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# Association of some virulence genes with antibiotic resistance among uropathogenic *Escherichia coli* isolated from urinary tract infection patients in Alexandria, Egypt: A hospital-based study

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ABSTRACT

Uropathogenic *Escherichia coli* (UPEC) is the infecting agent most frequently involved in urinary tract infections (UTIs) worldwide. UPEC resistance to commonly used antibiotics represents a major health problem all over the world. Several factors have been associated with UPEC resistance to antibiotics. The present study deployed a molecular approach to explore the association between some UPEC virulence genes and antibiotic resistance among patients with UTI in Alexandria, Egypt. The study revealed a significant association between presence of the *pap* gene and resistance to gentamicin; however, it was not significantly associated with resistance to β-lactam antibiotics, quinolones, aminoglycosides, nitrofurantoin and trimethoprim/sulfamethoxazole. The genes *sfa*, *aer* and *cnf1* were not significantly associated with UPEC resistance to any of the tested antibiotics. In conclusion, resistance of UPEC isolates in the present study could be attributed to other virulence factors.


## 1. Introduction

*Escherichia coli* is the most common infecting agent of the urinary tract, most frequently targeting neonates, pre-school girls, and sexually active and elderly women [1]. Urinary tract infection (UTI) is a major cause of morbidity and mortality, with *E. coli* frequently being isolated in uncomplicated UTI [2]. *E. coli* is the most common bacteria isolated from urine samples in outpatients and inpatients of both sexes [3,4]. Certain uropathogenic *E. coli* (UPEC) serotypes have been consistently associated with uropathogenicity [2].

The most common antibiotics used to treat UTI are trimethoprim/sulfamethoxazole (SXT), cephalosporins, semisynthetic penicillins with or without β-lactamase inhibitors, and quinolones [5]. The selection of antibiotic depends on the health condition of the patient and the type of incriminated bacteria. However, antibiotic resistance is a major problem hindering the treatment of UTIs [6,7]. Virulence of UPEC has been associated with certain virulence factors, including aerobactin (*aer*), cytotoxic necrotising factor 1 (*cnf1*), pyelonephritis-associated pili (*pap*) and S fimbrial adhesin (*sfa*) [8]. Therefore, the aim of the present study was to isolate UPEC, to determine their susceptibility to commonly used antibiotics and to study the association of some virulence genes with resistance to these antibiotics.

## 2. Materials and methods

### 2.1. Bacteria

This study included 35 UPEC isolated from patients with UTI attending the Microbiology Department of Alexandria Medical Research Institute (Alexandria, Egypt) during the period April–November 2009.

### 2.2. Antibiotics

Antibiotic disks (Oxoid Ltd., Basingstoke, UK) used in this study included ampicillin (AMP), amoxicillin/clavulanic acid, ampicillin/sulbactam, cefradine (CED), cefalexin (CEX), cefuroxime (CXM), cefoperazone (CFP), ceftazidime (CAZ), imipenem (IPM), meropenem (MEM), nalidixic acid (NAL), norfloxacin (NOR), SXT, gentamicin (GEN), amikacin (AMK) and nitrofurantoin (NFT).

### 2.3. Collection of specimens

Midstream urine samples were collected from patients suspected of having UTI in sterile containers and were immediately

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**Table 1**
Sequence of the primers used for detection of the various urovirulence factors by PCR [9].

| Gene | Primer | Primer sequence (5′ → 3′) | Amplicon size (bp) |
|---|---|---|---|
| *pap* | pap1 | 5′-GACGGCTGTACTGCAGGGTGTGGCG-3′ | 328 |
| | pap2 | 5′-ATATCCTTTCTGCAGGGATGCAATA-3′ | |
| *sfa* | sfa1 F1 | 5′-CTCCGGAGAACTGGGTGCATCTTAC-3′ | 410 |
| | sfa2 R2 | 5′-CGGAGGAGTAATTACAAACCTGGCA-3′ | |
| *aer* | aer1 F1 | 5′-TACCGGATTGTCATATGCAGACCGT-3′ | 602 |
| | aer2 R2 | 5′-AATATCTTCCTCCAGTCCGGAGAAG-3′ | |
| *cnf1* | cnf1 F1 | 5′-AAGATGGAGTTTCCTATGCAGGAG-3′ | 498 |
| | cnf1 R2 | 5′-CATTCAGAGTCCTGCCCTCATTATT-3′ | |

**Table 2**
Antibiotic susceptibility of uropathogenic *Escherichia coli* isolates from Egyptian patients.

| | AMP | CED/CEX | CXM | CFP | CAZ | IPM/MEM | NAL | NOR | SXT | GEN | AMK | NFT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Resistant strains (%) | 89 | 63 | 74 | 49 | 40 | 0 | 66 | 63 | 74 | 57 | 11 | 11 |

AMP, ampicillin; CED, cefradine; CEX, cefalexin; CXM, cefuroxime; CFP, cefoperazone; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; NAL, nalidixic acid; NOR, norfloxacin; SXT, trimethoprim/sulfamethoxazole; GEN, gentamicin; AMK, amikacin; NFT, nitrofurantoin.

transferred to the laboratory for microbiological examination. This study was approved by the Ethics Committee of Alexandria Medical Research Institute, and informed consent was obtained from patients participating in the study.

### 2.4. Isolation and identification of E. coli isolates

*E. coli* was isolated from urine samples using MacConkey and blood agars (Oxoid Ltd.) and colonies were identified by biochemical tests, including triple sugar iron, indole, methyl red, Voges–Proskauer, citrate utilisation, urease and phenylalanine deaminase tests. Antibiotic susceptibility was determined on Mueller–Hinton agar (Oxoid Ltd.) by the Kirby–Bauer disc diffusion method.

### 2.5. Detection of virulence genes of uropathogenic E. coli by PCR

Bacterial DNA was extracted from UPEC isolates subcultured overnight at 37 °C on MacConkey agar. Briefly, a few colonies were emulsified in 200 μL of sterile distilled water to produce a heavy suspension that was heated at 100 °C for 15 min and centrifuged at 14,000 rpm for 5 min. The resulting supernatant was used as a template for subsequent amplification. PCR amplification of virulence-associated genes of UPEC (*pap*, *sfa*, *aer* and *cnf1*) was performed using the protocol of Le Bouguenec et al. [9] with the modification that DreamTaq® Green Master Mix 2× (Fermentas, Hilden, Germany) was used in a final volume of 25 μL using the primers in Table 1. Amplicons were detected by electrophoresis in 2% agarose gel stained with ethidium bromide and visualised in an ultraviolet transilluminator.

## 3. Results

Among 97 urine samples from UTI patients, only 35 isolates (36%) were found to be UPEC based on biochemical tests.

### 3.1. Antibiotic susceptibility

Table 2 shows that 40–89% of UPEC strains in the present study were resistant to β-lactam antibiotics (AMP, CED/CEX, CXM, CFP and CAZ). The highest resistance was to AMP (89%), followed by 63–66% resistance to quinolones (NAL and NOR), whilst resistance to aminoglycosides (GEN and AMK) ranged from 11% to 57%. Forty percent of the isolates were multidrug-resistant, being resistant to at least two different groups of antibiotics. UPEC isolates showed a high susceptibility to NFT, and all isolates were 100% sensitive to carbapenems (IPM/MEM).

**Table 3**
Presence of virulence genes among uropathogenic *Escherichia coli* isolated from Egyptian patients.

| Gene | *n* (%)[a] |
|---|---|
| *pap* | 19 (54) |
| *aer* | 18 (51) |
| *sfa* | 16 (46) |
| *cnf1* | 6 (17) |

[a] Total number of examined isolates was 35; *n*, number of isolates with resistance genes.

### 3.2. Virulence genes in uropathogenic E. coli isolates

The virulence genes *pap* (328 bp), *sfa* (410 bp), *aer* (602 bp) and *cnf1* (498 bp) were successfully amplified for UPEC isolates (Fig. 1). *pap* and *aer* genes were detected in approximately one-half of the UPEC isolates, whereas *sfa* and *cnf1* were detected in 46% (16/35) and 17% (6/35) of the isolates, respectively (Table 3).

### 3.3. Association of uropathogenic E. coli virulence genes with antibiotic resistance

Table 4 shows that there was a statistically significant ($P = 0.013$) association between presence of the *pap* gene in UPEC

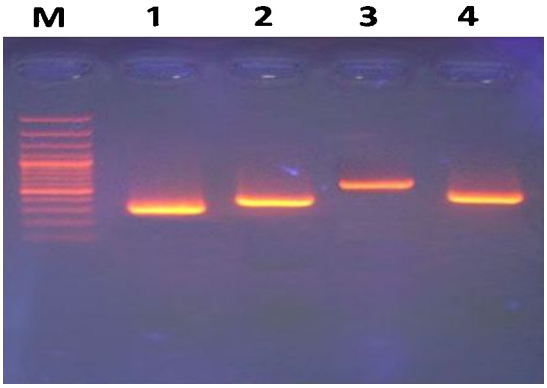


**Fig. 1.** Representative gel electrophoresis of uropathogenic *Escherichia coli* virulence genes isolated from urinary tract infection patients. Lane M, 100-bp ladder; lane 1, *pap* (328 bp); lane 2, *sfa* (410 bp); lane 3, *aer* (602 bp); and lane 4, *cnf1* (498 bp).