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Antibiotic Susceptibility Spectrum in UPEC from Urine in Children with UTI in Mofid Children Hospital

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Urinary tract infection due to UPEC with antibiotic resistance is one of the most important problems in infants and children. Prevalence of UPEC isolated from children urine samples and their antimicrobial susceptibilities were considered in this study. Urine samples of children were studied during one year. *E.coli* strains in urine samples were identified by conventional methods. The UPEC strains was confirmed by the gene including *by* decteting *papC*, *papGII* , *papGIII* , *sfa*/*foc*, *hlyC*, *c nf1* , *iucC*, *fyuA*, *iron N* genes by PCR method. Antibiotic susceptibility testing was done for *E. coli* by disk-diffusion method based On CLSI protocol. 12572 urine samples of suspected children with urinary infections were studied and then 378 *E.coli* strains were detected in which 149 of strains were UPEC (39/7%). All of Uropathogenic *E.coli* were resistant to penicillin, Oxacillin, Bacitracin, Cloxacillin and Pipracillin. Resistant to other antibiotics were: Sulfametoxazole 92%, Nalidixic acid 53%, Ampicillin 89%, Nitrofurantoin 9%, Cephotaxime55.3%, Cefixime67%, Gentamicin72%, Cephalexin75.6%, Ciprofloxacin17.5%. The prevalence of *papC*12.37%, *papGII*15.06%, *papGIII*13.17%, *sfa*/*foc*17.23%, *hlyC*39.41%, *c nf1* 23.4%, *iucC* 7.35%, *fyuA*18.12%, *iron N*22.13% genes by PCR method. Of the putative uropathogenic Virulence Factors examined *papC*, *papGII* , *papGIII* , *sfa*/*foc*, *hlyC*, *c nf1* , *iucC*, *fyuA*, *iron N* were frequently associated with urinary tract infection. Especially *iroN* was most frequently associated with Cystitis and Pyelonephritis. Some VF genes were closely associated with a specific anatomical site of infection. The strong associations between several virulence factors (VFs) might indicate not only well-known genetic linkages, but also unknown functional linkages among these VF genes. Periodic review and formulation of antibiotic policy are needed for control of Acquisition of drug resistance. Further studies on better understanding of interaction of different virulence factors at molecular level are necessary as most urovirulent strain express multiple virulence factors Simultaneously[1].

Key Words: Uropathogenic *E.coli* virulence genes, Antibiotic susceptibility pattern, UTI.

Escherichia coli is by far the most common pathogen isolated from urinary tract infection (UTI), and frequently originates from the patients' own intestinal flora. However, only some members of the normal flora elicit an infection in persons without local or general predisposing conditions to UTI. *E. coli* clones present in the large intestine are not equally able to initiate and maintain the infectious process in the urinary tract. Special components or products, called virulence factors, enable *E. coli* cells to colonies selectively the mucosal uro-epithelium, evoke an inflammatory reaction and eventually proceed from the lower urinary tract to the renal cavities and tissues[2].

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Persistent urinary tract infections usually emerge in early childhood. Approximately 1 to 8% of children between the ages of 1 month and 11 years have experienced at least one Urinary tract infection [3, 4, 5, 6] Recurrent urinary tract infection (RUTI) endangers renal function; even the first episode of acute Pyelonephritis can lead to renal scarring in 9.5% to 57% of cases, according to Hoberman *et al*.[7] and *Lin et al*[8]., respectively. In childhood the most important risk factor for RUTI has been considered to be the presence Of vesicourinary reflux, alone or combined with dysfunctional voiding. The second important risk factor is closely associated with antimicrobial therapy for RUTI. In children, as in adults, The most frequent urinary pathogen is *Escherichia coli*, and the prevailing treatment schemes include the beta-lactam antibiotics, Trimethoprim-Sulfamethoxazole (SXT), and aminoglycosides. However, increased resistance among urinary *E. coli* strains to some beta- lactam antibiotics and SXT has been reported in different countries. Furthermore, children with vesicourinary reflux require long-term antimicrobial prophylaxis usually with SXT or Nitrofurantoin. This, in turn, can select bacteria with increased resistance during the course of RUTI[9].

Most UTIs in children result from ascending infections, although hematogenous spread may be more common in the first 12 weeks of life. Most UTIs in children are monomicrobic, often Caused by *Escherichia coli* (60 to 80 percent of cases), Proteus (more common in boys and in Children with renal stones), Klebsiella, Enterococcus, and coagulase-negative staphylococci. Evidence on risk factors for UTI in children is limited. UTIs were associated with Constipation, encopresis, bladder instability, and infrequent voiding, but not in a cohort of Febrile children younger than two years. Bathing and back-to-front wiping have not been demonstrated to be risk factors[10].

MATERIALS AND METHODS

Patients

This was a cross-sectional study of the current clinical practice over a 1-year period from September 2008-2009 in urban children's hospital emergency department. Boys younger than 1 year and girls younger than 2 years of age were eligible for the study if they had a rectal Temperature 8.5°C, were not taking antibiotics, were not immunosuppressed, and did not have a definite source of fever on examination. Clean catch midstream Urine were obtained On these children as part of routine clinical practice. This questionnaire was completed by the examining physician and nurse at the time.

The diagnostic threshold depends on the method of urine collection. Compilation of studies comparing counts of colony-forming units from various collection methods with the probability of UTI has established the recommendations of Sarah Curry, 2005.

For urine collection from infants and young children, or transurethral catheterization generally is recommended. Urethral catheterization is more likely than aspiration to obtain a sufficient sample of urine. Collection from bags or pads leads to high contamination rates. Clean-catch urine collection from infants requires more patience and effort than the use of pads or bags, but this method is reasonably accurate and rates of contamination are low.

Diagnostic thresholds for UTI in children, (Sarah Curry, 2005)

Method of urine collection	Diagnostic threshold
Clean catch voiding in girls	10^5 CFU/ml Repeat testing if 10^4 to 10^5 CFU/ml
Clean catch voiding in boys	10^4 CFU/ml
Catheter	10^4 CFU/ml Repeat testing if 10^3 to 10^4 CFU/ml

Bacteria

A total of 378 *E. coli* strains obtained in counts of $>10^5$ cfu/ml and in pure growth, from routine urine cultures were isolated from the urine of children with UTI (n=155), acute Pyelonephritis (n = 97), cystitis (n = 126). Clinical symptoms and signs, ages, and previous histories of the patients have been described[11].

These strains were previously described, and their adhesion properties were determined by phenotypic and genotypic approaches. *E. coli* K-12 strain HB101 was used as a negative control

(absence of adhesin). *E. coli* J96 carrying at least three separate adhesin-encoding operons (*pap*, prs [pap-related sequences], and foc) *E. coli* KS52 carrying an afa operon , and *E. coli* K-12 strain HB101 carrying recombinant plasmid pANN801-13, which contains the entire sfa gene cluster, were used as positive controls for detection of adhesins. Bacteria were Grown in Luria broth medium without glucose (10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter [pH 7.0]) for 18 h at 37°C.

Antibiotic Susceptibility Testing

E. coli isolates were screened for susceptibility to penicillin, Oxacillin, Bacitracin, Cloxacillin, Pipracillin, Sulfametoxazole, Nalidixic acid, Ampicillin, Nitrofurantoin, Cephotaxime, Cefixime, Gentamicin, Cephalexin, and Ciprofloxacin with the use of a standard disk-diffusion assay by CLSI protocol. *E. coli* strain 25922 ATCC (from the American Type Culture Collection) was used as the reference strain[12].

Preparation of bacterial DNA

DNA to be amplified was released from whole organisms by boiling. Bacteria were harvested from 1 ml of an overnight broth culture, suspended in 200 µl of sterile water, and incubated at 100°C for 10 min and transfer to -20 C (three times). Following ultracentrifugation of the lysate, a 150 µl sample of the supernatant was stored at -20°C as a template DNA stock.

Amplification procedure

Nine putative virulence factor genes characteristic of extraintestinal pathogenic *E. coli* (*papC*, P fimbriae; *papGII*, adhesin *pap*G class II; *papGIII*, adhesin *Pap*G class III; *sfa/foc*, S fimbriae; *hlyC*, hemolysin; *cnf1*, cytotoxic necrotizing factor; *iucC*, iron uptake system [IUS] aerobactin; *fyuA*, IUS yersiniabactin; and *iroN*, IUS salmochelin) were identified by using a new multiplex PCR method adapted from our previous studies.[13,14,15]

The Uropathogenic *E.coli* strains was

Table 1. Primers used in PCR to amplify specific fragments for uropathogenic *E.coli*

Gene	Primer	Size of product(bp)	Reference
eae	Facgttgcagcatgggtaact Rgatcggcaacagtttcacctg	815	Jesus Blanco (1996)
papA	Fatggcagtggtgttttggtg Rcgtcccaccatacgtgctctt	717	Johnson & Stell (2000)
afa	F ggcagagggccggcaacaggc R cccgtaacgcgccagcatctc	594	Johnson & Stell (2000)
sfa	F ctccggagaactgggtgcatcttac R cggaggagtaattacaaacctggca	410	Johnson & Stell(2000)
hlyD	F ctccggtacgtgaaaaggac R gccctgattactgaagcctg	904	J. R. Johnson protocols
iha	F ctggcggaggctctgagatca R tccttaagctcccgcggctga	829	J. R. Johnson protocols
papC	F gtggcagtatgagtaatgaccgtta R atatcctttctgcagggatgcaata	205	Johnson & Stell (2000)
papGII	F gggatgagcgggcctttgat R cgggcccccaagtaactcg	190	Johnson & Stell (2000)
papGIII	F ggcctgcaatggatttacctgg R ccaccaaatgaccatgccagac	258	Johnson & Stell (2000)
cnf1	F atcttatactggatgggatcatcttgg R gcagaacgacgttcttcataagtatc	1105	Johnson & Stell (2000)
iucC	F cgccgtggctggggtaag R cagccggttcaccaagtatcactg	541	Skyberg *et al*. (2003)
fyuA	F tgattaaccccgcgacgggaa R cgcagtaggcacgatgttgta	787	Johnson & Stell (2000)
iron N	F aagtcaaagcaggggttgcccg R gacgccgacattaagacgcag	667	J. R. Johnson protocols

confirmed by the gene including *by detecting pap**C**, papGII , papGIII , sfa/foc, hlyC, c nf1 , iucC, fyuA, iron N* genes by PCR method. PCR was done in a total volume of 50 µL containing 10 µA of the template DNA, each of the primers at 0.45 µM, the four deoxynucleoside triphosphates (each at 200 µM), 10 mM Tris hydrochloride (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, and 1.2 U of Taq DNA Polymerase (GENET BIO T^M Taq DNA polymerase).

The reaction mixture was overlaid with 3 drops of mineral oil. PCR amplifications consisted Of 25 cycles of denaturation at 94°C for 2 min, annealing at 65°C for 1 min, and extension at 72°C for 2 min in a Thermal Cycler (Eppendorff). Five microliters of the reaction mixture was then analyzed by electrophoresis on 2% agarose gels, and the reaction products were visualized by staining with ethidium bromide[16].

Statistical Analysis

Prevalence rates with 95% confidence intervals (CIs) were calculated for the study sample. Comparisons were made between categorical variables using x2 test of proportions or, in the case of small samples, Fisher's exact test, with $P \leq 0.05$ being the a priori significance level. Multiple logistic regressions were used to evaluate the possibility of confounding in the relationship between race and UTI.

RESULTS

12572 urine samples of suspected children to have urinary infections were studied and then 378 E.coli strains were detected in which 149 of strains were UPEC (39/7%). All of Uropathogenic *E.coli* were resistant to penicillin, Oxacillin, Bacitracin, Cloxacillin and pipracillin .pattern of resistancy to other antibiotics were: Sulfametoxazole 92%, Nalidixic acid 53%, Ampicillin 89%, Nitrofurantoin 9%, Cephotaxime55.3%, Cefixime67%, Gentamicin72%, Cephalexin75.6%, Ciprofloxacin17.5 %. The prevalence of *papC*12.37%, *papGII*15.06%, *papGIII*13.17%, *sfa/foc*17.23%, *lyC*39.41%, *c nf1* 23.4%, *iucC* 7.35%, *fyuA*18.12%, *iron N*22.13% genes by PCR method.

DISCUSSION

E.coli is the most common cause of acute Pyelonephritis which is the most serious form of UTI, particularly harmful to newborns and small children. In *E.coli* strains, the P fimbriae is considered as an essential virulence factor causing Pyelonephritis. The pap family group encodes for the p fimbriae adhesion which has been shown to mediate attachment to specific cell surface glycopeptides present throughout the urinary tract. They facilitate colonization and invasion of the renal parenchyma. The ability to adhere to epithelial surface has been shown to be a prerequisite for E.coli strains to colonize the urinary tract and cause UTI in the absence of urological abnormalities.[18, 19, 20, 21]

The ability to adhere to epithelial surfaces has been shown to be a prerequisite for *E. coli* strains to colonize the urinary tract, i.e., to cause UTI in the absence of urological abnormalities.

Periodic review and formulation of antibiotic policy are needed for control of Acquisition of drug resistance. Further studies on better understanding of interaction of different virulence factors at molecular level are necessary as most urovirulent strain express multiple virulence factors simultaneously. In conclusions, our findings indicated that pap Adhesion–encoding genes have an important role in the development and severity of UTI. Many cases of serious urogenital disease are caused by a limited number of uropathogenic *E.coli* strains that generally possess special virulence factors such pap operon[22].

In conclusions, our findings indicated that *papC*, *papGII*, *papGIII*, *sfa/foc*, *hlyC*, *c nf1*, *iucC*, *fyuA*, *iron N* especially pap Adhesion–encoding operon has an important role in the development and severity of UTI. Many cases of serious urogenital disease are caused by a limited number of uropathogenic *E.coli* strains that generally possess special virulence factors. Periodic review and formulation of antibiotic policy are needed for control of Acquisition of drug resistance. Further studies on better understanding of interaction of different virulence factors at molecular level are necessary as most urovirulent strain express multiple virulence factors simultaneously. Some Virulence Factors genes were closely associated with a specific anatomical site of infection. The strong associations between several VFs might indicate not only well-known

genetic linkages, but also unknown functional linkages among these VF genes.

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