

Antimicrobial susceptibility pattern of Extended Spectrum of Beta Lactamase (ESBL) producing uropathogens from pregnant women

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Abstract

Extended spectrum of beta lactamase (ESBL) producing organisms create a major problem in clinical therapeutics. Antimicrobial resistant pattern of uropathogens were isolated from Government hospital, Thiruvanamalai district, Tamilnadu, India, during the period of January to June 2008. Total of 54 ESBL producing clinical isolates of gram negative bacilli were obtained from urine samples of 50 pregnant women. ESBL producers were detected of which 24(44.4%) isolates were *E.coli*, 20(37%) isolates were *K.pneumoniae* and 10(18.5%) isolates were *P.aeruginosa*. Out of 54 isolates 57.4%, 46.6% and 33.3% were resistant to Ampicillin, Amoxycillin and Ceftriaxone respectively. Of all isolates 100% susceptible to Ampicillin, Amoxycillin and Ceftriaxone combined with sulbactam and clavulanic acid showed synergistic effect. These combined drugs were found to be the choice for UTI infection to pregnant women. This study shows the frequency of drug resistant and ESBL producing *E. coli*, *K. pneumoniae* and *P.aeruginosa* is high in pregnant women. Advance drug resistance surveillance of ESBL isolates is need for perceptible use of antimicrobial agents.

Keywords: Uropathogens, UTI, ESBL, Antimicrobial susceptibility, drug resistant

1. Introduction

Urinary tract infections (UTIs) are the most common infections, with an annual global incidence of at least 250 million cases (Ronald *et al.*, 2001). It was estimated that one half of all women in their lifetime are affected by uropathogens and recurrence is more common (Ronald, 2003). Resistance to antibiotic treatment in patients with urinary tract infection is a representative example of the increasing problem of antimicrobial resistance. *Escherichia coli* accounts for most uncomplicated pathogens among uropathogens (Hoepelman *et al.*, 2003; Marchese *et al.*, 2003) accounting for 75-95% of all positive culture in uncomplicated cystitis (Grude *et al.*, 2005; Kahlmeter, 2003). Increasing trends of antimicrobial resistance to uropathogens from UTIs require alternative treatment options.

Control of antibiotic resistant gram negative bacteria including extended spectrum beta- lactamase (ESBLs) producing bacteria require the understanding of the relative casual importance of the organism (Paterson, 2006). To date only a few studies have been reported about the relationship between ESBL production and clinical outcomes with inconclusive results (Metan *et al.*, 2005; Du *et al.*, 2002; Kim *et al.*, 2002). To our knowledge no studies have been focused specially on ESBL producing uropathogens to pregnant women. The purpose of this study was to determine the clinical outcome and risk factor for mortality among pregnant women with uropathogens including those that produce ESBLs caused by the strains. In the present study, the *in vitro* activity of antimicrobial resistance against uropathogens are isolated from pregnant women outpatients in South India are reported.

2. Materials and Methods

2.1 Sample collection

A total of 50 pregnant women's urine samples suspected of urinary tract infection (UTI) between the ages of 25 to 30 were collected in a sterile screw cap tube from Government hospital, Thiruvanamalai district, Tamilnadu, India during the period of January to June 2010. These women were presented to outpatient urological clinics and were not hospitalized. A clean catch midstream urine sample was used for the isolation of uropathogens.

2.2 Microorganisms

Gram negative uropathogens such as *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were recovered from suspected urine sample by standard microbiological procedure. The uropathogens were identified by standard biochemical test (Collee *et al.*, 1996).

2.3 Culture media

Culture media such as Blood agar, MacConkey agar, Muller Hinton agar, Antimicrobial susceptibility discs and other chemicals were procured from Himedia, Mumbai, India.

2.4 Antimicrobial Susceptibility testing

Antimicrobial susceptibility and potential ESBL producing isolates were confirmed by disc diffusion method (Bauer *et al.*, 1996) and interpreted according to the Clinical Laboratory Standard Institute guidelines (CLSI, 2006) using *E.coli* 25922 ATCC as a control strain. Antimicrobial susceptibility of Ampicillin (20 µg), Amoxicillin (20 µg) and Ceftriaxone (20 µg) were determined by disc diffusion method on Muller Hinton Agar (MHA).

2.5 Detection of ESBLs producing bacteria

Production of ESBL in the strains was screened by the double disc synergy test (Ho *et al.*, 1998). An agar plate was inoculated as described for the standard disc diffusion method. Antibiotic disc of four different Cephalosporine (Ceftazidime, Cefataxime, Ceftriaxone and Cefepime with the concentration of 30 µg each) were placed at center to center distances of 30mm apart from an Amoxicillin – Clavulanic acid disc (20/10 µg). A clear extension of greater than 5 mm inhibition zone between any of the four Cephalosporins towards the disc containing Clavulanic acid was interpreted as positive for ESBL production.

3. Results and Discussion

Antibiotic resistance is an important issue affecting public health drastically. Rapid detection in clinical laboratories is essential for the prompt recognition of antimicrobial resistant organisms, especially those producing ESBL. ESBL producing gram negative bacteria are the major cause of urinary tract infection. During past decade ESBL producing gram negative bacteria especially *E.coli*, *K. pneumoniae* and *P. aeruginosa* have emerged as serious pathogens both in hospital and community acquired infections. A total of 54 isolates were obtained from pregnant women outpatients during study period.

The distributions of isolated uropathogens are showed in Table 1. Among the uropathogens isolated, the most frequent strain was found to be *E.coli* (44.4%) followed by *K. pneumoniae* (37.0%) and *P. aeruginosa* (18.5%). The antimicrobial resistant pattern of isolated uropathogens to various antimicrobial agents is represented in Table 2. The test revealed that, out of 54 bacterial uropathogens studied, 31 (57.4%) strains were resistant to Ampicillin followed by 21 (46.6%) strains to Amoxicillin indicating maximum resistance to these drugs. Ceftriaxone constitute 18(33.3%) resistant to the tested uropathogens. Ceftriaxone was found to be the antibiotic showed least resistant to uropathogens.

Table 1. Distribution of extended – spectrum beta lactamase producing uropathogens from pregnant women out patient. (N= 50), January – June 2010.

S.No.	Bacterial species	Number of isolates
1	Escherichia coli	24 (44.4%)
2	Klebsiella pneumoniae	20 (37.0%)
3	Pseudomonas aeruginosa	10 (18.5%)
Values in parenthesis showed percentage of isolates.		

The prevalence of ESBL producing organism among clinical isolates vary greatly worldwide and geographically and rapidly changing over time (Babypadmini & Appalaraju, 2004). The present study results revealed that, the incidence of ESBL producing uropathogens of *E.coli* was 44.4%, *K. pneumoniae* was 37% and *P. aeruginosa* was 18.5% isolated from pregnant women. The differences were statistically significant (P< 0.05). The frequency of ESBL producing *E.coli* and *K. pneumoniae* has been reported from the Latin America (54.4% and 8.5%), the Western Pacific (24.6% and 7.9%) and Europe (22.6% and 5.3%) respectively (Aminzadeh *et al.*, 2008). The highest isolation rate of ESBL producing clinical isolates of *E.coli* vary from 45.2 to 67.2% (Mehrgan & Rahbar, 2008) *K.pneumoniae* from 44.4% to 52% (Feizabadi *et al.*, 2006).

Alipourfard and Nili (2010) have studied in Bangladesh, the occurrence of ESBL producers in urinary isolates of *E.coli* and *K.pneumoniae* was found to be 60% and 40 % respectively. Behroozzi *et al.*, (2010) have reported that the frequency of ESBL pro-

ducing *E.coli* and *K.pneumoniae* was found to be 21% and 12% respectively isolated from urine sample in tertiary care hospital. A study from north India on ESBL producing uropathogens showed 26.6% ESBL producers belonged to *E. coli*, *Klebsiella*, *Enterobacter*, *Proteus* and *Citrobacter* species (Khurana *et al.*, 2002). Supriya *et al.*(2004) reported that ESBL producing isolates of *E.coli* was 49.8%, *K.pneumoniae* was 37.8% and *P. aeruginosa* was 6.5%.Taneja *et al.*,(2008) reported that the highest ESBL positivity of *Klebsiella* 51.2% followed by *E.coli* 40.2%, *Enterobacter* 33.4% and *P.aeruginosa* 29.9% from urine samples. Ramesh *et al.*, (2008) have reported that ESBL producing isolates of *E.coli* was 60.07%, *Klebsiella* was 78.7% and *Pseudomonas* was 84.67%. This is higher figure than the present study of ESBL producers in pregnant women.

Table 2. Antimicrobial resistant pattern of uropathogens from pregnant women out-patient.

S.No.	Antimicrobials	Antimicrobial resistant by the isolate in 2010 (%)			
		E.coli (n=24)	K. pneumoniae (n=20)	P. aeruginosa (n=10)	Total (n=54)
1	Ampicillin	15(62.5%)	11(55.0%)	5(50.0%)	31(57.4%)
2	Amoxycillin	11(45.8%)	7(35.0%)	3(30.0%)	21(46.6%)
3	Ceftriaxone	8(33.3%)	6(30.0%)	4(40.0%)	18(33.3%)

Values in parenthesis showed percentage of antibiotic resistant.

Antimicrobial susceptibility pattern of isolated ESBL producing uropathogens against tested antimicrobial agents is presented in Table 3. The antibiotics such as Ampicillin, Amoxycillin and Ceftriaxone combined with Sulbactam and Clavulanic acid were separately tested against all the uropathogens. The test result revealed that, synergistic effect of antibiotic showed 100% susceptibility against tested uropathogens. These combined drugs were found to be the choice for UTI infection to pregnant women. In the present study of the 54 bacterial uropathogens antibiogram studied revealed that 31(57.4%) and 21(46.6%) isolates were resistant to Ampicillin and Amoxicillin respectively indicating maximum resistance to these drugs. Ceftriaxone constitute the reasonable option for the treatment of UTI as 18(33.3%) isolates were sensitive to this antibiotic. The antibiotic such as Ampicillin, Amoxycillin and Ceftriaxone combined with Sulbactam and Clavulanic acid revealed synergistic effects against tested uropathogens. Hooton *et al.*, (2004) reported that Nitrofurantoin, Fosfomycin and Amoxicillin-Clavulanate have been most commonly used antibiotics because of their admirable *in vitro* activities against most uropathogens especially *Escherichia coli* (Ho *et al.*, 2007).

Table 3. Antimicrobial susceptibility pattern of ESBL producing uropathogens from pregnant women out patient.

S.No.	Antimicrobials	Antimicrobial susceptibility by the isolate in 2010 (%)			
		E.coli (n=24)	K. pneumoniae (n=20)	P. aeruginosa (n=10)	Total(n=54)
1	Ampicillin	9(37.5%)	9(45.0%)	5(50.0%)	23(42.6%)
2	Ampicillin + Sulbactam	24(100%)	20(100%)	10(100%)	54(100%)
3	Ampicillin + Clavulanic acid	24(100%)	20(100%)	10(100%)	54(100%)
4	Amoxycillin	13(54.2%)	13(65.0%)	7(70.0%)	33(61.1%)
5	Amoxycillin + Sulbactam	24(100%)	20(100%)	10(100%)	54(100%)
6	Amoxycillin+ Clavulanic acid	24(100%)	20(100%)	10(100%)	54(100%)
7	Ceftriaxone	16(66.6%)	14(70.0%)	6(60.0%)	36(66.7%)
8	Ceftriaxone + Sulbactam	24(100%)	20(100%)	10(100%)	54(100%)
9	Ceftriaxone+ Clavulanic acid	24(100%)	20(100%)	10(100%)	54(100%)

Values in parenthesis showed percentage of antibiotic susceptibility.

4. Conclusion

The ESBL producing organisms are increasing rapidly and becoming a major problem in the area of infectious diseases. Problems associated with ESBL producing isolates are difficulty in detection, treatment and thus increased mortality of patients. This is particularly true for ESBL producing bacteria. The spread of *E.coli*, *K.pneumoniae* and *P.aeruginosa* that produce beta lactamase will have important future implications for the empirical treatment of patients infected with UTI. We suggested that future investigations have to be undertaken to study the microbiological factors that make ESBL producing bacteria to reduce the mortality rate for pregnant women due to such successful pathogens. From this study we recommended that antibiotics such as Ampicillin, Amoxycillin and Ceftriaxone combined with Sulbactam and Clavulanic acid revealed synergistic effects. This will help for the treatment of UTI infections caused by ESBL producing bacteria infected with uropathogens especially for pregnant women.

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6. References

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