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Prevalence of Extended Spectrum Beta Lactamases in Uropathogenic *Escherichia coli* and *Klebsiella* Species in a Chennai Suburban Tertiary Care Hospital and its Antibiogram Pattern

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ABSTRACT

The prevalence of Extended Spectrum Beta Lactamases (ESBL) among uropathogenic bacteria have increased over time raising a global concern in the therapeutic management of infections caused by these organisms. ESBLs contribute to multidrug resistance among the organisms and detection of these enzymes is crucial in analyzing the antibiogram pattern of such isolates for empirical therapy. The aim of the study was to analyze the prevalence of ESBL producers among UTI patients of a tertiary care hospital in Chennai suburban. Among 131 clinical isolates obtained from patients, *E. coli* and *Klebsiella* species were identified to be the dominant uropathogens. Production of Extended Spectrum Beta Lactamases (ESBL) was detected among *E. coli* and *Klesiella* species following the methods recommended by the CLSI including the double disc diffusion test and phenotypic confirmatory test. About 47% of *E. coli* and 36% of *Klebsiella* species were identified as ESBL producers. Co-resistance to non-β-lactam antibiotics such as gentamycin, co-trimoxazole, nitrofurantoin and ciprofloxacin was demonstrated by Kirby-Bauer method. ESBL producing isolates of *E. coli* and *Klebsiella* species were found to be resistant to more than three antibiotics. Imipenem was found to be the most effective drug against most of the isolates tested followed by amikacin. It is concluded that there is an urgent need for the microbiology laboratories in the suburban of Chennai to include ESBL detection procedures as a routine along with conventional antibiogram analysis for obtaining better therapeutic options.

Key words: Uropathogens, β-lactams, multidrug resistance, ceftazidime, cefotaxime, minimum inhibitory concentration, extended spectrum beta lactamases

INTRODUCTION

Urinary Tract Infection (UTI) is one of the most common infectious diseases in humans (Mohammadi *et al*., 2010) that occur in both community and hospital environments. It presents a spectrum of clinical entities upon severity ranging from asymptomatic infection to acute pyelonephritis with sepsis (Fish, 2009).

The etiology of most of the uncomplicated bacterial UTI is by the pathogens such as *E. coli, K. pneumoniae, P. mirabilis, E. faecalis, S. saprophyticus* and those of complicated UTI by *E. coli, K. pneumoniae, E. faecalis, P. aeruginosa* and species of Enterobacter, *Staphylococcus, Serratia* and *Acinetobacter*. Among bacterial UTIs, *E. coli* is the most prevalent cause (Manikandan *et al*., 2011;

Sibi *et al*., 2011) accounting for greater than 80% of the infections and appears to be a true community pathogen (Pitout and Laupland, 2008).

UTIs are often treated with different broad-spectrum antibiotics (Akram *et al*., 2007) like penicillin and cephalosporins. However the emerging resistance among the uropathogenic strains, effective therapy with the above mentioned antibiotics has been hindered.

This resistance towards β-lactam antibiotics is mainly mediated by the production of a diverse set of enzymes, β- lactamases which are capable of hydrolyzing the β-lactam ring (Bradford, 2001).

The increased use of second and third generation cephalosporins to treat infections caused by such resistant strains has led to the development and selection of multiple resistant organisms (Pitout and Laupland, 2008). The production of a set of enzymes called the Extended Spectrum Beta Lactamases (ESBLS) may result in the development of multidrug resistance (Babypadmini and Appalaraju, 2004).

There are diverse types of ESBLs which include TEM, the first plasmid-mediated β lactamase in gram negative bacteria named after the patient Temoniera in Greece. SHV is another plasmid-mediated β-lactamase named for sulphydryl variable and CTX that preferentially hydrolyze cefotaxime. OXA is another β-lactamase belonging to molecular class D and functional group 2 day possess hydrolytic activity against oxacillin and cloxacillin. Other β-lactamases like PER that efficiently hydrolyzes penicillins and cephalosporins, VEB, a β-lactamase isolated from a patient from Vietnam, GES for Guiana extended spectrum (Lee *et al*., 2007) and IBC β-lactamases. The IBC-1 enzyme is a novel integron-associated class A extended-spectrum β-lactamase that hydrolyses all β-lactams except the cephamycins and the carbapenems (Bradford, 2001; Thomson, 2010). These infections are associated with increased morbidity and mortality (Aggarwal *et al*., 2009; Mansouri *et al*., 2011).

In India, the prevalence of ESBLs have been reported since 1990s (Revathi and Singh, 1997; Karim *et al*., 2001; Mathai *et al*., 2002; Gupta, 2007; Aggarwal *et al*., 2009; Narayanaswamy and Mallika, 2011) and very few reports of them existing in South India have been published (Babypadmini and Appalaraju, 2004; Menon *et al*., 2006; Kingsley and Verghese, 2008; Mano and Vasanthi, 2008; Kamatchi *et al*., 2009; Narayanaswamy and Mallika, 2011). Reports on the etiology and resistance pattern of community acquired UTIs in the rapidly developing Chennai Suburban are scarce. The objective of this study was to analyze the antibiotic susceptibility pattern and to find out the prevalence of ESBLs among uropathogenic *E. coli* and *Klebsiella* species isolated from patients with community-acquired UTIs from a tertiary care hospital in the southern suburban of Chennai.

MATERIALS AND METHODS

A total of 131 urine samples were collected from out patients clinically suspected to have UTI from local tertiary care hospital near Chennai between December 2009 and November 2010. The clinical and demographic details regarding the identification, age, sex of the patients were recorded. After processing the samples for significant bacteriuria, the isolates were identified by performing the routine bacteriological identification tests (Koneman *et al*., 1997) and their colony morphology on HiCrome UTI agar (Himedia Laboratories, India). Among the isolated strains (126), only the *E. coli* (58) and *Klebsiella* species (39) isolates identified were included in the study as they were dominant.

Antibiogram assay: A panel of thirteen antimicrobial agents comprising of β lactam and non-β lactam antibiotics (Himedia laboratories, India) were tested by the disc diffusion method against

the isolates of *E. coli* and *Klebsiella* species as recommended in the CLSI guidelines (CLSI, 2005). *E. coli* (ATCC 25922) and *K. pneumoniae* (ATCC 700603) were used as reference strains throughout the study.

The antibiotics included in the study were piperacillin/tazobactam (100 10 μg^{-1}), imipenem (10 μg), amikacin (30 μg), gentamycin (10 μg), co-trimoxazole (1.25/23.75 μg), cefuroxime (30 μg), ceftriaxone (30 μg), nitrofurantoin (300 μg), ciprofloxacin (5 μg), ceftazidime (30 μg), ampicillin (10 μg), cefotaxime (30 μg) and tobramycin (10 μg). Any isolate was considered as multidrug resistant (MDR), if it showed resistance to ≥3 antimicrobial agents (Aggarwal *et al*., 2009).

Detection of ESBL production: The methods used in this study involved the testing of the isolates for ESBL production against oxyimino β-lactam antibiotics following the recommendations of Clinical and Laboratory Standards Institute (CLSI) formerly NCCLS (CLSI, 2005).

Double Disc Synergy Test (DDST): In the DDST, synergy was determined between a disc of amoxyclav (20 μg amoxycillin and 10 μg clavulanic acid) and a 30 μg disc of each 3rd generation cephalosporin (3GC) (ceftazidime 30 μg mL^{-1}, cefotaxime 30 μg mL^{-1}) placed at a distance of 30 mm apart on Mueller-Hinton agar swabbed with the resistant isolates and incubated at 37°C for 18 to 24 h. The organisms were considered to produce ESBL, if the zone size around the 3GC disc extended towards the amoxyclav disc.

Cephalosporin clavulanate combination discs: The phenotype confirmatory test for ESBL production was performed with the use of ceftazidime (30 μg), cefotaxime (30 μg) with and without clavulanic acid against the isolates. Only the isolates that showed synergy in the DDST procedure were included for the test. The discs were placed on pre inoculated Mueller-Hinton agar and incubated at 37°C for 18 to 24 h. A difference of ≥5 mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/clavulanate disk is taken to be phenotypic confirmation of ESBL production.

Micro broth dilution method: The Minimum Inhibitory Concentration (MIC) values for ceftazidime and cefotaxime with and without clavulanic acid were determined with Mueller-Hinton broth by micro broth dilution test under sterile conditions in micro titer plates. The results were interpreted as per the CLSI guidelines. Phenotypic confirmation is considered as a ≥3-two-fold-serial-dilution decrease in MIC of either cephalosporin in the presence of clavulanic acid compared to its MIC when tested alone.

Agar supplemented with clavulanate test: Antibiotic discs containing ceftazidime (30 μg), cefotaxime (30 μg) were placed on to the Mueller Hinton agar supplemented with clavulanic acid (4 μg mL^{-1}) and also on regular clavulanate-free Mueller-Hinton agar plates and were incubated for 18 to 24 h at 37°C. A difference in the β-lactam zone width of ≥10 mm on the two media is considered positive for ESBL production (Paterson and Bonomo, 2005; Al-Jasser, 2006).

Disc replacement method: Two amoxyclav discs were placed on Mueller-Hinton agar inoculated with the test organisms. After one hour incubation at room temperature, the amoxyclav discs were replaced with ceftazidime and cefotaxime discs on the same spot along with control discs of

ceftazidime and cefotaxime placed at least 30 mm from the replaced discs and incubated at 37°C for 18 to 24 h. A positive test is indicated by a zone increase of ≥5 mm for the discs which have replaced the amoxyclav compared to the control discs (Paterson and Bonomo, 2005; Al-Jasser, 2006).

RESULTS

Out of 131 urine samples obtained, 126 samples were considered positive for UTI. The uropathogens isolated from the UTI positive samples included *E. coli* (58), *Klebsiella* species (39), *Proteus mirabilis* (13), *Pseudomonas aeruginosa* (9), *Staphylococcus* species (6) and *Citrobacter* species (1). Only *E. coli* and *Klebsiella* species were included in this study to detect ESBL production as they were the dominant strains among the other uropathogens.

The antibiogram pattern of the isolates was analyzed. Determination of the multidrug resistance showed that 59% of *E. coli* and 67% of *Klebsiella* species were MDR. All the *E. coli* and *Klebsiella* isolates showed 100% susceptibility to imipenem. Among the ESBL positive isolates, only 37% of *E. coli* and 36% of *Klebsiella* species were susceptible to amikacin and 26% of *E. coli* and 29% of *Klebsiella* species showed susceptibility to gentamycin. The non-ESBL producing isolates showed more than 50% susceptibility to other non-β lactams antibiotics with amikacin being the most effective drug (Fig. 1). Intermediate susceptibility to other antibiotics was also detected in the isolates of *E. coli* and *Klebsiella* species.

In the DDST procedure for ESBL detection, 27 *E. coli* (47%) isolates and 14 *Klebsiella* species (36%) showed a synergy and a clear zone of extension towards amoxyclav. Interestingly, three *E. coli* isolates (UTEC18, UTEC31 and UTEC55) showed susceptibility to ceftazidime in the routine antimicrobial susceptibility testing but revealed a synergy indicative of ESBL production.

All the 27 *E. coli* and 14 *Klebsiella* species including the three isolates which showed susceptibility to ceftazidime were confirmed to be ESBL producers by the cephalosporin/clavulanate combination method.

The Minimum Inhibitory Concentration (MIC) values of ceftazidime and cefotaxime of the ESBL positive *E. coli* and *Klebsiella* species displayed a ≥3 log 2 (two fold) dilution reduction in the presence of clavulanic acid in the broth micro dilution method. The MIC values of ESBL positive *E. coli* and *Klebsiella* species showed a high level resistance of ≥2 μg mL^{-1} to ceftazidime two fold dilution reduction of ≥1 μg mL^{-1} to ceftazidime/clavulanate combination. The MIC values of the ESBL positive isolates towards cefotaxime alone were ≥64 μg mL^{-1} and for cefotaxime/clavulanate combination was ≥1 μg mL^{-1} (Table 1, 2).

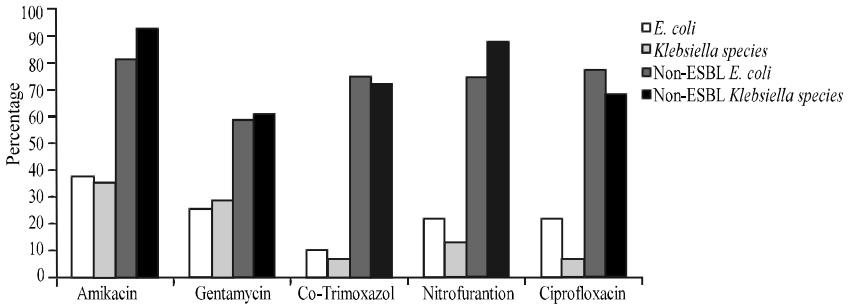

Fig. 1: % Susceptibility of the isolates to non-β lactam antibiotics

Table 1: Mic values of *E. coli* by microbroth dilution method (≥3 log 2 (two fold) decrease in values is significant)

Isolates	CE (0.25-64 μg mL^{-1}) CE/CL (0.25/4-128/4 μg mL^{-1})	CA (0.25-128 μg mL^{-1}) /CA/CL (0.25/4-64/4 μg mL^{-1})
UTEC1	32/2	8/1
UTEC2	8/1	64/1
UTEC3	64/2	16/2
UTEC4	32/2	32/2
UTEC5	32/1	16/2
UTEC18	16/1	16/2
UTEC20	32/2	64/8
UTEC22	64/2	64/1
UTEC24	64/1	128/1
UTEC27	32/2	64/0.5
UTEC29	64/1	32/0.5
UTEC31	16/0.5	8/1
UTEC32	32/2	64/2
UTEC35	16/1	32/1
UTEC38	16/2	64/2
UTEC40	32/2	32/1
UTEC41	64/2	128/2
UTEC43	64/1	64/0.5
UTEC45	64/2	32/1
UTEC47	32/1	64/1
UTEC48	64/2	32/0.5
UTEC51	32/1	32/1
UTEC53	32/2	32/1
UTEC54	32/1	32/0.5
UTEC55	8/0.5	16/2
UTEC56	16/2	16/1
UTEC57	64/2	64/0.5

Table 2: Mic values of *klebsiella* isolates by microbroth dilution method (≥ log 2 (two fold) decrease in values is significant)

Isolates	CE (0.25-64 g mL^{-1}) E/CL (0.25/4-128/4 g mL^{-1})	CA (0.25-128 μg mL^{-1}) CA/CL (0.25/4-64/4 g mL^{-1})
UTK1	32/4	4/0.5
UTK2	16/2	8/1
UTK3	16/2	8/2
UTK12	32/2	128/2
UTK15	32/1	64/0.5
UTK16	8/0.5	16/1
UTK20	16/2	64/2
UTK21	8/1	32/1
UTK22	8/1	32/1
UTK29	64/1	128/2
UTK30	32/0.5	64/1
UTK33	16/1	32/0.5
UTK36	8/0.5	32/1
UTK38	32/1	64/1

In the agar supplemented with clavulanate test all the ESBL producing *E. coli* and *Klebsiella* species displayed a zone difference of >10 mm when tested alone and in the presence of clavulanic acid (4 μg mL^{-1}). In the disc replacement method, a zone difference >5 mm was observed with all the ESBL positive isolates.

DISCUSSION

UTIs occur frequently in both community and hospital environments and are the most common bacterial infections in human beings. Extremes of age, female gender, sexual activity, contraception, pregnancy, instrumentation, UT obstruction, neurologic dysfunction, previous antimicrobial use and other such factors act as predisposing factors for UTI development.

Extended spectrum cephalosporins in addition to amino glycosides are important therapeutic agents in medicine and are often used for the therapeutic management of UTIs in India. The development of drug resistance could be attributed to the indiscriminate use of the antibiotics (Akram *et al*., 2007) and also to the availability of some of the drugs over the counter.

It has become very essential to treat and control the infections caused by the ESBL producing pathogens and hence the need for the development of novel antibiotics and the discovery of new antibacterial agents is very urgent. A report by the Infectious Diseases Society of America (IDSA) has mentioned about doripenem's activity to be similar to meropenem and was recently approved by Food and Drugs Administration (FDA) as a therapeutic agent for complicated urinary tract infections (Boucher *et al*., 2009).

The study revealed *E. coli* and *Klebsiella* species to be the dominant organisms among other uropathogens. This result coincides with the previous report on the predominance of these pathogens (Shareef and Yagoub, 2006; Selvakumar and Jasmine, 2007; Karou *et al*., 2009; Zinnat *et al*., 2011). The prevalence of ESBL producers among the 131 clinical isolates was found to be 47% with *E. coli* and 36% with *Klebsiella* species. Previous reports of ESBL occurrence among uropathogenic *E. coli* and *Klebsiella* species in India were known to be 40 and 54.54%, respectively (Aggarwal *et al*., 2009; Patel *et al*., 2010). The ESBL positive isolates of *E. coli* and *Klebsiella* species were found to be multidrug resistant. This finding is correlated with that of Bhowmick and Rashid (2004). In addition to that a higher level resistance to non-β-lactam antibiotics in addition to β-lactams in the ESBL producers than non-producers was also demonstrated. This is in accordance with the previous study in South India (Selvakumar and Jasmine, 2007). Amikacin was found to be the most effective antibiotic next to imipenem against the ESBL producers. These findings are in accordance with the previous results (Babypadmini and Appalaraju, 2004). Although, susceptibility of ESBL producers to amikacin was reported as 86% in the previous study (Babypadmini and Appalaraju, 2004) only around 36% of the ESBL positive isolates showed susceptibility to amikacin. All strains of ESBL positive *E. coli* and *Klebsiella* species showed 100% susceptibility to imipenem and these results had correlated with the findings of earlier studies (Kingsley and Verghese, 2008).

The DDST procedure was found to be a reliable and efficient method for the detection of ESBL in the isolates. Three isolates which showed susceptibility to ceftazidime in the conventional antibiogram testing were confirmed to be ESBL producers. This inhibitor based detection method is cost effective and can be easily performed in all laboratories.

The MIC values of ceftazidime and cefotaxime in all the ESBL positive *E. coli* and *Klebsiella* species displayed a ≥3 log 2 (two fold) dilution reduction in the presence of clavulanic acid by broth micro dilution method. In this study, the ESBL producing isolates exhibited high level resistance

to ceftazidime (up to 64 μg mL^{-1}) and to cefotaxime (up to 128 μg mL^{-1}). Previous report also demonstrated high level resistance to the above mentioned drugs (Babypadmini and Appalaraju, 2004; Kingsley and Verghese, 2008). The method was found to be reliable for ESBL detection and also to determine the appropriate drug concentration for therapy.

The agar supplemented with clavulanate test method confirmed the ESBL production in the clinical isolates but was found to be laborious. It also suffered from a drawback in preparing the clavulanate containing agar freshly before each use (Al-Jasser, 2006) and hence could not be used in a microbiology laboratory on a routine basis. However, it can be used in conjunction with DDST or cephalosporin/clavulanate combination test for ESBL detection.

The disc replacement method was a useful test in the confirmation of ESBL production in the isolates. It was found to be easy to perform and could be used in conjunction with the DDST for routine ESBL surveillance. The findings in this study have clearly indicated the high prevalence of ESBL producing organisms in the community acquired UTI among the patients in Chennai suburban.

Prevalence of ESBL producing organisms in South India have been reported in recent years (Menon *et al*., 2006; Kingsley and Verghese, 2008; Kamatchi *et al*., 2009; Narayanaswamy and Mallika, 2011) but the information on the antibiotic resistance pattern in the community acquired UTIs in the suburbs of Chennai is scarce.

Over the past few years Chennai has seen a spurt of development, both industrial as well as residential, in certain suburban localities. The pressure of an almost unstoppable increase in population in these areas has increased the risk of communicable diseases due to factors like improper sanitation and lack of hygiene.

To our knowledge this is the first report on the presence of ESBL producing uropathogenic *E. coli* and *Klebsiella* species in the southern suburban of Chennai and may be of significance because of the potential risk of transfer of ESBL producing pathogens to others in a population.

CONCLUSION

Microbial resistance to antibiotics has gained major concern because of difficulty in treating infections caused by drug resistant strains. All the ESBL producing isolates and some non-producers included in this study were found to be multidrug resistant, few being resistant to all the antibiotics tested with the exception of imipenem. Among the other antibiotics, amikacin was found to be effective. Because ESBL production in the organisms not only confers resistance to the β-lactams but also confers co-resistance to other classes of antibiotics there is a limited option in selection of antibiotics for therapy. Most of the laboratories in the suburbs do not perform regular ESBL screening procedures. Hence it can be concluded that routine and prompt ESBL screening along with conventional antimicrobial testing should be followed in all microbiology laboratories to aid the physician in the therapeutic management of infections caused by multidrug resistant strains. Also, measures to prevent and control the spread of ESBLs are the much needed approach. An antibiotic holiday could be declared by the physicians for the first few days until accurate diagnosis is made and the patients should complete the entire course of the recommended treatment. Other measures to control the spread of drug resistance include public awareness programmes involving prudent and judiciary use of antibiotics which in turn reduce the selective pressure on organisms and surveillance of infections, prompt medical compliance must be taken. Antibiotic stewardship program to enhance the clinical outcome and to control the emergence of resistance should be followed to prevent this global problem.

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