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Isolation, Identification, Molecular Characterization and Antibiotic susceptibility testing of UPEC isolated from Non-Hospitalized UTI patient from Baluchistan region

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ABSTRACT
Urinary tract infections (UTIs) caused by Uropathogenic E, coil (UPEC) considered as most serious infections with increased mortality and mobidity. The ability of UPEC to encode variety of virulence determinates correlated with high recurrence rates and antibiotic resistance. The present study focuses on DNA extraction from E. coil by rapid PCR method and also characterization of Emboli's molecules. E. Coil ability is carrying many mobile genes these mobile genes carry the virulence factory by present study using phenotypic method to deduct these virulence genetic factors like using multiplex PCR and detecting capsules synthesis, invasions toxin's Adhesions and siderophores, The study also focused on a specific gene CHUA gene having ability in heme iron acquisition system and investigation of various virulence determinates expressed by UPEC and their relationship with antibiotic resistance. Total 15 clinical isolates of UPEC were isolated, identified and screened for antibiotic susceptibility pattern. Kirby Bauer disc diffusion test and micro both dilution method was used to measure the antibiotic sensitivity testing of UPEC isolates. The susceptibility was tasted by measuring the zone size after impregnated with antibiotic discs. The interpretation of zone size was done according to clinical laboratory and standard institute (CLSI) guidelines. Majority of UPEC isolates (22%) were sensitive to tetracycline followed by norfloxacin (18%). However, last sensitivity was observed against ampicillin (2%) and no sensitivity was experienced against cephalosporin (0%) and penicillin (0%). 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*

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INTRODUCTION

Urinary tract Infections (UTIs) are categorize as most serious infections due to high recurrence rates and increased antibiotic resistance [19]. The UTI indicated by the presence of significant (>10^5 CFU/ml) number of pathogens in urine, however in certain cases, blood corpus cells (few to many) in urine can also act as good indicator [2;23]. Up to 150-250 million cases per year of UTI's with increased mortality and morbidity have been reported all over the globe [3; 20]. E. coli is one of the widespread etiologic agent that can cause both complicated and Uncomplicated UTI [4]. Among *E.coli* strains, uropathogenic *E. coli* (UPEC) is a well-known and excellent tolerance (tolerance excellent tolerance (rare hypersensitivity potential). However in pregnancy cautions should be taken before using fluoroquinolones as it effects fetus develop. Moreover, it not recommended as a first line drug for treating pregnant women suffering from Severe pyelonephritis. MDRUPEC strains are also responsible for relapse and recurrence of Urinary tract infection[17], 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 of 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. fluoroquinolones as well as third 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.

MATERIAL AND METHODS

Sample Collection

A total of 50-urine sample on the bases of selected criteria were taken from our patients. Sample was collected by applying standard microbiological methods under sterile aseptic conditions from tertiary care academic hospitals e.g. Bolan medical college and hospital as well as civil hospitals of Quetta city and then urgently transferred to laboratory at CASVAB (center for Advance studies in vaccinology and Biotechnology).

Specimen Processing

The urine samples were detected macroscopically for their color and turbidity. Wet mounts of the samples were prepared and examined for the presence of pus cells and organisms. Semi Quantitative cultures were done by inoculating thoroughly the mixed urine onto (CLED) agar (cytosine Lactose Electrolyte Deficient) and MacConkey agar and incubated at 37°C for overnight under aerobic conditions. The primary culture so obtained was the grown over MacConkey agar. Primary level identification was made on the basis colony morphology and gram staining, followed by secondary level identification based on biochemical tests and finally by molecular techniques.

DNA Extraction and Bacterial Confirmation using Polymerase Chain Reaction:

Pure E.coli colonies from MacConkey agar master plates were proceed with DNA extraction by using the standard phenol- chloroform DNA extraction protocol. Bacteria were cultured overnight on Luria-bertani broth (Merck, Germany) and genomic DNA was extracted from typical colonies using the DNA extraction kit (DNPTM, CinnaGen, Iran) according to the manufacturer's instructions. 450bp primers of the distal conserved and proximal flanking region of *E.coli* 165 rRNA were used for the molecular recognition [13]. All *E.coli* colonies were also conformed using the Polymerase Chain reaction (PCR) techniques. He reagents for the PCR were delivered by soils Bio Dyne and premiers used were of Montreal Quebec. The PCR reaction reagent comprised of 5X Master mix used contained Taq polymerase, 220uM dNTPs, 7.5 mM buffer and MGCI. Concentration of each premier used was 10ul.

Determination of Antibiotic Sensitivity Testing by Kirby Bauer disc Diffusion Test

In this method antimicrobial disc with known concentration and volume are placed on sensitivity testing agar plate containing the test organism. The antibiotic diffused into the medium and after overnight incubation at 37°C zone of inhibition is witnessed and measured in millimeters. The plates were incubated at 37°C for 18-24 hours. Zone diameter were measured in millimeters. The size of zone of inhibition was interrupted by referring to the CLSI (clinical Laboratory standard Institute)
Guidelines and organism was labeled susceptible, intermediate, or resistance accordingly [7].

RESULTS

A total number of 50 urine samples from UTI patients were processed, of which n=15 (30%) were confirmed as E.coli by Gram staining, biomechanical tests and molecular analysis. E.coli isolates were produced green colonies on eosin methylene blue agar (EMB) medium as 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 wile rest of these samples showed growth of *Klebsiella*, *staphylococcus* and *Bacillus* species. More number of isolates were isolated from female patient as compared To female patient.

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

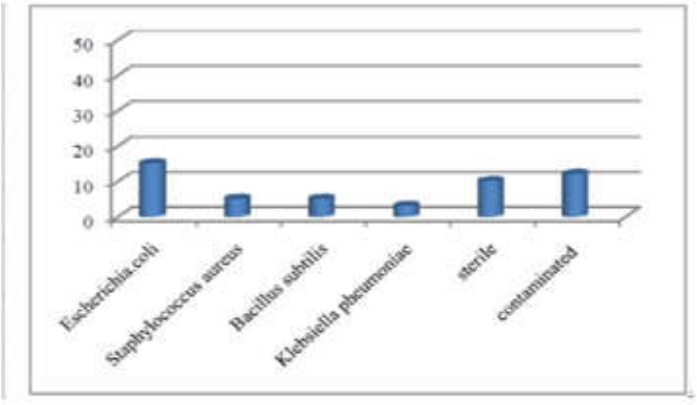

Figure 2. Graphical representation of percentages of different bacterial species isolated from UTI patients.

Biochemical Tests

All the E.coli isolates were positive for indole, methyl red catalase test and showed lactose-fermenting abilities. Figure 3. Depicted the biomechanical test 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 indicated of positive citrate test Fig.3c. A characteristics bubble was formed while performing catalase test shown in Fig.3D. These isolates were also showed lactose fermenting Abilities and coagulase production shown in Fig.3E and 3F all isolates have shown negative result for vogues- Proskuaer and urease tests. Hence biomechanical test have confirmed these isolates as *E.coli*.

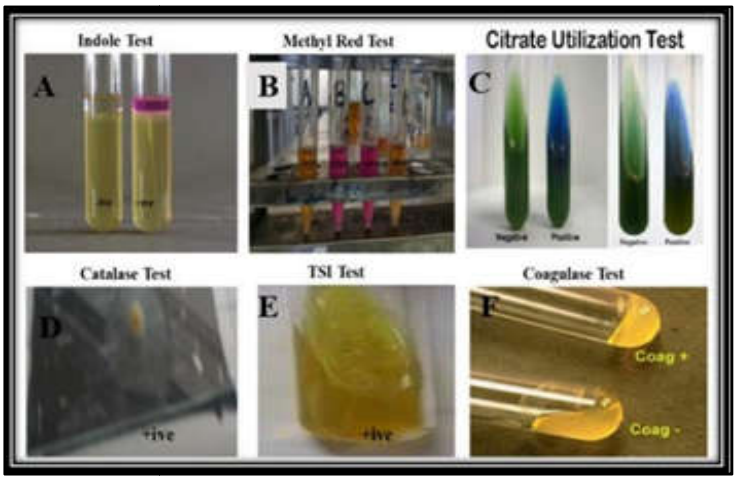

Figure 3: Different biomechanical test of Uro pathogenic E.coli strain (UPEC) e.g. indole test (A), EMB te (B), citrate utilization test (C), catalase test (D), TSI test (E), and coagulase test (F)

Antibiotic Sensitivity Testing

All UPEC isolates were tested for antibiotic sensitivity testing against drugs e.g ampicillin penicillin tetracycline cephalosporin's and nor floxacin. Majority of UPEC isolates (22%) were sensitive to Tetracycline followed by norfloxacin (18%). However, last sensitivity was observed against ampiciln (2%) and no sensitivity was experienced against cephalosporin (0%) and pencilin (0%) as shown in Fig 5. Majority of UPEC isolates were moderately sensitive against ampicillin while in case of cephalosporin majority of isolates were resistant against the class of antibiotic as shown in figure.

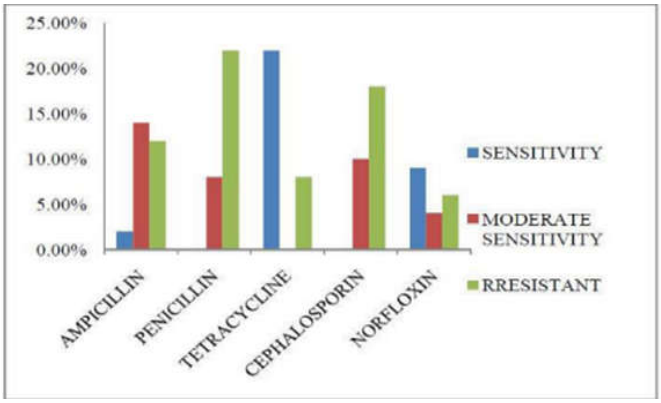

Figure 4 Graphical Representation of percentage sensitivity of UPEC against different antibiotics

DISCUSSION

Urinary tract infections are the major public health concern across the globe and one of the most common hospital- acquired infections [24]. UPEC strain express ubiquitous and complex plethora of virulent determinates that contributes to its effective colonization, increased persistence and pathogenesis of the disease [21]. Uro pathogenic *E.coli* (UPEC) is a major Etiological agent associated with both complicated and uncomplicated UTIs [4]. UPEC alone accounts for 90% of all the UTIs including both nosocomial and community acquired infections [9]. Our study had proved that majority of UTI patients were suffering from UPEC. Total 50 samples from UTI patients were processed of which 30% were positive for UPEC after being analyzed by Gram's staining biomechanical test and molecular techniques DNA was detected by using multiplex PCR and As according to my study also 30% Gene CHUA from *E.coli* detected from 15 samples of urine. These isomers detected by multiplex PCR. The UPEC isolates were further processed for antibiotic susceptibility testing against frontline drugs e.g.

Fluroquinolones, Aminoglycosides, Ampcillin and cephalosporins

Empirical treatment of UTI at different geographical locations relies on local susceptibility profile however, front line antibiotics such as Co-trimoxazole (trimethoprim/ sulfamethoxazole) fluoroquinolones (e.g. levofloxacin, Ciprofloxacin), aminoglycosides (gentamycin) and 3rd gen cephalosporin's (e.g. ceftazidime, Ceftriaxone) are widely used therapeutic options for the treatment of both un complicated and complicated UTIs. Because of their excellent penetration, trimethoprim and 2nd generation Fluoroquinolones such as levofloxacin are important choices for the treatment of male prostatis However, unfortunaitly resistance against this important class of antibiotic has been increasing steadily over the last few decades. For example, in Austria (15.9%), Greece (18.2%), Portugal (16.7%) Sweden (16.3%) UK (14.4%) Korea (35.9%) and Europe (25.4%) [25;15;16]. Alternatively fluoroquinolones have been widely used as treatments against different infections including UTIs and provide long –half life and excellent tissue penetration properties [6]. 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, Aminoglycossides are used in combination with B lactam or glycopeptides [22]. However Among 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 [1]. 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 Islamabad [13].The present study showed about uropathogenic E coli molecular and detection of primers and a specific gene by rapid multiplex polymerase chain reaction. Chua gene was detected from 15 samples .

In this study, we should high resistance of Cephalosporin (Cefixime) i.e. 63%. Higher number of isolates those are resistant to cephalosporin are ESBLs producers. It is known that extensive use of cephalosporin's leads to the co-selection of ESBLs producers and resistance to other antibiotics. There by rational and appropriate use of antibiotrecs is the only Way to save important therapeutics options

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