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ANTIBIOTICS SUSCEPTIBILITY PATTERNS AND CLONAL RELATEDNESS OF UROPATHOGENIC *ESCHERICHIA COLI* IN ABAKALIKI, EBONYI STATE

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ABSTRACT

Eight months (Feb-Sept. 2009) prospective study was carried out in Ebonyi State University teaching hospital (EBSUTH) Abakaliki to determine the distribution and antibiotic susceptibility pattern of uropathogenic *Escherichia coli* isolated from out-patients with community-acquired urinary tract infections (UTIs) in Abakaliki Ebonyi State, Nigeria. We consecutively collected one hundred and forty (140) non-duplicate isolates of *E. coli* from female out-patient with UTI. Urine samples were analyzed and organisms isolated using standard Microbiology technique, antibiotic susceptibility studies was carried out using Kirby and Bauer method of determining susceptibility. Bla $_{TEM}$ and $_{SHV}$ beta lactamases was determined in resistant isolates by specific PCR and clonal relatedness of strains was determined by randomly amplified polymorphic DNA (RAPD). Antibiotic susceptibility rates for *E. coli* were; aztreonam (86.1%), doxycycline (31%), ampicillin (5%), ceftazidime (99.1%), cefotaxime (95.6%), cefoxitin (91.9%, cefotaxime (96.5%), amoxicillin/clavulanic acid (82.9%), cefepime (89.1%), cefuroxime (89%), imipenem (99%), ciprofloxacin (65.4%), levofloxacin (69.1%), sulphamethoxazole/trimethoprim (6.4%), Nitrofurantoin (96.5%), gentamicin (72%), kanamycin (93.7%) and ticarcillin 39.1%. Bla $_{TEM}$ beta lactamase was polymerase chain reaction positive in all the strains while bla $_{SHV}$ was negative. RAPD analysis grouped our isolates into four clonal groups (A-D) with majority of the isolates belonging to clonal group A (85.7%). Our findings showed high rate of resistance of uropathogenic *E. coli* to ampicillin sulphamethoxazole/trimethoprim, tircarcillin and doxcycline. Uropathogenic *E. coli* resistance to ampicillin and sulphamethoxazole/trimethoprim which is the commonest oral drug of choice in treating UTIs, are worrisome and also the wide distribution of the majority of uropathogenic *E. coli* in one clonal group (A) may have a major public health implications.

Keywords: Uropathgenic *Escherichia coli*, urinary tract infections, susceptibility, random amplified, polymorphic DNA.

INTRODUCTION

The reservoir for urinary tract infections (UTIs) is the human bowel flora and most infections result from uropathogens moving into bladder via the urethra (Kunin, 1997). *E. coli*, a universal bowel inhabitant causes between 80 to 90 percent of out-patients UTIs (Zhang and Foxman, 2003), although only a small fraction of *E. coli* are uropathogenic (Foxman and Riley, 2001). Urinary tract infections are one of the most common bacteria infections in humans both in the community and hospital settings (Tice 1999; Clarridge *et al*., 1998; Sussman, 1998). The incidence of UTI is more frequent in women (17.5% incidence between 18 and 24yrs) (Foxman *et al*., 2000) than in men (0.5% incidence in the same age range) (Krieger *et al*., 1993). The gender difference in the incidence of symptomatic infection is attributed in part to the shorter urethra of women and the proximity of the urethra to the anal opening and vaginal introitus (Hooton *et al*., 1999).

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The clinical management of urinary tract infection is complicated by the increasing incidence of infections caused by strains of *E. coli* that are resistant to commonly used antimicrobial agents. Although UTI is not usually thought of as a disease associated with community wide outbreaks, certain multi-drug resistant uropathogenic lineages of *E. coli* have exhibited epidemic behavior (Philips *et al*., 1988). *E. coli* 015:K52:H1 is an endemic cause of urinary tracts infections in Barcelona Spain, (Prats *et al*., 2000). In almost all cases there is a need to start treatment before the final microbiological results are available because antimicrobial susceptibility testing of urinary tract isolates is usually achieved 48 hrs following sampling and therefore in the majority of community acquired UTI, the treatment decision is empirical, being influenced by available data reflecting antibiotic resistance (Blondeau and Tillotson, 1999).

Antibiotics are among the most frequently prescribed drugs in tertiary hospitals and the high consumption of often inappropriate prescribed antibiotics combined with crowding multiple pathology and frequent use of invasive

devices is a major factor contributing to high levels of resistance. In the present study, we determined the antibiotic susceptibility patterns and clonal relatedness of uropathogenic *E. coli* in patients attending Ebonyi State University teaching hospital, Abakaliki.

MATERIALS AND METHODS

Sample Collections

Mid-stream urine samples of 140 patients visiting out-patient department of EBSUTH with case of UTI was collected in sterile bottles and innoculated on MacConkey agar plate, incubated at 37^{o}C for 24h. Colonies that were positive for lactose and indole were presumptively identified as *E. coli*. One putative *E. coli* colony from each patient's culture was arbitrarily selected for further analysis. A case of *E. coli* UTI was defined as symptoms suggestive of infection and a culture of a clean-catch mid stream urine specimen with more than 10^2 colony forming units of *E. coli* per milliliter (Manual of Clinical Microbiology, 2002; Gupta *et al*., 1999; Hooton and Stamm, 1997).

Antibiotic Susceptibility Testing

Susceptibility of uropathogenic *E. coli* to 17 different types of antibiotics namely; aztreonam, doxycycline, ampicillin, ceftazidime, cefotaxime, cefepime, cefuroxime, cefoxitin, amoxicillin/clavulanic acid, imipenem, sulpamethoxazole/trimethoprim, Nitrofurantoin, ciprofloxacin, levofloxacin, gentamicin, kanamycin and ticarcillin were determined by Kirby and Bauer method for determining susceptibility. Exactly 0.5 MacFarland equivalent standards of test organisms was inoculated on the surface of sterile Mueller-Hinton agar plate and single antibiotic discs was placed on the surface of the agar plate and incubated for 18-24h at 37^{o}C. The radial zone of inhibition diameter in mm was taken after the incubation period (Bauer *et al*., 1966). All resistant isolates were further screened for the presence of bla $_{TEM}$ and $_{SHV}$ β- lactamases.

DNA Isolation

Genomic DNA was prepared using the Nucleospin Kit (Macherey & Nagel, Germany) following manufacturer's instructions. Briefly, an overnight culture in a fresh Luria Bertani broth incubated at 37^{o}C for 18-24h was prepared of all *E. coli*. 1.5ml of this overnight broth culture was transferred into a reaction tube and centrifuged for 5mins at 8,000rpm and supernatant discarded. Pre-lysis was carried out by re-suspending the pellet in 180µl of T_1 buffer and 25µl of proteinase K, mixed vigorously and incubated at 56^{o}C for 30mins with shaking. 200µl of B_3 buffer was added and incubated at 70^{o}C for 10mins, 210µl of 96-100% ethanol was added into tube containing 200µl of B_3 buffer and was mixed vigorously until all insoluble particles became soluble. This solution was transferred into a Nucleospin column and centrifuged for 1min at 11,000rpm, flow-through was discarded and the column was placed back into the collection tube. 500µl of BW buffer was added and centrifuged at 11,000rpm for 1min, flow-through was discarded and 600µl of B_5 buffer was added, centrifuged for 1min at 11,000rpm, then flow-through was discarded and the column was centrifuged again for 1min at 11,000. Elution buffer was pre-incubated for 5mins at 70^{o}C and 100µl was added into each column and centrifuged for 1 min at 11,000rpm to elute the total DNA. Eluted total DNA was stored at -20^{o}C for further analysis.

PCR Analysis of BLA $_{TEM}$ AND $_{SHV}$ β- Lactamases

Detection of bla $_{TEM}$ and $_{SHV}$ beta-lactamase genes was carried out using specific primers. Appropriate positive and negative controls were used in all cases. The PCR mixture contained 2µl each of buffer, 4.0mM each of dNTP, 2.5µM each of primer, 5µl each of genomic DNA, 1U each of Taq polymerase, and 11µl of water in a total volume of 25µl. The amplification protocol consists of the following steps; initial denaturation at 94^{o}C for 5 mins, followed by 34 cycles of denaturation at 94^{o}C for 30 sec and a final extension step at 72^{o}C for 5 min. Annealing temperatures differed according to the primer pair used and was 42°C for TEM and 47°C for SHV. Amplified PCR products were separated on 0.8% agarose gels at 100 Volts, stained with ethidium bromide and visualized under UV illumination (Schleslinger *et al*., 2005).

Randomly Amplified Polymorphic DNA (RAPD) analysis of uropathogenic *E. coli* strains

RAPD was performed with all uropathogenic *E. coli* strains using a single primer. The PCR mixture contained 2.5µl each of buffer, 4.0mM each of dNTP, 2.5µM each of primer, 5µl each of genomic DNA, 2U each of Taq polymerase, 1.5µl of $MgCl_2$ and 9.5µl of water in a total of 25µl with the following PCR amplification protocol; initial denaturation at 95^{o}C for 5min, followed by 34 cycles of denaturation at 94^{o}C for 5min, final extension step of 72^{o}C for 5min and 94^{o}C for 1 min, final extension step of 72^{o}C for 5min and annealing temperature at 37^{o}C. Amplified PCR products were separated on 1.5% agarose gels at 75Volts, stained with ethidium bromide and visualized under UV illumination (Pacheco *et al*., 1997).

RESULTS

The results of the in-vitro susceptibility testing of uropathogenic *E. coli* are presented in table 1a, 1b. *E. coli* was susceptible to thirteen antibiotics namely; aztreonam (86.1%), ceftazidime (99.1%), cefotaxime (95.6%), amoxicillin/clavulanic acid (82.9%), imipenem (99%), cefepime (89.1%), cefoxitin (91.9%), cefuroxime (89%), gentamicin (72%), kanamycin (93.7%), ciprofloxacin (65.4%), levofloxacin(69.1%) and Nitrofurantoin (96.5%) but resistant to sulphamethoxazole/trimethoprim (94.6%), ampicillin (95%), doxycycline (69%) and tircacillin

(60.9%). PCR analysis for the presence of bla $_{TEM}$ and $_{SHV}$ β- lactamase genes revealed that all uropathogenic *E. coli* (100%) strains were positive for bla $_{TEM}$ and negative for $_{SHV}$. Clonal classification of our isolates by RAPD grouped our isolates into four clonal groups (A-D) with the majority of the strains belonging to clonal group A (85.7%), B(8.3%), C(5.5%) and D(1.5%) (Table 2).

DISCUSSIONS

This paper describes the susceptibility patterns and clonal relatedness of uropathogenic *E. coli* isolated from UTIs out-patients in EBSUTH Abakaliki. We found that over 90% uropathogenic *E. coli* was susceptible to cefoxtin, ceftazidime, cefotaxime, imipenem, nitrofurantoin and kanamycin, while over 65% was susceptible to ciprofloxacin, levofloxacin, amoxicillin/clavulanic acid, cefepime, cefuroxime, aztreonam and gentamicin, while 95% were resistant to ampicillin, (94.6%) to sulpamethoxazole/trimethoprim, 60.9% to ticarcillin and 69% to doxycycline. Resistance of these bacteria to these drugs especially to ampicillin and sulphamethoxazole/ trimethoprim which are the most common oral drugs used in general practice calls for serious concern and therefore empirical treatment of urinary tract infections with these drugs should be avoided. Also regular monitoring is required in order to make reliable information available for optimal empirical therapy for patients with UTIs. Also the wide susceptibility of our *E. coli* to different classes of antibiotics are similar to data's obtained in other countries indicating that *E. coli* is still susceptible to many other antimicrobial agents (Fluit *et al*., 2000; Cunney *et al*., 1992; Jones *et al*., 1999).

Presence of bla $_{TEM}$ beta lactamase in all the strain could be suggested to be responsible to the overwhelming resistance of the uropathogenic *E. coli* to ampicillin and doxcycline, although no further study was done to substantiate this claim. But, it has been established that beta lactamase production is one major mechanism by which Gram- negative organism which *E. coli* is inclusive confers resistance to beta lactam drugs (Medeiros, 1997). Majority of the uropathogenic *E. coli* belong to single clonal group A. This clonal group (A) accounts for 95% of UTI infection caused by *E. coli* strains that were resistant to ampicillin sulpamethoxazole/trimethoprim tircacillin and doxycycline. Although a limited number of isolates were surveyed, this data may suggest that a single *E. coli* clonal group, may cause further increase in antibiotic resistant among *E. coli* isolates from patients with UTI in Abakaliki in the near future. That the *E. coli* isolates with resistance to ampicillin, sulpha-methoxazole/trimethoprim, tircacillin and doxycycline represent a phylogenetically distinct clonal group was suggestive of their similarities to one another. This finding indicates that clonal group A contributes substantially not only to drug-resistant UTIs but also to UTIs in general. Clonal group A appears to represent a new lineage of multi-drug resistant uropathogenic *E. coli* as this is the first reported from this hospital. Although UTIs is usually regarded as a sporadic disease caused by organisms from its hosts own fecal flora, transmission of *E. coli* between sex partners and house hold members had been reported (Foxman *et al*., 1997; Johnson *et al*., 1998). Nosocomial our-break of *E. coli* phylonephrities have also been reported (Tullus *et al*., 1984) and a community wide outbreak of UTI due to single strain have been reported in South London (Philips *et al*., 1988), in California and Minnesota (Manges *et al*., 2001). The presence of these uropathogenic *E. coli* in out-patients revealed prevalence of this infection in the community nevertheless this analysis does not show that this uropathogenic *E. coli* was

Table.1a. Percentage susceptibility of uropathogenic *E. coli* to the cephalosporins.

Amp (%)	Doxy (%)	Ceft (%)	Aztr (%)	Cefo (%)	Cefe (%)	Cefu (%)	Imp (%)	Cefx (%)	Amc (%)
5	31	99.1	86.1	95.6	89.1	89	99	91.9	82.9

Keywords: Amp: ampicillin, Doxy: doxycyxline, Ceft: ceftazidime, Aztr: aztreonam, Cefo: cefotaxime, Cefe: cefepime, Cefu: cefuroxime, Imp: imipenem, Cefx: cefoxitin, Amc: amoxyicillin/clavulanic acid.

Table.1b. Percentage susceptibility of uropathogenic *E. coli* to other antibiotics

Cip (%)	Lev (%)	Gen (%)	Kan (%)	Tic (%)	Nitr (%)	Sxt(%)
65.4	69.1	72	93.7	39.1	96.5	94.6

Keywords: Cip: ciprofloxacin, Lev: levofloxacin, Gen: gentamicin, Kan: Kanamycin, Tic: tircacillin, Nitr: nitrofurantoin, Sxt: sulphamethoxazole/trimethoprim

Table. 2. Percentage distribution of bla $_{TEM}$, bla$_{SHV}$ and Clonal relatedness of uropathogenic *E. coli*.

		Clonal Groups			
Bla TEM (%)	Bla SHV (%)	A (%)	B (%)	C (%)	D (%)
100	0	85.7	8.3	5.5	1.5

circulating in members of the community with cases of UTIs. The presence of majority of the strains in one clonal group could be possible as a consequence of increasing antimicrobial selection pressure or it could be possible that the strains were spread by one or more contaminated products, ingested by community residents which is similar to the way an enteric pathogen such as *E. coli* 015:H7 causes community-wide out break after being disseminated by the consumption of contaminated foods (Bender *et al*.,1997; Dorn, 1993) if a large population of urinary tract infections caused by drug-resistant *E. coli* to commonly used antibiotics in the out-patient settings were due to the ingestion of widely consumed, contaminated foods, this would cause a serious and novel public health problems. In conclusion, we hereby report uropathogenic *E. coli* with high resistance to ampicillin, sulpha-methoxazole/trimethoprim, tircacillin and doxycycline, that majority represent a single clonal group.

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REFERENCES

Bauer, AW., Kirby, WMM., Sherris, JC. and Turch, M. 1966. Antibiotic testing by standardized single disk method. Am. J. Clin. Path. 45:493-496.

Blondeau, JM. and Tillotson, GS. 1999. Formula to help select rational antimicrobial therapy (FRAT): its application to community and hospital-acquired urinary tract infections. Int. J. Antimicrobio. Agents. 12(2):145-50.

Bender, JB., Hedberg, CW., Besser, JM., Boxrud, DJ., MacDonald, KL. and Osterholm, MT. 1997. Surveillance for *Escherichia coli* 0157:H7 infections in Minnesota by molecular subtyping. N. Engl. J. Med. 33:388-94.

Clarridge, JE., Johnson, JR. and Pezzio, MT. 1998. Cumitech 2B Laboratory Diagnosis of urinary tract infections. Ed. Weissfeld, AS. American Society for Microbiology, Washington, DC, US.

Cunney, R., McNally, J., McNamara, RM., Al-Ansari, EM. and Smyth, EG. 1992. Susceptibility of urinary pathogens in a Dublin teaching hospital. Irish J. Med. Sci. 161:623-5.

Dorn. CR, 1993. Review of food borne outbreak of *Escherichia coli* 0157:H7 infection in the Western United States. J. Am. Vet. Med Assoc. 203:1583-7.

Foxman, B., Zhang, L., Tallman, P., Andree, BC., Geiger, AM., Kospman, JS., Gillespie, BW., Pallin, KA., Sobel, JD., Rode, CK., Bloch, CA. and Marris, CF. 1997. Transmission of uropathogens between sex partners. J. Infect. Dis. 175:989-92.

Foxman, B. and Riley, L. 2001. Molecular epidemiology: Focus on infection. Am. J. Epidemiol. 153: 1135-41.

Foxman, B., Barlow, R. and d' Arcy, H. 2000. Urinary tract infection: estimated incidence and association costs Ann. Epidemiol. 10:509-15.

Fluit, AC., Jonesc, ME., Schnitz, FJ., Acar, J., Gupta, R. and Verhoef, J. 2000. Antimicrobial resistance among urinary tract infection (UTI) isolates in Europe: results from the SENTRY Antimicrobial Surveillance Program 1997. Antonie van Leeuwenhoek. 77:147-52.

Gupta, K., Scholes, D. and Stamm, WE. 1999. Increase prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women JAMA. 281:736-8.

Hooton, T. and Stamm, W. 1997. Diagnosis and treatment of uncomplicated urinary tract infection. Infect. Dis. Clin. North. Am. 11:551-81.

Hooton, TM., Stapleton, AE., Roberts, PL., Winter, C., Scholes, D., Bavendam, T. and Stamm, WE. 1999. Perineal anatomy and urine-voiding characteristics of young women with and without urinary tract infections. Clin. Infect. Dis. 29:1600-1.

Johnson, JR., Brown, JJ., Carlino, UB. and Russo, TA. 1998. Colonization with and acquisition of uropathogenic *Escherichia coli* as revealed by polymerase chain reaction-based detection. J. Infect. Dis. 177:1120-4.

Jones, RN., Kugler, KC., Pfaller, MA., Winokur, PL. and the SENTRY Surveillance Group, North America. 1999. Characteristics of pathogens causing urinary tract infections in hospitals in North America: Results from the SENTRY Antimicrobial Surveillance Program, 1997. Diag. Microbio. Infect. Dis. 35:55-63.

Kunin, CM. 1997. Urinary tract infections. Detection, prevention and management. (5th ed.). Williams & Wilkins. Baltimore, MD, US.

Krieger, JN., Ross, SO. and Simonsen, JM. 1993. Urinary tract infections in healthy university men. J. Urol. 149:1046-8.

Manges, AR., James, MPH., Johnson, RB., Foxman, MD., O' Bryan, TT., Fullerton, KE. and Riley, LW. 2001. Widespread distribution of urinary tract infections by a multi-resistant *Esherichia coli* clonal group. N Engl. J. Med. 345:1007-1013.

Manual of Clinical Micorbiology (7th ed.). 2002. American Society for Microbiology Press, Washington, DC, USA. 442-452.

Medeiros, AA. 1997. Evolution and dissemination of beta- lactamases accelerated by generations of beta-lactam antibiotics. Clin. Infect. Dis. 24 Suppl1:S19-S45.

Pacheco, AB., Guth, BE., Soares, KC., Nishimura, L., de Almeida DF. and Ferreira, LC. 1997. Random amplification of polymorphic DNA reveals serotype-specific clonal clusters among enterotoxigenic *Escherichia coli* from humans J. Clin. Microbiol. 35(6):1521-5.

Philips, I., Eykyn, S., King, A., Gransden, WR., Rowe, B., Frost, JA. and Gross, RJ. 1988. Epidemic multiresistant *Escherichia coli* infection in West Lambert Health District. Lancet. 1038-41.

Prats, G., Navarro, F., Mirelis, B., Dalman, D., Margall, N., Coll, P., Stell, A. and Johnson, JR. 2000. *Escherichia coli* serotype 015:K52:H1 as a uropathogenic clone. J. Clin. Microbiol. 38:201-9.

Schleslinger J., Navon-Venezia, S., Chmelnitsky, I., Hammer-Munz, O., Leavilt, A., Gold, HS., Schwaber, MJ. and Carmeli, Y. 2005. Extended spectrum beta-lactamases among *enterobacter* isolates Obtained in Tel Aviv, Isreal, Antimicrob. Agents Chemother. 49(3):1150-1156.

Sussman, M. 1998. Urinary tract infections. In Topley and Wilsons Microbiology and microbial infections (9^{th} ed.). Eds. Collier, L., Balows. A. and Sussman, M. Arnold, London. 554-636.

Tullus, K., Horlin, K., Svenson, SB. and Kallenius, G. 1984. Epidemic outbreaks of acute pyleonephritis caused by nosocomial spread of P fimbriated *Escherichia coli* in children. J. Infect. Dis. 150:728-36.

Tice, AD. 1999. Short course therapy of acute cystitis: a brief review of therapeutic strategies. J. Antimicrob. Chemother. 43:85-93.

Zhang, L. and Foxman, B. 2003. Molecular epideiomiology o*f Escherichia coli* mediated urinary tract infections. Front Biosci. 8:e235-e244.

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