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ORIGINAL ARTICLE

Phenotypic Assays to Determine Virulence Factors of Uropathogenic *Escherichia coli* (UPEC) Isolates and their Correlation with Antibiotic Resistance Pattern

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Abstract
Objectives: Urinary tract infection caused by uropathogenic *Escherichia coli* (UPEC) strains is one of the most important infections in the world. UPEC encode widespread virulence factors closely related with pathogenesis of the bacteria. The purpose of this study was to evaluate the presence of different phenotypic virulence markers in UPEC isolates and determine their correlation with antibiotic resistance pattern.
Methods: UPEC isolates from patients with different clinical symptoms of UTI were collected and screened for biofilm and hemolysin production, mannose resistant, and mannose sensitive hemagglutination (MRHA and MSHA, respectively). In addition, antimicrobial resistance pattern and ESBL-producing isolates were recorded.
Results: Of the 156 UPEC isolates, biofilm and hemolysin formation was seen in 133 (85.3%) and 53 (34%) isolates, respectively. Moreover, 98 (62.8%) and 58 (37.2%) isolates showed the presence of Types 1 fimbriae (MSHA) and P fimbriae (MRHA), respectively. Our results also showed a relationship between biofilm formation in UPEC isolated from acute cystitis patients and recurrent UTI cases. Occurrence of UTI was dramatically correlated with the patients' profiles. We observed that the difference in antimicrobial susceptibilities of the biofilm and nonbiofilm former isolates was statistically significant. The UPEC isolates showed the highest resistance to ampicillin, tetracycline, amoxicillin, and cotrimoxazole. Moreover, 26.9% of isolates were ESBL producers.

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Conclusion: This study indicated that there is a relationship between the phenotypic virulence traits of the UPEC isolates, patients' profiles, and antibiotic resistance. Detection of the phenotypic virulence factors could help to improve understanding of pathogenesis of UPEC isolates and better medical intervention.

1. Introduction

Urinary tract infections (UTIs) including cystitis and pyelonephritis are considered to be the second most common type of infections in humans. They account for ~150−250 million cases globally per year [1,2]. More than 50% of women will have at least one episode of UTI during their lifetime [3,4]. Furthermore, after an initial episode of UTI, women are more likely (20−40%) to get recurrent UTIs [4,5]. UTI patients are classified as either asymptomatic or symptomatic cases. Asymptomatic bacteriuria (ABU) occurs in a small number of healthy individuals and may not need treatment, which makes it different from symptomatic bacteriuria. It often affects pregnant women, with varying prevalence among different populations, depending on factors such as age, sex, sexual activity, and the presence of genitourinary abnormalities [3,4,6]. Uropathogenic *Escherichia coli* (UPEC) is the most common cause of urinary tract infections (UTIs) both in community and hospital settings with significant morbidity and mortality worldwide [7−9]. Previous investigations have shown that UPEC strains encode widespread virulence factors closely related to colonization, persistence, and pathogenesis of bacteria in the urinary tract [8,10]. The most important of these factors include adhesins or fimbriae, biofilm formation, and toxins such as hemolysin [8,10]. Fimbriae are categorized serologically by their hemagglutination pattern and receptor specificities as mannose sensitive (MSHA) or mannose resistance hemagglutination (MRHA) [7,11]. Despite the vast subclass of adhesins that have been reported in UPEC, Type I (MSHA) and P (MRHA) are the most common fimbriae found in UPEC strains. They play an important role in binding and invasion to bladder (cystitis) and kidney (pyelonephritis) epithelial cells [12,13].

Biofilm of UPEC provides a nutrient-rich environment which promotes growth and persistence of microorganisms at the site of infection, and protects bacteria from antimicrobial substances [1,14]. Moreover, UPEC strains often express and secrete a labile pore-forming toxin known as α- hemolysin production that is mainly associated with more virulent UPEC strains [15,16].

Emergence of drug resistance to broad-spectrum beta lactams mediated by extended spectrum beta lactamases (ESBLs) and especially multi-drug resistant (MDR) clonal groups among UPEC strains increase the serious threat to global public health [1,17]. Therefore, to optimize the use of effective antibiotics for appropriate treatment of UTI patients, it is important for physicians to be aware of the etiological agents and antimicrobial resistance trends of UTI pathogens in their geographic area.

This investigation is aimed to determine the prevalence and correlation of phenotypic virulence traits and antibiotic resistance profile among the UPEC isolated from UTIs, with regard to patients' profiles in Tehran, Iran.

2. Materials and methods

2.1. Organism collection and identification

The present study was conducted in the Department of Molecular Biology, Pasteur Institute of Iran, Tehran, Iran. Random samples of 156 clinical isolates of UPEC were collected from urine samples of symptomatic and asymptomatic cases of UTIs with significant counts ($\geq 10^5$ CFU/mL) in various hospitals of Tehran, Iran between March 2013 and February 2014. Only patients aged $\geq$ 20 years who were not on antimicrobial therapy at sample collection or had not taken antibiotic drugs 1 month prior to sampling time, were included in this study. Isolated organisms were identified and characterized on the basis of standard microbiological tests such as Gram staining, catalase, indole, methyl red, voges-proskauer, citrate utilization, motility, fermentation, and utilization of glucose, lactose, and sucrose. All isolates were suspended in 15% glycerol-supplemented Luria-Bertani (LB) medium and preserved frozen at −80°C.

2.2. Patient demographics

Clinical symptoms, infection history, treatment summary, and all of the necessary relevant information from patients were obtained from hospital records, laboratory reports, and interviewing patients.

2.3. Biofilm formation assay

We analyzed the ability of the UPEC isolates to produce the biofilm according to the protocols described by O'Toole and Kolter [18] and Dusane et al [19]. We used the *E. coli* ATCC 25922 strain as a positive control. Briefly, UPEC isolates were grown in LB broth at 37°C overnight, then the cultures were diluted 1:100 with Tryptic Soy Broth (Merck, Darmstadt, Germany) and incubated in a 96-well polyvinyl chloride (PVC) microtiter plate at room temperature (RT) for 48 hours.