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Major Article

Determination of antibiotic resistance genes and virulence factors in *Escherichia coli* isolated from Turkish patients with urinary tract infection

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Abstract

Introduction*: Escherichia coli* ranks among the most common sources of urinary tract infections (UTI). **Methods:** Between November 2015 and August 2016, 90 isolates of *E. coli* were isolated from patients at Rize Education and Research Hospital in Turkey. Antibiotic susceptibility was determined for all isolates using the Kirby–Bauer disk diffusion method. These *E. coli* isolates were also screened for virulence genes, β-lactamase coding genes, quinolone resistance genes, and class 1 integrons by PCR. **Results:** With respect to the antibiotic resistance profile, imipenem and meropenem were effective against 98% and 90% of isolates, respectively. A high percentage of the isolates showed resistance against β lactam/β lactamase inhibitor combinations, quinolones, and cephalosporins. PCR results revealed that 63% (57/90) of the strains carried class 1 integrons. In addition, a high predominance of extended-spectrum β-lactamases (ESBLs) was observed. The *qnrA*, *qnrB*, and *qnrS* genes were found in 24 (26.6%), 6 (6.6%), and 3 (3.3%), isolates, respectively. The most common virulence gene was *fim* (82.2%).The *afa, hly*, and *cnf1* genes were detected in 16.6%, 16.6%, and 3.3% of isolates, respectively. Moreover, we observed eleven different virulence patterns in the 90 *E. coli* isolates. The most prevalent pattern was *fim,* while *hly-fim, afa-aer-cnf-fim, aer-cnf, afa-aer*, and *afa-cnf-fim* patterns were less common. **Conclusions:** Most of the *E. coli* virulence genes investigated in this study were observed in *E. coli* isolates from UTI patients. Virulence genes are very important for the establishment and maintenance of infection.

Keywords: Quinolones. Virulence genes. UTI.

INTRODUCTION

Escherichia coli are among the most common etiological agents that cause urinary tract infections (UTI); this makes *E. coli* infection an important public health issue[1-4]. Many virulence factors are responsible for the pathogenicity of *E. coli* strains[4,5]. There are two main types of *E. coli* virulence factors; these include (i) virulence factors that are produced within the cell and released at the site of action, and (ii) virulence factors that are displayed on the surface of the cell[6].

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The most important *E. coli* virulence factors are the surface virulence factors (adhesins). P fimbriae are encoded by *pap* genes and are the main adherence factors[7]. S fimbrial adhesion factors, encoded by *sfa* genes, represent another type of virulence factor[8]. A fimbrial adhesion factors in *E. coli* are encoded by *afa* genes[9]. In addition, the main fimbrial subunit of type 1 fimbriae is encoded by *fimA* in *E. coli*[10]. Toxins are another important type of virulence factor in *E. coli*. The a-hemolysin (HlyA) virulence factor, cytotoxic necrotizing factor, and aerobactin are encoded by the *hly*, *CNF1*[5], and *aer* genes, respectively[11,12].

The β-lactamases (enzymes that hydrolyze β-lactam antibiotics) are classified into four groups depending on their amino acid sequences: class A (e.g., KPC, CTX-M, and GES), class B (e.g., IMP, VIM, SPM, GIM, NDM, and SIM), class C (e.g., AmpC), and class D (e.g., OXA-type β-lactamase).

All four classes of β-lactamase have been identified in *E. coli*. Metallo-β-lactamases (MBLs) are disseminated worldwide[13] and have been mainly identified in *Enterobacteriaceae* of the IMP and VIM types[14-17].

Quinolones are widely used to treat UTIs caused by *E. coli*. This extensive use of quinolones has led to increased resistance in *E. coli*[18]. Target modification, and changes in membrane permeability can confer resistance to quinolones. Moreover, plasmid-mediated *qnr* (quinolone-resistance) genes can facilitate quinolone resistance, with the *qnrA, qnrB*, and *qnrS* groups comprising the major *qnr* determinants[19].

Integrons are mobile genetic elements that contribute to the spread of antibiotic resistance. Many gene cassettes emerged when class 1 integrons were first discovered in clinical strains. The role of integrons in promoting bacterial multidrug resistance is significant. A number of studies investigating the prevalence of integrons in *E. coli* isolates from UTI patients have reported a significant link between antimicrobial resistance and integrons[20,21].

The purpose of this study was to investigate the presence of virulence genes, β-lactamase coding genes, quinolone resistance genes, fosfomycin resistance genes, and class 1 integron gene cassettes in *E. coli* isolates from patients with UTI.

METHODS

A total of 90 *E. coli* isolates were investigated in this study. All strains were isolated at the Rize Education and Research Hospital in Turkey between November 2015 and August 2016. Urine samples were cultured on blood agar and Eosin Methylene Blue (EMB) agar, then incubated at 37ºC for 18–24 h. Bacteria were identified using colony morphology and biochemical tests in urine cultures with high levels of viable bacteria ($\geq 10^5$ CFU/mL). Antibiotic susceptibility of each isolate was determined by Kirby–Bauer disk diffusion and was based on the criteria recommended by the Clinical Laboratory Standards Institute (CLSI, 2014).

Genomic DNA was obtained from bacterial suspensions grown overnight in Luria Broth (LB) at 37°C. Bacterial suspensions were centrifuged. Pellets were resuspended in 500 µL of distilled water, then boiled in a water bath for 10 min. Boiled suspensions were centrifuged at 11,357 g for 5 min. Five hundred microlitres of each supernatant were used as a template for PCR assays[22].

All strains were isolated from adult patients with uncomplicated community-acquired UTIs. Ninety *E. coli* isolates were screened for genes encoding β-lactamases, quinolone resistance factors, fosfomycin resistance factors, and virulence factors via polymerase chain reaction (PCR). Primers for β-lactamase-encoding genes (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{CTXM-1}, bla_{CTXM-2}, bla_{GES}, bla_{SIM}, bla_{AmpC}, and bla_{SPM}), quinolone resistance genes (*qnrA, qnrB,* and *qnrS*), fosfomycin resistance genes (*fosA, fosC2,* and *fosA3*), and virulence genes (*pap, sfa, afa, hly, aer, cnf,* and *fim*) were used in these experiments. All PCR results were analyzed by electrophoresis in 1% agarose containing 0.5 µg/mL ethidium bromide, followed by examination under UV light.

PCR was performed on all isolates to detect class 1 integron gene cassettes using the primers 5′-GGCATCCAAGCAGCAAG-3′ (5′CS) and 5′-AAGCAGACTTACCTGA-3′ (3′CS). The PCR conditions were 3 min at 94°C for initial denaturation, followed by 34 cycles of 45 s at 94°C , 1 min at 55°C, and 3 min at 72°C, with a final extension at 72°C for 5 min.

RESULTS

Ninety *E. coli* isolates were investigated in this study. Of the 90 patients diagnosed with community-acquired UTIs, 62 (68.9%) were women and 28 (31.1%) were men. The extended-spectrum β-lactamase (ESBL) positivity rate was 18.9%. All 90 strains were isolated from urine samples. Results of antibiotic susceptibility test revealed that these isolates had low resistance rates for fosfomycin (2.7%), imipenem (3.2%), and meropenem (3.2%). However, resistance rates for ciprofloxacin (62.2%), trimethoprim sulfamethoxazole (75.6%), and ampicillin (61.1%) were high. Rates of resistance against amikacin, nitrofurantoin, ceftriaxone, ceftazidime, gentamycin, amoxicillin with clavulanic acid, aztreonam, cefazolin, and cefepimine were found to be 9.9%, 8.9%, 22.2%, 21.1%, 27.8%, 27.8%, 18.9%, 18.9%, and 20%, respectively.

More specifically, we found that *fim* was the most common virulence gene and was found in 74 isolates (82.2%). The *afa* and *cnf1* genes were detected in 16.6% of the isolates, and *hly* was found in only three (3.3%) of the 90 isolates. The *sfa* and *pap* genes were not detected. In addition, the *aer* gene was found in 33 (36.6%) of the isolates. PCR results revealed that 63% (57/90) of the strains carried class 1 integron gene cassettes. We also observed a high prevalence of ESBLs, with 52 strains (57%) carrying a CTX-M-2, and 52 isolates (57%) carrying a CTX-M-1 group β-lactamase. No other β-lactamase-encoding genes (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{GES}, bla_{SIM}, bla_{AmpC}, or bla_{SPM}) were identified. We also demonstrated that the *qnrA, qnrB,* and *qnrS* quinolone resistance genes—present on the plasmid—were present in 26.6% (24/90), 6.7% (6/90) and 3.3% (3/90) of the isolates, respectively. No fosfomycin resistance genes (*fosA, fosC2*, or *fosA3*) were found.

The prevalence of virulence factors differed among isolates that produced a class 1 integron, bla_{CTXM-1}, bla_{CTXM-2}, *qnrS, qnrA,* and *qnrB* (**Table 1**). Class 1 integron and CTX-M harboring isolates were more commonly positive for *fim* than for other virulence factors.

Eleven different virulence factors were observed among the 90 *E. coli* isolates. The most common virulence factor was *fim* (n = 35 isolates; 8.9%); *hly-fim, afa-aer-cnf-fim, aer-cnf, afa-aer,* and *afa-cnf-fim* were less commonly observed. No virulence factor was detected in fourteen of the isolates (**Table 2**).

DISCUSSION

Urinary tract infections (UTIs) are a major public health problem worldwide. *E. coli* is the most prevalent etiologic agent of UTIs. The virulence of UTI inducing *E. coli* strains is due to their expression of virulence factors[4,23].

P fimbriae (*pap*), a fimbrial adhesin I (*afaI*), hemolysin (*hly*), cytotoxic necrotizing factor 1 (*cnf1*), aerobactin (*aer*), S fimbriae (*sfa*) and type 1 fimbriae (*fimH*) are the most

TABLE 1: Prevalence of virulence factors and antibiotic resistance genes among strains.

Antibiotic resistance genes and integrons	Virulence factor genes				
	afa	***hly***	***aer***	***cnf***	***fim***
class 1 integron	9	3	25	11	48
bla_{CTXM-1}	7	-	11	7	42
bla_{CTXM-2}	7	-	16	7	44
qnrS	-	-	-	-	2
qnrA	4	1	12	3	23
qnrB	1	1	1	1	4

important virulence factor genes found in these *E.coli* strains[24-27]. The bacterial adhesin *fimH* (which plays an integral role in the pathogenesis of *E. coli*) is a virulence factor that is located on the type 1 pili of *E. coli*. Of the seven virulence genes examined in this study, the *fim* gene was detected most frequently (82.2%). Kot et al. (2016) reported similar results[28]. Moreover, the *fimH* adhesion gene was the most common virulence gene in both UTIs and asymptomatic bacteriuria (ABU) isolates studied by Yun et al. (2014). Their results showed that the *pap* gene family was also prevalent in UTI and ABU isolates[29]. In our study, we did not find the *pap* gene in any *E. coli* isolates. In another study[30], *sfa* was the most common virulence gene; by contrast, we found no *sfa* in our isolates. The presence of *afaI, hly*, and *cnf1* virulence factor genes was estimated to be 8.13%, 50.4%, and 50.4%, respectively, by another study[24]. In our study, we determined the presence of the *afa*, *hly*, and *cnf1* virulence genes to be 16.6%, 3.3%, and 16.6%, respectively. Among the seven virulence genes that we studied, *aer* (36.6%) was the second most common virulence factor. Similar to our results, another study reported *aer* as the second most frequently detected virulence factor coding gene after the highly prevalent *fimH* gene[31]. The elevated levels of type 1 fimbriae may be correlated with the pathogenicity of the isolated strains, as type 1 fimbriae (*fimH*) play a crucial role in the colonization of the urinary tract[32]. In addition, these results showed that the geographical region can affect the prevalence of these genes[12]. The studied strains exhibited 11 virulence gene patterns. The E2 was characterized by the presence of only the *fim* gene and was the most commonly seen pattern, found in 35 isolates. A small number of isolates with four virulence factors were detected, and *fim* was the most common virulence factor. Most of the isolates contained different combinations of resistance determinants.

Inappropriate and unnecessary application of quinolones has led to the emergence of resistant *E. coli* isolates that limit treatment options[19]. Our PCR results showed that *qnrA*, *qnrB,* and *qnrS* genes were present in 26.6% (24/90), 6.6% (6/90), and 3.3% (3/90) of studied isolates, respectively. In contrast to our results, one study reported that the most prevalent *qnr* determinant was *qnrB,* followed by *qnrS*[18]. In another study, 120 isolates of *E. coli* from UTIs were investigated for the presence of *qnrA*, *B*, and *S,* and *qnrB* (2.18%) and *qnrS* (1.12%) genes were detected, but *qnrA* was not found[33].

TABLE 2: Prevalence of virulence patterns among 90 *E. coli* isolates.

Pattern codes	Virulence Patterns	Number of Isolates
E1	*afa-aer-fim*	6 (6.7%)
E2	*fim*	35 (38.9%)
E3	*aer-fim*	12 (13.3%)
E4	*afa-fim*	6 (6.7%)
E5	*hly-fim*	1 (1.1%)
E6	*afa-aer-cnf-fim*	1 (1.1%)
E7	*hly-aer-cnf-fim*	2 (2.2%)
E8	*aer-cnf-fim*	10 (11.1%)
E9	*aer-cnf*	1 (1.1%)
E10	*afa-aer*	1 (1.1%)
E11	*afa-cnf-fim*	1(1.1%)
	No virulence factor	14 (15.6%)

Plasmid-mediated quinolone resistance (PMQR) genes are usually found in association with the ESBL genes[18]. CTX-M enzymes have been identified in both hospital and community settings and belong to one group of ESBLs[34]. Co-expression of bla_{CTXM} and PMQR genes has been reported in *E. coli* isolated from UTIs[18,35]. Multi drug resistance (MDR) rates were significantly higher in PMQR-positive *K. pneumoniae* and *E. cloacae* isolates (17-28 times) than in PMQR-negative isolates. This finding, which has been observed by other researchers, may indicate a link between *qnrB* and other antibiotic resistance genes. In this study, however, this association was not found in *E. coli* isolates retaining PMQR genes[36].

The pattern of virulence factors and antibiotic resistance genes is constantly changing in organisms isolated from UTIs, so this and similar studies are necessary to stay abreast of local and national antimicrobial resistance trends for the empirical treatment of UTIs[19].

Conflict of interest

The authors declare no conflicts of interest.

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