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ORIGINAL ARTICLE

Virulence factors, antibiotic resistance phenotypes and O-serogroups of *Escherichia coli* strains isolated from community-acquired urinary tract infection patients in Mexico

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Abstract *Background/Purpose:* Uropathogenic *Escherichia coli* (UPEC) strains isolated from patients with community-acquired urinary tract infections (UTIs) were assessed to determine the prevalence of virulence genes, antibiotic resistance, and the O-serogroup of the strains.
Methods: Consenting patients with community-acquired UTI were enrolled at Unidad Médica Familiar Number 64 (Instituto Mexicano del Seguro Social, Estado de Mexico, Mexico) and 321 urine samples were collected. Polymerase chain reaction (PCR) was used to assess 24 virulence genes and 14 O-serogroups. The Kirby-Bauer method was used to evaluate the antibiotic susceptibility of the isolated strains to 12 commonly used antibiotics.
Results: A total of 194 strains were identified as *E. coli* using standard biochemical tests, followed by PCR amplification of 16S ribosomal RNA gene. Only 58.2% of the strains belonged to the assessed 14 O-serogroups. The serogroups O25, O15, O8, and O75 were present in 20.6%, 17%, 6.1%, and 4.6% of strains, respectively. The most frequently occurring virulence genes among UPEC strains included *kpsMT* (92.2% strains), *usp* (87.1%), *irp2* (79.3%), *iha* (64.9%), *fim* (61.3%), *set* (36%), *astA* (33.5%), *pap* (24.7%), and *papGII* (21.1%). In addition, 97% of the strains were multi-drug resistant (coresistance to 3—11 antibiotics).

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Conclusion: The isolated UPEC strains predominantly belonged to three serogroups (O25, O15, and O8), harboured numerous virulence genes, and are multiresistant to antibiotics. The findings of this study could be used to orient UTI treatment strategies and in epidemiological studies in Mexico.

Introduction

Urinary tract infection (UTI) is one of the most commonly occurring bacterial infections[1] and 70—95% of the UTIs are caused by uropathogenic *Escherichia coli* (UPEC).[2] Virulence factors related to UPEC include genes encoding adhesins (*pap*, *papG* allele I, *papG* allele II, *papG* allele III, *sfa*, *afal*, *fim*, *iha*, and *tsh*); genes related to the iron acquisition systems (*iuc*, *iroN*, and *irp2*); protectin genes (*kpsMT* and *iss*); and genes encoding toxins (*set*, *astA cnf1*, *hlyA*, *vat*, *usp*, and *cva/cvi*).[3,4]

UPEC strains are routinely serotyped using the common O antisera and the serogroups O1, O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75, and O83 are preferentially associated with UPEC strains.[3,5] Serogroup assays are used for accurate *E. coli* identification and for epidemiological investigations of *E. coli* outbreaks.

The treatment of UTIs caused by UPEC often requires antimicrobial therapy. However, currently there has been a rise in the occurrence of antibiotic-resistant UPEC strains, and UTIs are considered a serious health concern.[1]

Given the clinical importance of UTIs, the aim of this work was to determine the prevalence of virulence genes, resistance to antibiotics, and the O-serogroup of UPEC strains isolated from patients with community-acquired UTIs in Mexico.

Methods

Sample collection and *E. coli* strains

Three hundred and twenty-one urine samples were collected from patients with community-acquired UTIs (247 women and 74 men; age range, 20—70 years) from Unidad Médica Familiar (UMF) Number 64 (Instituto Mexicano del Seguro Social), located in Estado de Mexico, Mexico, from August 2013 to December 2013. Patient inclusion criteria were as follows: persons with signs and symptoms of UTI and accepting to participate in the study by signing the informed consent letter were enrolled. Patients undergoing antibiotic treatment were excluded. The local ethics committee of UMF approved the study. The samples were cultured on blood agar and MacConkey agar (Bioxon, Mexico, Mexico) at 37°C overnight. The identification of *E. coli* was performed by IMViC tests, and confirmed by 16S ribosomal RNA (rRNA) gene polymerase chain reaction (PCR) amplification as described elsewhere.[6,7] For each test, *E. coli* ATCC 11775 was used as the control strain. An UTI caused by *E. coli* was recognized if it was accompanied by symptoms suggestive of infection and a culture of a clean-catch urine sample with at least 10^5 colony forming units of *E. coli* per milliliter.

DNA extraction

DNA extraction was performed using the boiling method. Bacteria were grown in brain-heart infusion broth (BHI; Bioxon) at 37°C overnight. The bacterial overnight growth culture (1.5 mL) was pelleted, the culture supernatant was discarded, and the cell pellet was suspended in 200 μL of sterile water. The resuspended pellet was then incubated at 100°C for 10 minutes and centrifuged at 10,000*g* for 5 minutes. The pellet was discarded and the DNA, present in the supernatant, was stored at −20°C until required for assays.

Antibiotic susceptibility testing

The standard disc diffusion method of Kirby-Bauer by using Mueller Hinton agar (Bioxon) was performed to evaluate antibiotic susceptibility. The antibiotic susceptibility of gram-negative bacteria was assessed by using 12 different antibiotic-loaded discs (Bio-Rad, Mexico). Results were interpreted in accordance with the Clinical and Laboratory Standards Institute guidelines.[8] The following antibiotics were used at the indicated concentrations: ampicillin (10 μg), cefalotin (30 μg), cefotaxime (30 μg), carbenicillin (100 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), pefloxacin (5 μg), gentamicin (10 μg), nitrofurantoin (300 μg), netilmicin (30 μg), amikacin (30 μg), and trimethoprim-sulfamethoxazole (25 μg). For each test, *E. coli* ATCC 11775 was used as the control strain. In accordance with the manufacturer of the discs (Bio-Rad), strains were considered resistant to carbenicillin if the diameter of inhibition halo was $\leq$ 19 mm; and resistant to pefloxacin if the diameter of inhibition halo was $\leq$ 14 mm.

Detection of virulence genes and serogroups of UPEC strains

The primer sequences and PCR cycling conditions described by Momtaz et al[9] were used to assess the prevalence of the following virulence genes: (1) adhesins: *pap* (pilus associated with pyelonephritis), *papG* allele I, *papG* allele II, *papG* allele III, *sfa* (S fimbriae), *afal* (afimbrial adhesin I), *fim* (type-1 fimbriae), *iha* (nonhemagglutinating adhesin), and *tsh* (temperature-sensitive hemagglutinin); (2) nutrition: *iuc* (aerobactin), *iroN* (iron), and *irp2* (iron-repressible protein); (3) protectins: *kpsMT* (K-antigen) and *iss* (increased serum survival protein); and (4) toxins: *set*

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