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Frequency and Antibiotic Resistance of Hybrid Entroaggregative/Uropathogenic *Escherichia coli* Isolated from Patients Hospitalized in Isfahan, Iran

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ABSTRACT

Backgrounds: This study aimed to analyse hybrid Entroaggregative/Uropathogenic *Escherichia coli* (EAEC/UPEC) isolates. To do so, the antibiotic resistance pattern and virulence genes were investigated in *E. coli* strains isolated from clinical specimens of patients hospitalized in Isfahan, Iran.

Materials & Methods: Disc diffusion method was used to determine the antibiotic susceptibility pattern of EAEC/UPEC isolates. Also, virulence determinants of these isolates were determinated by singleplex and multiplex PCR.

Findings: Overall, a total of 148 *E. coli* isolates were collected, of which 12 (8.1%) isolates were hybrid EAEC/UPEC strains, then antibiotic susceptibility examination was operated on these strains. The higest antibiotic resistance rate was related to ofloxacin (42%), followed by trimethoprim-sulfamethoxazole (41%), ceftriaxone and cefepime (33%), and cefoxitin (17%). All the isolates showed susceptibility to fosfomycin.

Conclusion: According to the current study, since resistance to fluoroquinolones has increased in hybrid strains, monitoring the drug susceptibility of hybrid strains seems critical in Iran. Fosfomycin is considered to be the drug of choise for infections caused by multidrug-resistant (MDR) Gram-positive and Gram-negative bacteria. Fortunately, 100% of the strains were sensitive to fosfomycin.

Keywords: Enteroaggregative *Escherichia coli*, Uropathogenic *Escherichia coli*, Hybrid strains.

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Introduction

Escherichia coli is a commensal bacterium that colonizes humans shortly after birth. It has also been reported to interact with its hosts [1]. However, they are circumscribed to the outside bed of the intestine, which allows them to sometimes cause enteric or extra-intestinal pathological processes in humans. Some extremely altered *E. coli* strains have appeared during their development. Gaining extensive virulence factors, *E. coli* is expected to change, colonize, and invade many body sites [2-3].

Enteroaggregative *E. coli* (EAEC) is amongst the foremost notable heterogeneous strains of *E. coli* and is a causative agent of persistent watery diarrhea in children and adults worldwide [4].

Uropathogenic *E. coli* (UPEC) is a strain recovered from urinary tract infections (UTIs) and is the leading cause of bacterial infections amongst humans, accounting for 100 million UTL cases per year. These strains are a heterogeneous category of *E. coli* strains harboring a significant number of well-recognized virulence factors (VFs) expressed in various genotypes [5].

In EAEC, the transcriptional activator *aggR*, involved in the launch of a minimum of 44 genes, regulates both plasmid and chromosomal AAF gene expression [6-7]. Some virulence factors of UPEC strains include aerobactin (*aer*), P fimbriae (*pap*), hemin receptor (*chuA*), yersiniabactin siderophore receptor (*fyuA*), hemolysin (*hly*), type 1 fimbriae, afimbrial adhesin I (*afaI*), cytotoxic necrotizing factor 1 (*cnf1*), S fimbriae (*sfa*), adhesins, and fimbriae [8-10].

Recently, the frequency of antibiotic resistance in urinary tract pathogens has increased [11-12]. Mobile genetic elements (MGEs) including transposons, plasmids, and integrons are the most important factors for transferring resistance genes among bacteria. *E. coli* strains may be intrinsically resistant to antibiotics and may harbor genes responsible for resistance to antibiotics like aminoglycosides, fluoroquinolones, and β-lactams [13-14].

Interestingly, hybrid EAEC/UPEC pathotypes are considered as the causative agents of UTIs, afterward resulting in bacteremia and sepsis in patients [15-17]. Studies have shown concerns about the increased isolation of EAEC and UPEC pathotypes carrying virulence genes. Also, they have shown resistance of these isolates to several antimicrobials commonly used for treatment [18-19]. Some studies have investigated the genomic characterization of hybrid EAEC/UPEC and virulence factors among different pathotypes isolated.

Objectives: This study aimed to investigate hybrid EAEC/UPEC strains isolated from clinical samples to identify their virulence genes and antibiotic susceptibility patterns in Isfahan, Iran.

Materials & Methods

sample collection and identification: In this study, a total of 148 *E. coli* isolates were recovered from patients admitted to three hospitals (Imam Hossain, Shariati, and Al-Zahra hospitals) in Isfahan from July to November 2019. Samples were collected from urine, stool, biopsy, blood, and wounds from different hospital wards, such as pediatric, surgical, ICU, emergency, internal, and outpatient. Then the collected samples were immediately transported to the laboratory and stored at 4 °C for further analysis.

Microbial Identification: Collected samples were cultured on selective media such as MacConkey agar, Eosin methylene blue (EMB), and xylose lysine deoxycholate (XLD) (Merck KGaA, Darmstadt, Germany) at 37 °C for 24-48 hours. After overnight incubation, all lactose-positive colonies were re-cultured, and then standard phenotypic

and biochemical tests were performed and analyzed on triple sugar iron (TSI) agar (Merck KGaA, Darmstadt, Germany), SIM (Condalab, Madrid, Spain), MR-VP, urea broth, Simmon citrate agar, and Lauryl sulfate broth (LSB) (Biolife, Milan, Italy) to identify the isolated bacteria. Confirmed *E. coli* isolates were stored in brain heart infusion (BHI) broth (HiMedia Laboratories, Mumbai, India) comprising 20% glycerol (Merck KGaA, Darmstadt, Germany) at -20 °C for detecting EAEC and UPEC pathotypes [20]. Reference strain *E. coli* ATCC 25922 was used as quality control in each assay. The reference strain was supplied from the Bacterial Cell Bank of Pasteur Institute of Iran (CSBPI).

Examining hybrid EAEC/UPEC and virulence agents: In order for detecting hybrid EAEC/UPEC strains, PCR methods was carried out. A singleplex PCR was executed to amplify *fimH, chu, fyuA,* and *papc* genes, and multiplex PCR was operated to detect *app*, *aggR*, and *aatA* genes. PCR amplification was performed in a PCR mixture containing 10 pmol of each primer, 12.5 µL of Qiagen HotStar Taq polymerase Master Mix 1X, 3 µL of extracted DNA, and sterile distilled water. PCR methods were performed as previously described [21-23]. To verify the presence and sequencing of VGs in hybrid EAEC/UPEC pathotypes, PCR amplicons were sent to Niagenenoor Company (Iran, Tehran). Online BLAST software was employed to examine the sequences in NCBI database (https://www.ncbi.nlm.nih.gov/BLAST/).

Antimicrobial susceptibility experiment: The disc diffusion method was employed for *in vitro* antimicrobial susceptibility testing of *E. coli* isolates as suggested by the Clinical and Laboratory Standards Institute (CLSI) [24]. The following antibiotic disks on Mueller Hinton agar (MAST Categories Ltd., Merseyside, U.K.) were used: cefoxitin (30 µg), ofloxacin (5 µg), ceftriaxone (30 µg), fosfomycin (200 µg), cefepime (30 µg), and trimethoprim-sulfamethoxazole (25 µg). they were then diluted to 0.5 McFarland turbidity standards. A sterile cotton swab was inserted into the standardized inoculum and inoculated evenly on Mueller-Hinton agar (Merck, Germany). The plate was then incubated at 37 °C for 24-18 hours. The zones of growth inhibition were investigated. Multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains were isolated according to criteria characterized by the standardized international terminology explained by the Centre for Disease Prevention and Control (CDC) and therefore the European Centre for Disease Prevention and Control (ECDC) [25]. The reference strain *E. coli* ATCC 25922 was used as a control in all steps.

Statistical analysis methods: Data were analysed in SPSS software, Version 18.0 (IBM Corp., Armonk, USA). Descriptive statistics were carried out on the data collected. Categorical determinants were analysed using the Chi-square ($\chi 2$) or Fisher's exact test. The p values <.05 were considered statistically significant.

Findings

In the present study, a total of 148 *E. coli* strains were isolated and verified using confirmatory tests. Of which 62 (41%) strains were collected from patients with extra-intestinal infections, including bacteremia (4; 6.4%), UTI (57; 91%), and wound infection (1; 1.6%). Besides, 86 (58%) isolates were collected from colon biopsies (8; 9.3%) and stool specimens (78; 90.7%) of patients with enteric infections (Figure 1).

To detect hybrid EAEC/UPEC strains, singleplex PCR was carried out to amplify the *fimH*, *chu*, *fyuA*, and *papC* genes, and multiplex PCR was done to detect the *app aggR*, and *aatA* genes.

There was a significant relationship between

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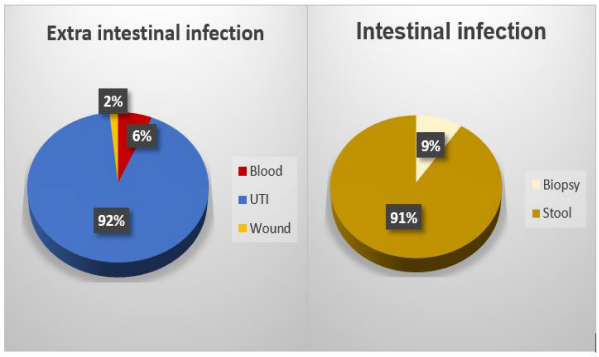

Figure 1) Percentage of *E. coli* strains isolated among different samples

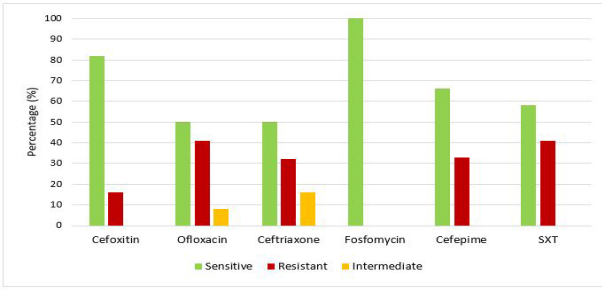

Figure 2) Phenotypic antibiogram profile of the hybrid EAEC/UPEC strains isolated from clinical specimens. SXT: Trimethoprim/Sulfamethoxazole

VGs and hybrid EAEC/UPEC pathotypes (p<.05). There was also a remarkable relation between the presence of *papC* and *fyuA* genes and *E. coli* isolates (p<.05); however, no significant relationship was found for the presence of *chuA*, *fimH*, *aatA*, *aggR*, and *app* genes (p>.05). BLAST sequencing analyses confirmed the presence of *aatA*, *app*, *aggR*, *papC*, *fimH*, *chuA*, and *fyuA* genes. In addition to the *aggR*, *aap*, and *aatA* genes, isolates carryed the UPEC-specific genes, including *chuA*, *fyuA*, *fimH*, and *papC*, highlighting their hybrid nature as hybrid EAEC/UPEC isolates.

In this study, 148 *E. coli* were collected, among them 12 (8.1%) isolates were hybrid strains. The hybrid EAEC/UPEC strains were tested against the antibiotic disks mentioned above on Mueller Hinton agar (Merck KGaA, Darmstadt, Germany). Most hybrid strains were resistant to ofloxacin (42%) and trimethoprim-sulfamethoxazole (41%), many were resistant to ceftriaxone and cefepime (33%), and lower than 20% were resistant to cefoxitin. More details are represented in Figure 2.

In addition to the antibiotic resistance pattern, XDR and MDR strains were identified; 8.3% (1 of 12) and 16.6% (2 of 12) of the isolates were MDR and XDR.

Discussion

This study analyzed the prevalence of virulence determinants and antibiotic susceptibility pattern of the hybrid EAEC/EUEC isolates obtained from patients referreing to three major hospitals in Isfahan. Many studies have evaluated the prevalence of cefepime-resistant *E. coli* strains in Iran. The lowest (25%; 95%CI: 21.67-28.55) and highest (61.95%; 95%CI: 56.62-67.07) prevalence of hybrid EAEC/UPEC strains has been observed in Mazandaran and East Azerbaijan, respectively [26-27].

In recent years, the increasing rates of antibiotic resistance imply a severe health problem with limited empirical treatment options, especially for UTIs. Therefore, the challenge of antibiotic resistance requires an imperative response to reduce the overuse of antibiotics.

The resistance rate of these hybrid strains to antibiotics, such as ampicillin, tetracycline, amikacin, and nitrofurantoin, has been reported to be high; therefore, these antibiotics should not be prescribed as first-line therapeutic drugs for *Enterobacteriaceae* [28-29]. In this study, antibiotic susceptibility testing was performed against cefoxitin, ofloxacin, ceftriaxone, fosfomycin, cefepime, and trimethoprim-sulfamethoxazole.

Interestingly, despite the high antimicrobial resistance amongst the hybrid EAEC/EUEC strains, these organisms were susceptible to fosfomycin (100%), cefoxitin (83%), and Trimethoprim-sulfamethoxazole (58%), which are the drugs of choice in many countries [30].

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The prevalence of cefepime-resistant *E. coli* isolates in Iran has been shown to vary from 15.3 to 100% [30-32]. Also, the prevalence of cefepime-resistant *E. coli* strains differs in various countries globally. Overall, the estimated frequency of cefepime resistance amongst *E. coli* isolates is as follows: 10.3% in the U.S., 8.8% in Europe, 6% in Argentina, and 13% in India [33-34], which is consistent with this study result.

In this study, 50% of the isolates were susceptible to fluoroquinolones, such as ofloxacin, commonly used as first-line antibiotics to treat diarrhea caused by hybrid EAEC/EUEC strains. Indeed, several studies have examined the spread of fluoroquinolone-resistant intestinal pathogens [35-36]. Thus, evaluation of antibiotic susceptibility of EAEC/EUEC hybrid strains seems to be a significant matter in Iran. Also, fosfomycin has been successfully figured out as a drug of choice for infections caused by MDR Gram-negative and Gram-positive organisms [37-38]. A study conducted on clinical MDR *Enterobacteriaceae* isolates, including extended-spectrum *β*-lactamase (ESBL) producing *E. coli* isolates, showed that over 90% of isolates were susceptible to fosfomycin [38]. Similarly in the present research, 100% of the hybrid EAEC/UPEC isolates were susceptible to fosfomycin. The rate of resistance to this antibiotic in some studies conducted in Iran varies from 6.6% [39] to 15% [40]. The rate of fosfomycin resistance is low worldwide. In England, resistance to fosfomycin has been reported to be 9.5 % [41]. Moreover, about less than 1% of *E. coli* strains isolated in clinical laboratories in Canada were shown in a study to be resistant to fosfomycin [42].

Conclusion

According to this study, drug resistance and multidrug resistance among *E. coli* strains causing UTIs have increased in recent years. This increase may be due to misuse and overuse of antibiotics without antibiotic susceptibility testing and without considering several factors such as age, sex, and health conditions in various geographical regions. In the present study, the hybrid EAEC/UPEC strains showed high sensitivity to cefoxitin (83%) and fosfomycin (100%). These two antibiotics still seem to be appropriate drugs for the treatment of patients with infections caused by hybrid EAEC/UPEC strains.

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Ethical Permission: This work was approved by Ethical committee of our University.

Conflict of interest: The authors announce that they have no competing interests.

Author's contribution: Conceptualization: TA and AD; Data curation and formal analysis: TA and SF; Investigation: TA, SF and AD; Methodology and project administration: AD; Supervision: AD; Validation: AD; Writing of original draft: AE and TA; Writing, reviewing and editing: AD and AE.

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