Research Article

Isolation, Identification, Molecular Characterization and Antibiotic Susceptibility Testing of Uro Pathogenic E.Coli (UPEC) Isolation from Non-Hospitalized Urinary Tract Infections (UTI)

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Abstract

Background of the study: Urinary tract infections (UTIs) caused by Uro-pathogenic *E.coli* (UPEC) considered as most serious infections with increased mortality and morbidity. The ability of UPEC to encode variety of virulence determinants correlated with high recurrence rates and antibiotic resistance.

Purpose of the study: The present study focuses on DNA Extraction from *E.coli* by rapid PCR method and also characterization of Emboli's molecules. *E. coli* has the carrying ability of many mobile genes, these mobile genes carry the virulence factors. The present study was designed to detect these virulence genetic factors, using phenotypic method like multiplex PCR and detecting capsule synthesis, invasions toxin's, Adhesions and side-rophores, The study also focused on a specific gene CHUA having ability in heme iron acquisition system and investigation of various virulence determinants expressed by UPEC and their relationship with antibiotic resistance.

Methods: Total 15 clinical samples of UPEC were isolated, identified and screened for antibiotic susceptibility pattern. Kirby Bauer disc diffusion test and micro broth dilution method were used to measure the antibiotic sensitivity testing of UPEC isolates. The susceptibility was tested by measuring the zone size after impregnated with antibiotic discs.

Results: The interpretation of zone size was done according to the proposed protocol of Clinical Laboratory and Standard Institute (CLSI). Majority of UPEC isolates (22%) were sensitive to tetracycline followed by Norfloxacin (18%). However, least sensitivity was observed against ampicillin (2%) and no sensitivity was experienced against cephalosporin (0%) and penicillin (0%).

Conclusion: It has been concluded that majority of UTI patient were suffering from UPEC. Resistance of UPEC against frontline drugs increasing rapidly. Thereby rational and appropriate use of antibiotics is the only way to save important therapeutic options.

Keywords: Urinary tract infection, UPEC, CLSI, tetracycline, norfloxacin, ampicillin

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INTRODUCTION

Urinary tract infections (UTIs) are categorized as most serious infections due to high recurrence rates and increased antibiotic resistance (10). The UTI is indicated by the presence of significant ($\geq 10^5$ CFU/ml) number of pathogens in urine, however in certain cases, blood or significant pus cells (few to many) in urine can also act as good indicator (2,13). Up to 150-250 million cases per year of UTIs with increased mortality and morbidity have been reported all across the globe (3, 12).

E.coli is one of the widespread etiologic agent that can cause both complicated and uncomplicated UTIs (4). Among *E.coli* strains, Uropathogenic *E.coli* (UPEC) is a well-known pathogen responsible for approximately 90% of all the UTIs including nosocomial (50%) and community acquired UTI's (70-95%) (9). UPEC strains act as an opportunistic intracellular pathogen that can colonize the bladder of urinary

tract having variable clinical manifestation ranging from cystitis to severe pyelonephritis (5, 8).

Earlier studies have reported that UPEC strains express highly ubiquitous virulent determinants such as fimbriae, biofilms and toxins (Hemolysins) that are known for their effective colonization, increased persistence and pathogenesis (11). Bacterial attachment and invasion is primarily facilitated by fimbriae, crucial for developing cystitis and pyelonephritis (18). The improved virulence of UPEC is also attributed to the secretion of a labile pore-forming toxin known as α - Hemolysins (4).

UTIs are usually treated with antibiotics. Globally they are the second predominant reason for antibiotic prescription (24).

In uncomplicated UTI cases, antibiotic nitrofurantoin is used for a short time period. And in complicated UTI many antibiotics such as intravenous antibiotics for long time or

trimethoprim/sulfa-methoxazole is used as a treatment option (22).

Due to the beginning of Multi Drug Resistant (MDR) UPEC strains by the expression of various virulence attributes and resistance mechanisms such as biofilm production has led to prolonged treatment of UTIs against beta lactams & fluoroquinolones class of antibiotics. Co-trimoxazole (trimethoprim/sulfa-methoxazole), fluoroquinolones (e.g. levofloxacin, ciprofloxacin), aminoglycosides (gentamycin) and 3rd gen cephalosporins (e.g. ceftazidime, ceftriaxone) are the most widely used frontline drugs for the treatment of both uncomplicated and complicated UTIs. Fluoroquinolones provide a good option for the treatment of serious UTIs because of their broad spectrum invitro efficacy and excellent tolerance (rare hypersensitivity potential). However, in pregnancy cautions should be taken before using fluoroquinolones as it effects fetus development.

Moreover, it is not recommended as a first line drug for treating pregnant women suffering from severe pyelonephritis. MDR UPEC strains are also responsible for relapse and recurrence of urinary tract infection (7) which is a serious global public health concern. To design appropriate therapy for UTIs, physician must have knowledge about the resistance profile of etiologic agent of his geographic region. Since the UPEC have evolved several mechanisms to evade antimicrobial therapy that contribute to the rise in antimicrobial resistance against the front-line drugs i.e. Fluoro-quinolones as well as third and fourth generation Cephalosporins. This research methodology is a novel approach that will provide greater insight for the treatment of UTIs. The present study focuses on scrutinizing the clinical isolates of UPEC and their antibiotic sensitivity pattern of UPEC.

METHODOLOGY

Sample Collection

A total of 50 urine samples on the bases of selected criteria were taken from out patients. Samples were collected by applying slandered microbiological methods under sterile aseptic conditions from tertiary care academic hospitals viz. Bolan Medical Complex (BMC) and Sandmen Civil Hospitals of Quetta city and then urgently transferred to Laboratory at Center for Advance Studies in Vaccinology and Biotechnology (CASVAB).

Specimen Processing

The urine isolates were identified through microscope for color and turbidity. Pus cells and organisms presence were detected in the samples by preparing Wet mounts. Semi quantitative cultures were done by inoculating, thoroughly the mixed urine onto Cytosine Lactose Electrolyte Deficient (CLED) and Mac-conkey agar, for overnight incubation at 37°C under aerobic conditions. The primary culture so obtained was then grown over Mac-conkey agar. Morphology and gram staining were performed for primary level identification, whereas, biochemical tests and molecular techniques were done for secondary level identification (13).

Polymerase Chain Reaction (PCR)

DNA was extracted from the pure *E.coli* colonies from Mac-conkey agar, master plates were proceeded by using the standard phenol-chloroform protocol. The overnight bacterial cultured on Luria-Bertani broth were used for genomic DNA extraction, using the DNPTM, CinnaGen, Iran kit with a little modification in manufacturer's protocol. 450bp primers of the

distal conserved and proximal flanking region of *E. coli* 16S rRNA were used for the molecular recognition (13). Polymerase Chain Reaction (PCR) was done for the confirmation of *E. coli* colonies. The reagents for the PCR were delivered by Solis Bio Dyne and primers used were of Montreal Quebec. The PCR reaction reagent comprised of 5X Master mix of FIREPOL®. The master mix used contained Taq polymerase, 200µM dNTPs, 7.5mM buffer and MgCl. Concentration of each primer used was 10µl.

Determination of Antibiotic Sensitivity Testing by Kirby Bauer Disc Diffusion Test

In this method antimicrobial discs with known concentration and volume are placed on sensitivity testing agar plate containing the test organism. The antibiotics diffused into the medium and after overnight incubation at 37°C zone of inhibition was observed and measured. These media plates were again incubated for about 24 hours and zone diameters were further measured in millimeters. The zone inhibition was interrupted by denoting to the Clinical Laboratory Standard Institute (CLSI) procedures and organisms were labeled as susceptible, intermediate, or resistance (22).

Determination of Minimum Inhibitory Concentration (MIC)

MIC is considered as a gold standard for determining antimicrobial susceptibility pattern of bacteria. n=13 UPEC strains were randomly selected that initially had shown susceptibility to multiple drugs via disc diffusion method. MICs of the drug susceptible strains were resolute by "broth micro dilution method" using cation-adjusted Mueller-Hinton broth against Ceftazidime, Levofloxacin, gentamicin and trimethoprim based on CLSI guidelines (17).

RESULTS

A total number of 50 urine samples from UTI patient were processed 30% of which (n=15) were found as *E.coli*, confirmed by Gram staining, biochemical tests and molecular analysis. *E.coli* isolates were produced green colonies on Eosin Methylene Blue agar (EMB) medium shown in Fig.1. Fig.2. depicted the number of bacterial species isolated from non-hospitalized UTI patient.

Majority of the samples were positive for UPEC while rest of these samples showed growth of *Klebsiella*, *Staphylococcus* and *Bacillus* species. More number of isolates were isolated from female patient as compared to male patient.

Fig. 1. UPEC isolates cultured on EMB agar. A. negative result, B. Positive result.

Fig. 2. Graphical representation of percentages of different bacterial species isolated from UTI patients.

Fig. 4. Graphical representation of percentage sensitivity of UPEC against different antibiotics

Biochemical Tests

Through biochemical tests it was confirmed that all the *E.coli* isolates were positive for indole, methyl red, and catalase test and lactose fermenting abilities were also found. Figure 3 depicted the biochemical tests of *E.coli* isolates. Characteristic ring formation was observed at the top of test tube for indication of positive indole test Fig.3A. UPEC isolates were produce red coloration for methyl red test Fig.3B while formation of blue coloration was indicative of positive citrate test Fig.3C. A characteristic bubble was formed while performing catalase test shown in Fig.3D. These isolates were also showed lactose fermenting abilities and coagulase production as shown in Fig.3E and 3F. All isolates have shown negative results for Voges-Proskauer, citrate and urease tests. Hence biochemical tests have confirmed these isolates as *E.coli*.

Fig. 3. Different biochemical tests of Uropathogenic *E.coli* strain (UPEC) e.g. Indole test (A), EMB test (B), citrate utilization test (C), catalase test (D), TSI test (E) and coagulase test (F).

Antibiotic Sensitivity Testing

Antibiotic sensitivity were tests for all UPEC samples against Ampicillin, Penicillin, Tetracycline, Cephalosporin and Norfloxacin. Majority of UPEC isolates (22%) were sensitive to tetracycline followed by Norfloxacin (18%). However, least sensitivity was observed against ampicillin (2%) and no sensitivity was experienced against cephalosporin (0%) and penicillin (0%) as shown in Fig 3. Majority of UPEC isolates were moderately sensitive against ampicillin while in case of Cephalosporin majority of isolates were resistant against this class of antibiotic (Fig 4).

Fig. 5. Bands in gels through PCR in Transilluminator.

DISCUSSION

Urinary tract infections are the major public health concern across the globe and represents one of the most common hospital-acquired infections (3). UPEC strains express ubiquitous and complex plethora of virulent determinants that contributes to its effective colonization, increased persistence and pathogenesis of the disease (12). Uropathogenic *E. coli* (UPEC) is a major etiological agent associated with both complicated and uncomplicated UTIs (14). UPEC alone accounts for 90% of all the UTIs including both nosocomial and community acquired infections (19).

Our study had proved that majority of UTI patients were suffering from UPEC. Total 50 samples from UTI patients were

processed by Gram's staining, biochemical tests and molecular techniques, DNA was detected by using multiplex PCR, which revealed 30% positive for UPEC, 30% of Gene CHUA from *E. coli* detected. These isomers detected by multiplex PCR. The UPEC isolates were further processed for antibiotic susceptibility testing against frontline drugs e.g. fluoro-quinolones, amino-glycosides, ampicillin and cephalo-sporins. Empirical treatment of UTI at different geographical locations relies on local susceptibility profile however, frontline antibiotics such as co-trimoxazole (trimethoprim /sulfa-methoxazole) fluoroquinolones (e.g. levofloxacin, ciprofloxacin), aminoglycosides (gentamycin) and 3rd gen cephalosporins (e.g. ceftazidime, ceftriaxone) are widely used therapeutic options for the treatment of both uncomplicated and complicated UTIs. Because of their excellent penetration, trimethoprim and 2nd generation fluoro-quinolones such as levofloxacin are important choices for the treatment of male prostatitis. However, unfortunately resistance against this important class of antibiotics has been increasing gradually over the last few decades. Country wise data was calculated as (15.9%), (18.2%), (16.7%), (16.3%), (14.4%), (35.9%) and (25.4%) in Austria, Greece Portugal, Sweden, UK, Korea and Europe, respectively (17, 13, 15).

Alternatively, Fluoroquinolones have been widely used as treatments against different infections including UTIs and provide long-half life and excellent tissue penetration properties (16). Likewise, third and fourth generation Cephalosporins such as ceftriaxone and Ceftazidime provide reliable therapeutic options for the eradication of co-Trimoxazole resistant uropathogens. In addition, aminoglycosides are used in combination with β -lactam or glycopeptides (12). However, resistance against these drugs is also increasing rapidly. Our study proved that highest resistance was observed against Cephalosporins and penicillin e.g. 100%, followed by ampicillin 98% while tetracycline and Norfloxacin showed little efficacy against UPEC. These results were in accordance with previous studies (17). Greater frequency of the UPEC in the females has been reported in few of the research studies being conducted in the different vicinities of Pakistan. 87.5% in Lahore, 71% in Karachi, 60% in Gilgit Baltistan, 79% in Hazara region and 63% in Islamabad (13).

CONCLUSION

Molecular Characterization of Uropathogenic *E. coli*, was performed during the present study, after the DNA extraction, with the help of specific primers, detection of a specific gene CHUA was done by rapid Multiplex Polymerase Chain Reaction. It was found that out of 50 isolates CHUA gene was present in the 15 samples of Uropathogenic *E. coli* (30%). Furthermore, the results of the present study showed high resistance of Cephalosporin (Cefixime) i.e. 63%. However, majority of Isolates, which are resistant to Cephalosporin, were ESBLs producers. Which revealed that the extensive use of cephalosporin leads to the co-selection of ESBLs producers and resistance to other antibiotics. Therefore, it can be suggested that the balanced and appropriate use of antibiotics is the only way to save important therapeutic options.

DECLARATION

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or

comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

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