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

1,2 Padmavathy Kesavaram, 1 Padma Krishnan and 3 Rajasekaran Sikhamani
1 Department of Microbiology, Dr. ALM PGIBMS, University of Madras, Chennai
2 Department of Microbiology, Sree Balaji Dental College and Hospital, Chennai
3 Medical Superintendent, Government Hospital of Thoracic Medicine, Chennai

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/AmpC 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
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 µg(Ca), ceftazidime+clavulanic acid 10 µg (Cac); cefotaxime 30 µg (Ce), cefotaxime+clavulanic acid 10 µg (Cec); cefipime 30 µg (Cpm), cefipime+clavulanic acid 10 µg (Cfp). In addition, cefoperazone 75 µg + sulbactam 10 µg (Cfs); piperacillin 100 µg + tazobactam 10 µg (Pt) and monobactam (aztreonam 30 µg (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 µg (Cn) and cefoxitin + Boronic acid (Cn+BA) were used. Disc with cefotaxime + clavulanic acid 10 µg (Cf); piperacillin 100 µg + tazobactam 10 µg (Pt) and monobactam (aztreonam 30 µg (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.

**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** |
| ESBL negative, 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 (Fulks 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 (as empirical therapy) only in E. coli. ago.
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 be ESBL positive. Another Indian study group had reported still lower incidence (30%) 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. The distribution of the ESBL positive isolates was highest (76.9%) among the urinary isolates. A recent South Indian study has shown 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.

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 bactaeremia 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.
Acknowledgement

This study was supported by a research grant provided by the Indian Council of Medical Research, New Delhi, India.

References

Agarwal, P., A.N. Ghosh, S. Kumar, B. Basu and K. Kapil. 2008. Prevalence of extended-spectrum beta-lactamases among Escherichia coli and Klebsiella pneumoniae isolates in a tertiary care hospital. Indian J. Pathol. Microbiol., 51: 139-142. PMID: 18417887

Ayyagari, A. and G. Sushma, 2009. Detection of antimicrobial resistance in common gram negative and gram positive bacteria encountered in infectious diseases—an update. ICMR Bull., 39: 1-20.

Black, J.A., E.S. Moland and K.S. Thomson, 2005. AmpC disk test for detection of plasmid-mediated AmpC (beta)-lactamases in enterobacteriaceae lacking chromosomal AmpC (beta)-Lactamases. J. Clin. Microbiol., 43: 3110-3113. PMID: 16000421

Brenner, B.M. and F.C. Rector, 1991. The Kidney. 4th Edn., Saunders Company, Philadelphia, ISBN: 0721626173, pp: 2443.

Coudron, P.E., 2005. Inhibitor-based methods for detection of plasmid-mediated AmpC beta-lactamases in Klebsiella spp., Escherichia coli, and Proteus mirabilis. J. Clin. Microbiol., 43: 4163-4167. PMID: 12543543

Deokar, S.D. and M.G. Bodhankar, 2009. Studies on Emergence of drug resistance in HIV associated bacterial urinary tract infections. Am. J. Infect. Dis., 5: 183-187. DOI: 10.3844/ajidsp.2009.183.187.

Evans, K., A. McOwan, R.J. Hillman and G.E. Forster, 1995. Incidence of symptomatic urinary tract infections in HIV seropositive patients and the use of cotrimoxazole as prophylaxis against Pneumocystis carinii pneumonia. Genitourin Med., 71: 120-122. PMID: 7744401

Falks, S., H.L. Schmidts, H. Muller, K. Berger and M. Schneider et al., 1987. Autopsy findings in AIDS—a histopathological analysis of fifty cases. Klin. Wochenschr., 65: 654-663. PMID: 3626432

Grabe, M., T.E. Bjerklund-Johansen, H. Botto, M. Cek and K.G. Naber et al., 2010. Guidelines on urological infections. Eur. Assoc. Urol., 146-162.

Hawkey, P.M., 2008. Prevalence and clonality of extended-spectrum beta-lactamases in Asia. Clin. Microbiol. Infect., 14: 159-165. PMID: 18154540

Hemalatha, V., M. Padma, U. Sekar, T.M. Vinodh and A.S. Arunkumar, 2007. Detection of AmpC beta lactamases production in Escherichia coli and Klebsiella by an inhibitor based method. Indian J. Med. Res., 126: 220-223. PMID: 18037717

Khurana, S., N. Taneja and M. Sharma, 2002. Extended spectrum beta lactamase mediated resistance in urinary tract isolates of family Enterobacteriaceae. Indian J. Med. Res., 116: 145-149. PMID: 12674828

Kumar, M.S., V. Lakshmi and R. Rajagopalan, 2006. Occurrence of extended spectrum beta-lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute. Ind. J. Med. Microbiol., 24: 208-211. PMID: 16912442

Mathai, D., P.R. Rhomberg, D.J. Biedenbachb and R.N. Jones, 2002. Evaluation of the in vitro activity of six broad-spectrum beta-lactam antimicrobial agents tested against recent clinical isolates from India: A survey of ten medical center laboratories. Diagn. Microbiol. Infect. Dis., 44: 367-377. PMID: 12543543

Naveen, R and E. Mathai, 2005. Some virulence characteristics of uropathogenic Escherichia coli in different patient groups. Indian J. Med. Res., 122: 143-147. PMID: 16177472

Nichols, L., K. Balogh and M. Silverman, 1989. Bacterial infections in the acquired immune deficiency syndrome. Clinicopathologic correlations in a series of autopsy cases. Am. J. Clin. Pathol., 92: 787-790. PMID: 2556018

Niedt, G.W. and R.A. Schinella, 1985. Acquired immunodeficiency syndrome. Clinicopathologic study of 56 autopsies. Arch Pathol. Lab. Med., 109: 727-734. PMID: 2990378

Padmini, S.B., B.A. Raju and K.R. Mani, 2008. Detection of Enterobacteriaceae producing CTX-M extended spectrum beta-lactamases from a tertiary care hospital in south India. Ind. J. Med. Microbiol., 26: 163-166. PMID: 18445956

Patel, M.H., G.R. Trivedi, S.M. Patel and M. Vegad, 2010. Antibiotic susceptibility pattern in urinary isolates of gram negative bacilli with special reference to AmpC β-lactamase in a tertiary care hospital. Urol. Ann., 2: 7-11. PMID: 20842250

Paterson, D.L. and R.A. Bonomo, 2005. Extended-spectrum beta-lactamases: A clinical update. Clin. Microbiol. Rev., 18: 657-686. PMID: 16223952

Philippon, A., G. Arlet and G.A. Jacoby, 2002. Plasmid-determined AmpC-type beta-lactamases. Antimicrob. Agents Chemother., 46: 1-11. PMID: 11751104
Pinho, A.M., G.S. Lopes, C.F. Ramos-Filho, O.D.R. Santos and M.P.B. De Oliveira, 1994. Urinary tract infection in men with AIDS. Genitourin Med., 70: 30-34. PMID: 8300097

Pitout, J.D., 2008. Multiresistant Enterobacteriaceae: New threat of an old problem. Expert Rev. Anti-Infect. Ther., 6: 657-669. PMID: 18847404

Ranga, U., A. Banerjea, S. Chakrabarti and D. Mitra. 2010. HIV/AIDS research in India: Past, present and future. Curr. Sci., 98: 335-345.

Schonwald, S., J. Begovac and V. Skerk, 1999. Urinary tract infections in HIV disease. Int. J. Antimicrob. Agents., 11: 309-311. PMID: 10394989

Shiju, M.P., R. Yaashavanth and N. Narendra, 2010. Detection of Extended spectrum beta-lactamase production and multidrug resistance in clinical isolates of E. coli and K. pneumoniae in Mangalore. J. Clin. Diag. Res., 4: 2442-2445.

Singhal, S., T. Mathur, S. Khan, D.J. Upadhay and S. Chugh et al., 2005. Evaluation of methods for AmpC beta-lactamase in gram negative clinical isolates from tertiary care hospitals. Indian J. Med. Microbiol., 23: 120-124. PMID: 15928443

Taneja, N., P. Rao, J. Arora and A. Dogra, 2008. Occurrence of ESBL and Amp-C beta-lactamases and susceptibility to newer antimicrobial agents in complicated UTI. Indian J. Med. Res., 127: 85-88. PMID: 18316858.

Tankhiwale, S.S., S.V. Jalgaonkar, S. Ahamed and U. Hassani, 2004. Evaluation of extended spectrum beta lactamase in urinary isolates. Indian J. Med. Res., 120: 553-556. PMID: 15654142

Wikler, M.A., 2008. Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement. 18th Edn., Clinical and Laboratory Standards Institute, Wayne, USA., ISBN-10: 1562386530, pp: 181.

Wilkie, M.E., M.K. Almond and F.P. Marsh, 1992. Diagnosis and management of urinary tract infection in adults. Br. Med. J., 305: 1137-1141.