30mm apart on the inoculated plates. The same procedure was carried with aztreonam, ceftazidine and ceftriaxone. Plates were incubated at 37◦C for 24 hours and result were noted **(**Jarlier Nicolas *et al*.,1988)

2.8 Disk Replacement Method

Three amoxicillin disks are applied to a plate inoculated with test organism in Muller Hinton agar. After one hour incubation at room temperature these antibiotic disk are replaced bycefotaxime, ceftrazidime and aztreonam. Then the plates were incubated at 37◦C for 24 hours and the zone diameter was measured (Schooneveldt *et al*., 1998)

2.9 Antimicrobial Susceptibility Test

Muller Hinton agar plates are prepared and test organism was inoculated. antibiotic disk of Ampicillin, Amikacin, Ceftazidime, Ciprofloxacin, Piperacillin, Tetracyclin, Gentamicin, Imipenem, Meropenen are place and incubated. After incubation zone of inhibition was measured.

III. RESULTS

3.1 Sample collection and identification

Urine sample were collected and isolated 7 strains. The isolated strains were characterized as *Shigella* sp., *Salmonella* sp., *Pseudomonas* sp., *Escherichia coli, Klebsiella* sp., and *Proteus* sp., based on their macroscopic, microscopic, biochemical and physiological characters. After biochemical characterization the bacterial pathogens were confirmed by growing in selective media.

3.2 Disk diffusion method

The plates were observed for the zone formation. The zone diameter ≤ 22mm is considered as ELBL producers. From the seven isolates three strains had a zone diameter less than 22mm.The three isolates were *Klebsiella* sp., *E.coli*., *Pseudomonas* sp.,. The zone diameter for these organism to different organism are represented in table 1.

3.3 Screening by dilution antimicrobial susceptibility tests

Growth at or above the screening antibiotic concentration was suspicious of ESBL production and it was an indication for the organism to be tested by a phenotypic confirmatory test. *E.coli* and *Klebsiella* showed positive result.

3.4 Phenotypic confirmatory tests for ESBL production

A difference of ≥5 mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/ clavulanate disks is the phenotypic confirmation of ESBL production. *E.coli* and *Klebsiella sp* showed a difference of ≥5mm in zone diameter (Table 2). *Pseudomonas* sp., *Escherichia coli, Klebsiella sp* developed a zone width greater than 10mm which confirms that these organisms are ESBL producers (Table 3).

3.5 Disk approximation test

E.coli, Pseudomonas and *K.pneumonia* showed a flattening of the zone of inhibition of the cephalosporin disk towards inducer disk by >1 mm which evidenced that these organism gives positive result (Table 4). A clear extension of the edge of the inhibition zone of cephalosporin towards clavulanate disk was interpreted as positive for ESBL production .The same was reported while using aztreonam, ceftazidime, ceftriaxone.

3.6 Disk replacement method

A positive test is indicated by a zone increase of≥ 5 form disks which was replaced the amoxicillin disk compared to control disk. This revealed that the isolated strain were ESBL producers (Table 5).

3.7 Antimicrobial susceptibility test

E. coli showed susceptibility for nitrofuratoine, gentamicin, imipenem,meropenem, piperacillin, amikacin and ciprofloxacin. *K. pneumonia* showed susceptibility towards ciprofloxacin, imipenem, meropenem, piperacillin, gentamicin, amikacin. *Pseudomonas* sp., showed susceptibility towards gentamycin, vancomycin,aztreonam,ciprofloxacin (Table 6).

IV.DISCUSSION

In this study of isolated Gram-negative bacilli was ESBL-positive with *Pseudomonas, Klebsiella*, and *E. coli* strains being the most frequent agents. Similarly, in a study performed in India, 48.3% of isolated uropathogens were found to be ESBL producers (Tankhiwale *et al.*, 2004). In contrast to our results, in the study of Hosain Zadegan *et al*.,2009 in an Iranian hospital, 23.5% of isolated Gram-negative microorganisms (53 of 222 isolates) were ESBL producers with the most frequent isolates being *K. pneumoniae* (8.9%), *E. coli* (4.4%), and *P. aeruginosa* (4.4%); also, of nine isolated *Acinetobacter* spp. strains, 2 (0.9%) were ESBL-positive (Hosain Zadegan, *et al.*, 2009). In another Iranian study conducted by Irajian *et al*., 2009 on different clinical specimens, ESBL was detected among 18.1% of all isolated *E. coli* and *K. pneumoniae* strains. Frequency of ESBL production was 17.45% and 19.6% for these two organisms respectively (Irajian, *et al*., 2009). The present study reveals the decreased values than Iranian study which is about 10%. This may be due to the fact that our study was performed only on urine samples as in the above-mentioned works, the most ESBL producing organisms were found in urine samples (39.6% and 88.4%, respectively) Saffar Enayti *et al*., 2008). Also, difference in the origin of isolated pathogens may be another contributing factor. Other studies have reported higher rates of ESBL production in *K. pneumonia* isolates (Durmaz *et al*., 2001).

ESBLs have become widespread in hospital as well as community settings (Pfaller and Segreti, 2006; Shakil, *et al*., 2010). These enzymes are becoming increasingly expressed by many strains of pathogenic bacteria with a potential for dissemination. Presence of ESBLs compromises the activity of a wide spectrum of antibiotics creating major therapeutic difficulties with a significant impact on the outcome of patients. The continued emergence of ESBLs presents a serious diagnostic challenge to the clinical microbiology laboratories (Meeta *et al*., 2013).

In the present study, we observed that *E. coli K. pneumoniae* and *Pseudomonas* isolates were ESBL producers. ESBL production was more common among the *E. coli* isolates as compared to the *K. pneumonia* isolates which is in harmony with the finding of other studies (Tankhiwale *et al*., 2004, Babypadmini and Appalaraju, 2004;Umadevi *et al*., 2011).

The length of hospital stay (>3 days) and prior exposure to beta lactam and aminoglycosides antibiotics were also found as significant risk factors (p = 0.01 and 0.02, respectively) for acquisition of ESBL producing isolates. This is coherent to the finding of Shanthi and Sekar, 2010. Underlying illness, presence of an invasive device and prolonged disease were not found to be significantly associated with the acquisition of ESBL-producing *E. coli* and *K. pneumoniae* among the patients studied. During the last several decades, the prevalence of MDR organisms in hospitals and medical centers has increased steadily. The prevalence of Gram negative bacteria resistant to third generation cephalosporins, fluoroquinolones, carbapenems, and aminoglycosides has also increased (Kritu *et al*., 2013).

Although ESBL activity is inhibited by clavulanic acid, β-lactam inhibitor combinations are not considered optimal therapy for serious infections due to ESBL producers as their clinical effectiveness against serious infections due to ESBL-producing organisms is controversial (Paterson *et al.,* 2005). The majority of ESBL-producing organisms produce more than one β-lactamase, often in different amounts. Additionally, it is well known that ESBL-producing organisms may continue to harbor parent enzymes. Hyperproduction of these non–ESBL-producing β-lactamases or the combination of β-lactamase production and porin loss can also lead to a reduction in activity of β-lactamase inhibitors.

This study showed good consistency between the results of disk diffusion and E-test methods for antimicrobial susceptibility testing of ESBL-producing Gram-negative bacilli. Most inconsistencies were observed for amikacin against *E. coli* and *P.aeruginosa* strains. Therefore, as also shown in similar comparative studies it seems that the agreement level for these two methods depends on both antibiotic and microorganism tested.

This present study showed that large numbers of Gram-negative bacteria causing nosocomial UTIs produce ESBL with most being multi-drug resistant (MDR). Therefore, routine ESBL detection testing and subsequent antibiogram with disk diffusion method could be useful to determine the best treatments for UTI.

Table 1 Disk- Diffusion Method

Sl. No	Organism	Antibiotic disk	Zone of diameter(mm)
1	*E.coli*	Cefpodoxime Ceftazidime Ceftriaxone Cefotaxime Azetreonam	22mm 20mm 18mm 16mm 15mm
2	*Pseudomonas sp*	Aztreonam Cefpodoxime Ceftriaxone Ceftazidime Cefotaxime	20mm 16mm 22mm 18mm 20mm
3	*K.pneumoniae*	Aztreonam Cefpodoxime Ceftriaxone Ceftazidime Cefotaxime	15mm 18mm 20mm 10mm 16mm

Table 2 Cephalosporin combination disks

Sl. No	Micro organism	Zone diameter (mm)	
		Cephalosporin disks	Cephalosporin Clavulanate disks
1	*E.coli*	10mm	15mm
2	*Pseudomonassp*	8mm	14mm
3	*K.pneumoniae*	12mm	19mm

Table 3 Inhibitor – Potentiated disk-diffusion test

Sl. No	Microorganisms	Zone diameter (mm)	
		Clavulanate containing plates	Regular Clavulanate free plates
1	*E.coli*	12mm	4mm
2	*Pseudomonas sp*	11mm	3mm
3	*K.pneumoniae*	15mm	6mm

Table 4 Disk approximation test

SL No	Microorganisms	Inducer disk	Zone of inhibition of Cephalosporin disk
1	*E.coli*		2mm
2	Pseudomonas	Cefoxitin	1.5mm
3	*K.pneumoniae*		3mm

Table 5 Disk replacement method

Table 6

Sl. No	Microorganisms	Antibiotics	Zone diameter	
			Control disk	Replaced disk
1	*E.coli*	Aztreonam Cefotaxime Cefazidime	10m 7mm 6mm	18mm 15mm 12mm
2	*Pseudomonas sp*	Aztreonam Cefotaxime Ceftazidime	8mm 10mm 7mm	15mm 14mm 13mm
3	*K.pneumonia*	Aztreonam Cefotoxime Ceftazidime	9mm 6mm 5mm	15mm 16mm 10mm

Antimicrobial Susceptibility Test

Sl. No	Microorganisms	Antibiotic disks	3 One diameter
1	*E.coli*	Amikacin Ciprofloxacin Cefotaxime Gentamicin Imipenem Meropenem Fosfomycin Nitrofuratonie Piperacillin	19mm 18mm 19.5mm 20mm 17mm 18mm 15mm 22mm 15.5mm
2	*Pseudomonas sp*	Amikacin Ciprofloxacin Gentamicin Imipenem Meropenem Nitrofuratonie Vamcomycin	12mm 15mm 17.5mm 16mm 18mm 15mm 6mm

		Penicillin Tetracycline Piperacillin	R R 16mm
3	*K.pneumonia*	Ciprofloxacin Amikacin Ampicillin Gentamicin Imipenem Meropenem Piperacillin Aztreonam	15mm 12mm 15.5mm 19mm 20.8mm 18mm 16mm 15mm

CONCLUSION

Nosocomial infection is a significant complication of hospitalization. Urinary tract infection is the most common type of nosocomial infection. Large number of gram negative bacteria causing nosocomial urinary tract infection produce ESBL with most of them being multidrug resistant. Cases of urinary tract infection caused by ESBL producing *E.coli*, *Klebsiella pneumonia* as well as *Pseudomonas aeruginosa* are included in multidrug resistant strain. Problems associated with ESBL producing isolates include multidrug resistance, difficulty in treatment and increased mortality of patients. Most of our study isolates were found to be resistant to many antibiotics. Imipenem, Gentamicin and Meropenem can be suggested as drug of choice in our study. Length of hospital stay andprior exposure to antibiotics were found to be significant risk factor associated with ESBL producing *E.coli*, *Klebsiella pneumonia* and *Pseudomonas sp* acquisition status of patient. Therefore restricting the 3rd generation cephalosporin along with implementation of infection control measures are the most effective means of controlling and decreasing the spread of ESBL producing pathogen.

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