

Detection of Extended-Spectrum β Lactamases and AmpC β -Lactamase Production in *Escherichia coli* Causing Urinary Tract Infection among HIV and Non-HIV Patients

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Abstract: Problem statement: Opportunistic infections have been documented as a leading cause of morbidity among HIV patients. Gram negative pathogens that elaborate beta-lactamases have been reported to be associated with increased morbidity and mortality, especially amongst immunocompromised patients on intensive care and high-dependency units. The most common mechanism of β -lactam drug resistance in *Escherichia coli* include ESBL, AmpC production. **Approach:** In this study, we assessed the prevalence of UTI in two groups of individuals which included patients with HIV/AIDS and non-HIV antenatal patients. **Results:** *E. coli* was the predominant pathogen associated with bacteriuria in both the HIV group and the non-HIV group. *In vitro* sensitivity revealed that 96.2 and 31.8% of the *E. coli* isolates from the HIV patients and non-HIV patients were resistant to co-trimoxazole. Also, 72.7 and 4.5% of *E. coli* strains produced ESBL and/or AmpC among the HIV and the non-HIV antenatal patients respectively. **Conclusion:** Our data suggests that UTI may represent a relevant cause of morbidity among the HIV/AIDS patients.

Key words: HIV patients, UTI, *Escherichia coli*, ESBL, AmpC

INTRODUCTION

Escherichia coli is the etiological agent in vast majority of UTI, causing both cystitis and pyelonephritis. The antimicrobial susceptibility pattern of the uropathogens has been changing over the years and is influenced by the changing patient population, especially the immunocompromised HIV/AIDS patients, the extensive use and misuse of antimicrobial agents, alterations in the microbial profile of urinary tract isolates. Over the last 20 years, β lactam antibiotics (penicillins, cephalosporins, carbapenems) are the most commonly used drugs. The predominant mechanism of acquired resistance to β lactams in *E. coli* is the production of Extended Spectrum β -Lactamases (ESBLs) and AmpC β lactamases (Paterson and Bonomo, 2005). *E. coli* is intrinsically susceptible to 7- α -methoxy-cephalosporins such as cefoxitin and cefotetan because of the low level expression of the non-inducible species specific *ampC* gene (Philippon *et al.*, 2002). *E. coli* strains producing Extended-Spectrum β Lactamases (ESBLs) and/or AmpC are associated with increased morbidity and mortality especially amongst the immunocompromised patients. Although

there are enormous data on the prevalence of ESBL producing strains in South India (Mathai *et al.*, 2002), there is hardly any data on the occurrence of ESBL producing uropathogenic *E. coli* isolated from the HIV patients in our region. Hence, we performed a comparative study to assess the prevalence of ESBL/AmpC producing uropathogenic *E. coli* among the HIV patients and non-HIV patients with UTI.

MATERIALS AND METHODS

Enrollment of patients and sample collection: A total of 50 HIV patients hospitalized at the Government Hospital of Thoracic Medicine, Chennai. and 50 symptomatic Non-HIV patients attending the antenatal clinic of a private hospital in Chennai, South India were enrolled in the study following institutional ethical clearance. Mid stream clean catch urine samples were collected in sterile screw capped containers and transported to the laboratory in ice and processed immediately. Urine culture and identification of the bacterial isolates were performed as per standard protocols. Antibiotic susceptibility testing was done for forty eight *E. coli* strains isolated from the urine of both

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HIV patients (n = 26) and non-HIV patients (n = 22) by the Kirby Bauer disc diffusion method as per CLSI guidelines.

Phenotypic detection of ESBL and AmpC producers: ESBL production was confirmed for the *E. coli* isolates (HIV isolates = 22, Non-HIV isolates = 22) using sensitivity discs containing third and fourth generation cephalosporins with and without β -lactam inhibitor, viz., ceftazidime 30 μ g(Ca), ceftazidime+clavulanic acid 10 μ g (Cac); cefotaxime 30 μ g (Ce), cefotaxime+clavulanic acid 10 μ g (Cec); cefipime 30 μ g (Cpm), cefipime+clavulanic acid 10 μ g (Cfc). In addition, cefoperazone 75 μ g + sulbactam 10 μ g (Cfs); piperacillin 100 μ g + tazobactam 10 μ g (Pt) and monobactam (aztreonam 30 μ g (Azt)) were placed on Mueller Hinton agar streaked with the test organism and incubated at 35°C for 18 h at ambient atmosphere. *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603 was used as negative control and positive control respectively.

For AmpC detection, Cefoxitin 30 μ g (Cn) and cefoxitin + Boronic acid (Cn+BA) were used. Disc with cefotaxime + clavulanic acid 10 μ g (Cec) + Boronic acid was used for AmpC masked ESBL detection for each isolate (Coudron, 2005). AmpC enzyme production was confirmed by the AmpC disc test (Black *et al.*, 2005).

Statistical analysis: One way ANOVA was performed using MINITAB statistical software to assess the significance of the results.

RESULTS

Among the 100 urine samples (HIV = 50, Non HIV antenatal = 50) that were screened, *E. coli* was the predominant pathogen isolated from both the HIV (66.7% among male and 73.3% among the female) and the non-HIV antenatal group (63.3%). Antibiotic susceptibility testing done for forty eight *E. coli* strains isolated from the urine of both HIV patients (n = 26) and non-HIV patients (n = 22) showed that 25(96.2%) and 7(31.8%) of *E. coli* isolates from HIV and non-HIV group respectively, exhibited co-trimoxazole resistance. Of the 22 *E. coli* isolates from the HIV patients that were screened, 16(72.7%) produced ESBL and/or AmpC. Among the 22 isolates, 13(59.1%) were both ESBL & AmpC positive while, 3(13.6 %) were pure AmpC producers). In contrast, only one *E. coli* isolate (4.5%) among the non-HIV antenatal patients exhibited ESBL and AmpC β lactamase production (Table 1).

Table 1: ESBL and/or AmpC production among the *E. coli* isolates

	HIV(n = 22)	Non-HIV(n = 22)	p value
ESBL positive	0	0	-
AmpC positive	3(13.6%)	0	0.076*
ESBL positive, AmpC positive	13(59.1%)	1(4.5%)	0.000**
ESBLnegative, AmpC negative	6(27.3%)	21(95.5%)	0.000**

*: Not statistically significant; **: Statistically significant

DISCUSSION

Unlike industrialized countries, HIV epidemics in developing countries like India and Africa are characterized by heavy incidence of several OIs (Ranga *et al.*, 2010). A majority of the infectious complications in HIV/AIDS patients are due to common bacterial pathogens that represent additional causes of morbidity and mortality (Falks *et al.*, 1987; Niedt and Schinella, 1985; Nichols *et al.*, 1989). A recent study by Wikler (2008) on OIs among HIV seropositive patients from Eastern India revealed that infections caused by *E. coli* ranks seventh. As has been observed in a previous study, UTI is very common among both men and women with HIV disease (Pinho *et al.*, 1994; Schonwald *et al.*, 1999). The results of our study reveal that *E. coli* was the leading cause of UTI among hospitalized HIV patients and Non HIV antenatal patients which is in agreement with the previous reports (Deokar and Bodhankar, 2009; Pinho *et al.*, 1994; Brenner and Rector, 1991; Wilkie *et al.*, 1992).

Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. As per the current EAU guidelines (2010), co-trimoxazole 160/800 mg bid for 3 days is recommended (as empirical therapy) only in areas with resistance rate <20% for *E. coli*. However, if co-trimoxazole resistance >20%, 160/800 mg bid for 14 days is recommended only if the susceptibility of the pathogen is known, not for empirical therapy (Grabe *et al.*, 2010). Co-trimoxazole is currently being used in the prophylaxis against *Pneumocystis Carinii* Pneumonia (PCP) in immunocompromised (HIV) individuals. In accordance with the results of another study (Evans *et al.*, 1995), our data shows that majority of the *E. coli* strains (96.2%) from the HIV patients were resistant to co-trimoxazole. This suggests that co-trimoxazole does not appear to reduce the incidence of UTI in this population. Pinho *et al.* (1994) demonstrated an in-hospital mortality rate of 20% among HIV patients with symptomatic UTI primarily due to the rapid development of sepsis, despite the empirical antibiotic therapy. An earlier study in our region (Naveen and Mathai, 2005) has reported 57.6% resistance to co-trimoxazole among

the *E. coli* isolates from pregnant women with symptomatic UTI. However in our study, a relatively low level of co-trimoxazole resistance was exhibited against this first line antibiotic which makes it useful in the treatment of UTI in antenatal women.

Resistance to antimicrobial agents (AMR) has resulted in increased morbidity and mortality due to treatment failures and increased healthcare costs. Currently the mechanisms of β -Lactam resistance include class A Extended Spectrum β -Lactamases (ESBL), class B (Metallo β -lactamases), class C AmpC β -lactamases (cephalosporinases) (Ayyagari and Sushma, 2009). However, the Indian literature lacks evidence on the incidence of UTI and the antibiotic susceptibility patterns of the UPEC (especially the ESBL/AmpC producing *E. coli*) amongst HIV patients.

Hawkey (2008) had reported that ESBLs were found in 80-90% of *Enterobacteriaceae* in Asia, especially in India and were primarily due to mobile genes carried on plasmids, which can readily spread through bacterial populations. A multicentric Indian study reported 55-61% of ESBL prevalence among clinical isolates of *Enterobacteriaceae* from various hospitals (Mathai *et al.*, 2002).

ESBL producers have been reported with increasing frequency in India. Khurana *et al.* (2002) have reported that overall 26.6 % of the uropathogens were ESBL producers. Tankhiwale *et al.* (2004) reported that 48.3% of urinary isolates tested were ESBL producers. In another South Indian study, 41% of the urinary *E. coli* isolates were found to elaborate ESBLs. Singhal *et al.* (2005) reported that 62% of the *E. coli* isolates included in their study were ESBL positive. Another Indian study group had reported an incidence of 63.7% of ESBL positivity among the *E. coli* isolates studied, of which majority (39%) were urinary isolates (Kumar *et al.*, 2006). Also, Taneja *et al.* (2008) have reported that 40.2 % of *E. coli* urinary isolates were found to be ESBL producers and Agarwal *et al.* (2008) reported a still lower incidence (30%) of ESBL positivity among the *E. coli* isolates studied and the distribution of the ESBL positive isolates was highest (76.9%) among the urinary isolates. A recent South Indian study has showed that 51.47% of the *E. coli* isolates from various clinical specimens, especially the urinary isolates were found to be ESBL producers (Shiju *et al.*, 2010). However, in our study the incidence of ESBL producing *E. coli* was found to be 59.1%.

A recent study by Patel *et al.* (2010) showed that *E. coli* was the predominant uropathogen, and 32.2% were AmpC producers. In our study, a relatively high percentage of the isolates from HIV patients (72.7%)

exhibited an AmpC phenotype whilst among the isolates from Non – HIV antenatal patients, only 4.5% were AmpC producers. Another Indian study showed that 6.97% of the *E. coli* isolates were found to harbor AmpC enzymes (Singhal *et al.*, 2005). Among the *E. coli* isolates from HIV patients that expressed an AmpC phenotype, 59.1% produced ESBL and AmpC enzymes, while 13.6% were pure AmpC producers. (Hemalatha *et al.*, 2007) reported that 47.3% of the study isolates harboured AmpC enzymes, of which 86.1% co-produced ESBLs while only 9.2% were pure AmpC producers.

Another important finding was the high level of resistance exhibited by the ESBL + AmpC producing *E. coli* towards co-trimoxazole (100%). However, another South Indian study has reported the incidence of high level of co resistance among CTX-M producers towards co-trimoxazole (82.6%) (Padmini *et al.*, 2008). The percentage variations between these two studies could possibly be due to the differences in the patient status as well as frequency of antibiotic exposure. Also, Pitout (2008) had reported that CTX-M producing *E. coli* strains isolated from hospital and community sites often exhibit co-resistance to trimethoprim-sulfamethoxazole, tetracycline, gentamicin, tobramycin, and ciprofloxacin.

Our data shows a significant difference in the incidence of ESBL and AmpC production among the *E. coli* isolated from the urine of the HIV patients when compared to the Non-HIV antenatal patients. Plasmid-mediated AmpC β -lactamases typically produced by isolates of *E. coli*, are associated with multiple antibiotic resistance that leaves very few therapeutic options. Hence, accurate and prompt detection of β -lactamase producers are essential for therapeutic decision making.

CONCLUSION

The relatively high frequency of UTI found in HIV patients in this study and the occasional non-specificity of its clinical manifestations suggest that UTI could be a cause of PUO which is very common among the HIV population. Also, co-trimoxazole resistance along with β -lactam resistance exhibited by these *E. coli* urges the need for prompt diagnosis and effective treatment of UTI in order to avoid haematogenous spread of these pathogens leading to bacteraemia associated morbidity and mortality among the HIV patients. However, a larger prospective controlled study is required to confirm these results and to assess the importance of HIV infection as a predisposing factor for UTI in these patients.

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