Antimicrobial Susceptibility Pattern of ESBL and Non-ESBL Producing Uropathogenic Escherichia coli (UPEC) and Their Correlation with Biofilm Formation

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Abstract: Extended Spectrum β-Lactamase (ESBL) producing Escherichia coli strains is reported to be the cause of community and hospital acquired infections. E. coli responsible for urinary tract infection (UTI) have capability to produce ESBLs in huge amounts. Biofilm are group of microorganisms encased in an exopolymer coat. The present study was undertaken over a period of one year from October 2009 to September 2010 to study the prevalence of ESBL and biofilm producer E. coli among UTI patients attending the tertiary care hospitals. Hundred isolates of E. coli were obtained from 166 urine sample and thereafter were subjected to susceptibility testing according to the CLSI guidelines using 12 different antibiotics. They were further screened for ESBL production by screening test (62%), double disc approximation test (43%) and NCCLS confirmatory test (34%) respectively. Of the 100 isolates of E. coli, 34 (34%) were found to be ESBL producer. E. coli exhibited 100% susceptibility only to imipenem and resistant to amikacin, amoxyclv, erythromycin, ampicillin and norfloxacin were observed among ESBL producers. The multiple drug resistance patterns of E. coli and the correlation between ESBL and biofilm producing E. coli was also determined. The results of present study indicated a need for continued surveillance of antimicrobial resistance among ESBL and biofilm producing uropathogens causing UTI, so as to increase positive outcomes of clinical interventions.

Key words: Escherichia coli · Urinary Tract Infections · Extended Spectrum β-Lactamase · Biofilm · Drug Resistance · CLSI Guidelines Antimicrobial Resistance

INTRODUCTION

Escherichia coli is the most frequent agent of urinary tract infection (UTI) and are considered to be the most common bacterial infections [1]. Long-term exposure to antimicrobial agents directly increases the selection pressure for resistance [2]. The enzymes, known as extended-spectrum b-lactamases (ESBL) hydrolyses expanded-spectrum cephalosporins, such as ceftazidime (CAZ), cefotaxime (CTX) and/or the monobactam aztreonam [3]. In recent years, ESBL-producing Enterobacteriaceae have emerged in hospitals worldwide [4, 5] and are associated with nosocomial outbreaks caused by single enzyme-producing strains. Infections caused by extended-spectrum (ESBL) producing Enterobacteriaceae in non hospitalized patients seems to be emerging in different countries [6-9].

However, recent studies have revealed more complex situations giving rise to a significant increase of ESBL-producing strains in the community [8-13]. The appearance of extended-spectrum β-lactamase-producing strains (ESBLs) and the severely limited therapeutic options currently available for this organism has increased its importance in the last few years [14].

Urinary tract infections (UTIs) are the most frequently community acquired bacterial infections which is associated with significant morbidity and mortality especially in children [15, 16]. The pathogenic potential of
*E. coli* strains is thought to be dependent on the presence of virulence factors (VF’s) [17-19]. As extra-intestinal pathogenic *E. coli*, uropathogenic *E. coli* (UPEC) are the most etiologic agent that constitutes a major target for antimicrobial therapy [20]. In fact, antimicrobial therapy is the main method of treatment. Different antibiotics can be used to treat UTI infections, but quinolones are an important class of antibiotics, because they have wide spectrum activities with excellent bioavailability, good penetration and low incidence of side effect [16, 21, 22].

The most prescribed antibiotics to combat a variety of UTI infections, had higher the rate of resistance among bacterial UPEC [23]. This resistance had become a major trouble in the treatment and management of the infections in different countries [24, 25]. The presence of integrons is associated with antimicrobial resistance and is being increasingly reported worldwide [26-28].

There is a paucity of data on the prevalence of ESBL and biofilm producer *E. coli* among UTI patients in South India. The present study was undertaken to detect the ESBL and biofilm producing *E. coli* in UTI patients and also determine their antibiogram profile which assumes a great significance.

**MATERIALS AND METHODS**

**Collection of Urine Sample:** A total of 100 consecutive non-duplicate clinical isolates of *E. coli* from 166 urine specimens were studied for ESBL and biofilm production were collected from tertiary care hospitals in and around Coimbatore. Identification of the isolates was done based on cultural characteristics and reactions in standard biochemical tests [29]. All *E. coli* isolates were included in the study and were analyzed for the correlation between susceptibility pattern of biofilm production and β-lactam antibiotics in ESBL producers.

**Detection of Biofilm Formation:** All the 100 *E. coli* isolates were subjected to biofilm production. Numbers of tests are available to test biofilm production in *E. coli*. The methods include Tissue Culture Plate method, Tube method (TM) Christensen et al. [33] and Congo Red Agar (CRA) methods.

**Test for ESBL Production**

**Screening Test [30]:** The organism was swabbed on to a Mueller-Hinton agar plate. Antibiotic discs of amoxicillin / clavulanic acid (20/10 µg) and cefotaxime (30 µg) were placed and incubated. The clear extension of cefotaxime inhibition zone towards the disc containing clavulanate was considered as ESBL producer.

**Double Disc Approximation Test [31]:** The organism was swabbed on to a Mueller-Hinton agar plate. Antibiotic discs of amoxicillin / clavulanic acid (20/10 µg) and cefotaxime (30 µg) were placed and incubated. The clear extension of cefotaxime inhibition zone towards the disc containing clavulanate was considered as ESBL producer.

**NCCLS Confirmatory Test [30]:** While performing antibiotic testing, ceftazidime (30 µg) and ceftriaxime plus clavulanic acid (30/10 µg) were placed on Mueller-Hinton agar and incubated. Organism was considered as ESBL producer, if there was a > 5mm increase in zone diameter of ceftazidime / clavulanate disc and that of ceftriaxime disc alone. *Escherichia coli* ATCC 25922 was used as negative control.

**Antibiotic Susceptibility Testing:** The above isolates were tested for antimicrobial susceptibility by disc diffusion technique according to Clinical and Laboratory Standards Institute guidelines [32] with commercially available discs (Hi-Media, Mumbai). The following antibiotic discs (drug concentration in µg) were used: amikacin (30), ampicillin (10), co-trimoxazole (25), chloramphenicol (30), tetracycline (30), tobramycin (10), gentamicin (15), imipenem (10), norfloxacin (10) and piperacillin/ tazobactam (100/10).

**RESULTS AND DISCUSSION**

Of the 166 urine specimens of urinary tract infection processed 146 (87.9%) specimens showed culture positive and the rest 20 (12.0%) were negative. Among the isolates, aerobic gram negative *E. coli* was 100 (68.5%) and other organisms were 46 (31.5%).

**Extended Spectrum β-Lactamases in *E. coli* isolates of UTI:** The augmented occurrence of ESBL producing *Enterobacteriaceae* forms an immense need for testing methods that correctly identifies the enzymes in urine samples. The detection of ESBL strains is of imperative significance as they are responsible for the spread of resistant genes in hospitals and community settings. From 100 *E. coli* isolates, we detected 34%, 43% and 62% of ESBL producer by screening test, DDAT test, NCCLS followed to detect ESBL’S in isolate of *E. coli*. 

for ceftazidime and ≤ 27 mm for cefotaxime was recorded, the strain was considered as ‘suspicious’ for ESBL producer.
Antibiotic Susceptibility Pattern of ESBL Producers:
The antibiotic susceptibility patterns in ESBL producer isolated from urinary tract infected patients were found to be as follows: the high percentage of susceptibility to imipenem (100%), chloramphenicol (97%) and gentamicin (42%) were observed among ESBL producers (Table 1).

Antibiotic Resistance in ESBL and Non-ESBL Producers:
The antibiotic resistance patterns of the ESBL producing E. coli isolates have showed maximum resistance to amoxyclv (95%), gentamicin (86%), tetracycline (83%) and piperacillin / tazobactam (81%). Both ESBL producer and Non-ESBL producer were resistant to amoxyclv and gentamicin. However, resistance to other four antibiotics such as tetracycline (83% vs 66%), amikacin (79% vs 64%), ampicillin (79% vs 52%) and tobramycin (67% vs 52%) was comparatively higher among ESBL producer than non ESBL producer. Resistance among ESBL producer to norfloxacin was also high (67% vs 51%) when compared with non-ESBL producers. 100% sensitive was noticed only for Imipenem. In the present study, markedly moderate resistances to aminoglycosides were observed among clinical isolates of E. coli (Table 2).

Correlation Between Biofilm and ESBL Formation:
Thirty four isolates of E. coli producing ESBL and sixty six non-ESBL producing isolates were compared for their ability to form biofilm. Striking difference was observed among ESBL positive and ESBL negative isolates with regard to the biofilm formation ability based on the tube method results. The isolates were classified according to [33] to three groups as follows: 4 (+), 21 (++) , 9 (+++) (Figure 1). Grouping the biofilm formation to weak (+), moderate (++) and strong (+++) showed that lower number of isolates with ESBL positive group had weak reaction (11.76%), in comparison with the ESBL negative group (53.03%) and the difference in the biofilm formation in all three group was significant. When comparing the potential of ESBL and Non ESBL producer among uropathogenic E. coli with regard to the production of biofilm formation. We followed that 26.47% and 12.12% ESBL and Non ESBL producing uropathogenic E. coli exhibited moderate biofilm formations. This shows that the ESBL producer have greater ability is producing biofilm among UPEC their by increase the antibiotic resistance.

In the community, bacterial infection of the urinary tract is one of the common causes for seeking medical attention. Demonstration of bacteria by appropriate
culture methods is one of the methods in diagnosis of UTI [34]. Increase in the spread of ESBL-producers is noticeably rapid globally, indicating the need in continuous monitoring systems and effective infection control measures [35]. Current studies on ESBL production among Enterobacteriaceae isolated from clinical specimens, shows an increase in the incidence of ESBL producers [36].

Earlier studies have reported the incidence of 97% [37]; 58% [38]; 50.7% [39]; 50% [40] and 71% [41] uropathogenic E. coli strains isolated from urine sample which is in accordance with our present study where a higher incidence of 68.5% was observed.

According to Naik and Desai [38], 96.8% of E. coli was recorded to be sensitive to imipenem which is the drug of choice against UTI infections. Chaudhary and Agarwal [42]; Luzzaro et al.[43] have reported that multi drug resistance was observed in ESBL and non ESBL producing strains of uropathogenic E. coli and also reported that 100% sensitivity was observed to imipenem. Even in the present study, all the uropathogenic strains of ESBL and non ESBL producers were found to be 100% sensitive to imipenem respectively. Since, Tankhiwala et al. [44] reported 48.3% and Ritu Aggarwal et al. [45] reported 40% of ESBL strains of uropathogenic E. coli were sensitive which is comparatively lower than our present study findings.

All ESBL positive isolates were found to be 79% resistant to Ampicillin and 100% sensitive to Imipenem [46]. Similarly, Shiju et al. [47] have reported that ESBL positive E. coli strains were 100% sensitive to imipenem and piperacillin-tazobactam and other antibiotics such as gentamicin, ciprofloxacin, co-trimoxazole, nalidixic acid and netilmicin were not suitable for empirical selection. Even in the our study, we report that the ESBL positive isolates were found to be 79% resistant to ampicillin, 100% sensitive to imipenem, 97% sensitive to chloramphencol whereas other antibiotics were found to be less sensitive towards the bacteria.

Ramesh et al. [48] reported the resistance rate of Amikacin and Tobramycin to be 59.5% and 81.31% respectively. Even in the present data, Amikacin resistance rate was found to be 95% as compared to Tobramycin resistant rate which was 67%. Neelam Taneja et al. [49] reported ESBL producing E. coli with a high degree resistance to Piperacillin/Tazobactam and Amoxyclav to be 93.1%, 93.4% as compared to non ESBL producers Piperacillin/Tazobactam and Imipenem to be 31.06% and 11% respectively. Even in the present study, ESBL producing strains were resistant to Piperacillin/Tazobactam (81%) and Amoxyclav (95%) and non ESBL producers showed resistance towards Piperacillin/Tazobactam (70%) and Imipenem (0%).

Agrawal et al. [46]; Basavaraj et al. [35].Naik and Desai [38] reported the prevalence of ESBL producer to be 22%, 32.1% and 66%. Other studies from India have reported the ESBL production varying from 6% to 87% respectively [50- 53]. In recent years increase in ESBL production was reported from several countries such as USA, Canada, China and Italy [54-57]. Similarly, in large survey of 1610 E. coli isolates from 31 centers, 10 European countries found that the prevalence of ESBL in these organism ranged from as low as 1.5% in Germany to high in 39-47% Russia, Poland and Turkey respectively [58]. Arabian Gulf region, high ESBL producer 31.7% in Kuwait and 41% in the United Arab Emirates [59, 60]. Similarly Husam et al. [61] have reported that prevalence the ESBL producer 60% in Saudi Arabia. Babypadmini et al. [62] reported that in Coimbatore (India) ESBL production was 41% in E. coli. Similarly, in our study, prevalence of ESBL producing E. coli was found to be 34% respectively.

Murugan et al. [41] have reported the correlation between biofilm and multiple drug resistance towards uropathogenic E.coli. Even our study showed similar correlation between biofilm and ESBL producing uropathogenic E.coli. Foremost occurrence of ESBL strains have been reported globally, thus making them emerging pathogens [63]. In US, a number of nosocomial outbreaks caused by ESBL producing organisms have been reported [64]. Among the available antimicrobial agents, carbapenens are the most consistent treatment options for infections caused by the ESBL producing isolates [36]. Similar observations were reported by highly prevalence in different regions such as Gujibarga [65] and Bangalore [66] which was lower than the reports from Hubli [67] and Davangere [68].

Kripke [69] have reported combinations of aminoglycoside modifying enzymes were found to be responsible for aminoglycoside resistance. In the present study, among the amino glycosides, tobramycin showed best activity (resistance rate 67%) as a compared to amikacin (resistance rate 79%). The regional variations of resistance to antibiotics have been explained in a constricted manner by different local antibiotics [70].

In conclusion result of this study, we found a significant difference in the biofilm of the ESBL producing E.coli clinical isolates in comparison with non ESBL producing isolates. The results of the present study are in accordance with Samaha-Kfoury and Araj [71].
Biofilm formation is an important virulence factor in many nosocomial infections, so that in 65% of nosocomial infections biofilm are formed [72]. Also in the present study, we found that ESBL producing isolates had a higher ability to form biofilm in comparison with non ESBL producing isolates. It has been suggested that a number of chromosomal gene re-arrangement occurs upon acquisition of the ESBL plasmid. It is possible that higher mortality and severity of infection caused by ESBL producing isolates is due to the expression of several virulence genes simultaneously, rather than gaining new virulence genes [73].

The reason for low detection in this study might be related to interpretation of results because authors used the ratio of the zone diameters with and without clavulanate to infer ESBL production rather than the difference in diameters as recommended by CLSI and organisms of note in this regard are ESBL producing Gram-negative bacteria. UTI patients infected with these strains cannot be treated with beta lactam antimicrobial agent and mono bactums. Amikacin and imipenem are found to be alternative treatment for ESBL producer. Multidrug resistance and ESBL is a common problem in hospital which emphasizes the need for judicious use of antimicrobial agent and their continuous in vitro monitoring.

REFERENCES


