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ORIGINAL ARTICLE

Occurrence of Pilus Associated with Pyelonephritis (*papEF*) Gene among Quinolone- and Fluoroquinolone- Resistant *Escherichia coli* Urinary Isolates

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Key words:

Escherichia coli, Quinolone (Q)resistance, Fluoroquinolone (FQ) resistance, papEF gene

ABSTRACT

Background*: Escherichia coli (E. coli) isthe main bacterial cause of uncomplicated urinary tract infection (UTI). Acquisition of quinolone (Q) and fluoroquinolone (FQ) resistance in E. coli urinary isolates could be associated with decreased pathogenicity.* ***Objectives:*** *This work aimed to determine the current levels of nalidixic acid (NA) and ciprofloxacin resistance in urinary tract isolates of E. coli and to assess the occurrence of pilus associated with pyelonephritis (papEF) gene among NA- and ciprofloxacin-resistant isolates, compared to their susceptible counterparts, from inpatients and outpatients.* ***Methodology:*** *E.coli isolates were tested for antibiotic susceptibility, hemolysin production and the presence of papEF gene.* ***Results:*** *The frequency of E. coli isolation was 86.36% and 62.04% from outpatients and inpatients respectively. Resistance to NA was detected in 77.38% E. coli isolates (71% and 85.8% of outpatient and inpatient isolates, respectively), while 26.13% of E. coli isolates were resistant to ciprofloxacin (17.5% and 37.6% of outpatient and inpatient isolates, respectively). E. coli isolates obtained from inpatients exhibited a significant higher resistance to NA (P=0.01) and ciprofloxacin (P=0.001) than inpatient one. Isolates resistant to NA had significantly lower prevalence of hemolysin production and papEFgene, compared to their susceptible counterparts (P< 0.0001 &P<0.0001, respectively). Similarly, a significantly lower ratio of ciprofloxacin-resistant E. coli isolates expressed beta-hemolysis (P<0.0001) and harbored papEF gene (P<0.0001), compared to ciprofloxacin-susceptible isolates.* ***Conclusion:*** *NA- and ciprofloxacin- resistant E. coli urinary isolates showed less virulence factors than their susceptible counterpart did, yet resistance itself may constitute a virulence factor that allows for the survival of a bacterium within the urinary tract of treated patients. Given the frequency with which UTIs are treated empirically, compounded with the speed that E. coli acquires resistance, prudent use of antimicrobial agents remains crucial.*

INTRODUCTION

Urinary tract infections (UTIs) are the most frequent bacterial disease in humans, affecting both inpatients and outpatients. *Escherichia coli* (*E. coli*) is considered, by far, the main cause of UTI, particularly, in uncomplicated cases [1].

In recent years, management of UTIs has become increasingly problematic due to the emergence of resistance to first-line antibiotics among the causative bacteria, particularly among uropathogenic *E. coli* (UPEC) strains. This phenomenon involves quinolones (Q) and fluoroquinolones (FQ)[2], drugs of paramount importance in the treatment of several other infectious diseases. Indeed, the renal excretion of these molecules and the availability of oral and parenteral formulations have allowed them to compete with aminoglycosides and beta lactams in the therapy of complicated UTIs, especially in hospital setting. Their appropriate spectrum and good tolerability have also led to increased empirical adoption in uncomplicated infections[3].

Mutations in chromosomal genes encoding quinolone targets, DNA gyrase and topoisomerase IV, can confer resistance to Q and FQ in *E. coli.* More recently, plasmid-mediated mechanisms have been reported [4].

Resistant *E. coli* isolates are associated with decreases in clinical cure rates and higher risk of recurrence, which significantly increases patient morbidity, costs of treatment, rates of hospitalization, and use of broad-spectrum agents[5].

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Several virulence factors (VFs), such as hemolysin, cytotoxic necrotizing factor–1 (CNF-1), aerobactin, and different adhesins, have been described in UPEC. These VFs are located on large plasmids and/or in particular regions, called "pathogenicity islands" (PAIs), on the chromosome. Previous *in vitro* studies have suggested that the acquisition of Q and FQ resistance is associated with decreased pathogenicity of UPEC[6].Most of these studies have focused on the relation of Q and FQ resistance with P fimbriae, being the primary adherence factor isolated from UPEC [7]. They can trigger the host immune response [8]; and fulfil Koch-Henle molecular postulates by conferring on an avirulentnonfimbriated strain the ability to induce a host response in the human urinary tract [9].

Since the gene for hemolysin and that of P fimbriae are often located on the same PAI [10], hemolysis was considered as a phenotypic indicator, in the current study, for the presence of the gene coding for the P fimbriae (pilus associated with pyelonephritis "*papEF*" gene).

This study aimed to determine the current levels of NA and ciprofloxacin resistance in urinary tract isolates of *E. coli* in Doha, Qatar and to assess the occurrence of *papEF* gene among NA and ciprofloxacin-resistant isolates, compared to their susceptible counterparts, from inpatients and outpatients.

METHODOLOGY

1- Patients and bacterial isolates.

E. coli isolates were collected from January 2014 to May 2015 from urinary specimens that were either sent to the Clinical Microbiology Laboratory of Qatar Armed Forces by internal medicine, surgery and day care wards or collected from outpatients, directly referred to laboratory. A single isolate from each patient was analyzed. Samples were derived either from clean-catch, midstream urine or from urinary catheters. Specimens (10 µl) were cultured on MacConkey agar. Cultures yielding growth $>10^5$ CFU/ml were considered. Lactose fermenting, indole-positive colonies were evaluated by the Vitek2 system (bioMe′rieux, Marcy I′Etoile, France) to identify *E. coli*. An I-UTI (inpatient UTI from the hospital) was defined as a case in which a urine sample was obtained for cultivation 48 h after hospitalization and subsequently exhibited microbial growth. In contrast, O-UTI (outpatient UTI from the hospital clinic) was used to designate cases in which a microbe was detected in a urine specimen that was cultivated within 48 h of the patient's visit [11].

2- Susceptibility testing with Vitek2 system.

Susceptibility to NA and ciprofloxacin was performed using Vitek2 system cards (AST-N103) (bioMe′rieux, Marcy I′Etoile, France).

3- Hemolysin production testing.

All obtained *E. coli* isolates, whether resistant or susceptible, were screened for beta-hemolysis by culturing on 5% sheep blood agar for 24 h at 35°C.

4- PCR for the presence of *papEF*.

All obtained *E. coli* isolates were tested for the presence of *papEF*gene by PCR. Bacteria were grown overnight in Luria-Bertani broth at 37°C. PCR was performed in a total volume of 50 µl. Each reaction mixture consisted of 5 µ1 of bacterial broth culturetreated at 94°C for 10 min, 1 µl (20 pmol) of each *pap*1 (5′-GCAACAGCAACGCTGGTTGCATCAT-3′; positions 490-514, *pap* E) and *pap*2 (5′-AGAGAGAGCCACTCTTATACGGACA-3′; positions 229-205, *pap* F) primers which amplifya DNA fragment of 336-bp, 1.25 µ1 of deoxynucleoside triphosphate mixture (containing 10 mMdATP, 10 mMdCTP, 10 mMdTTP, and 10 mMdGTP), 5 µ1 of buffer solution (100 mMTris. HCI, pH 8.8, 500 mMKCl, 15 mMMgCl, 1% Triton X-100), 1.25 µ1 of 50 mMMgCl, and 0.3 µl (1.5 unit) of *Taq* DNA polymerase (Roche). The reaction mixture was overlaid with 50 µ1 of mineral oil. PCR cycling conditions were as follows; heating at 94°C for 3 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 63°C for 30 s, and extension at 72°C for 3 min, and final extension for 7 min at 72°C. Ten microliters of each reaction mixture were then analyzed by electrophoresis on 2% agarose gel. Amplicons were visualized by staining with ethidium bromide [12].Reagent control was included in each PCR run which consisted of all PCR components except for the template DNA. Universal bacterial primers targeting 16S rRNA[13] were used in a separate PCR step, preceding that targeting *papEF* gene, to assess the quality of DNA extracts as well as the amplification process.

Statistical analysis:

Data was analyzed using windows SPSS version 17.0 and descriptive statistics were used. Statistical significance was set at $p \leq 0.05$.

RESULTS

Two hundred and sixty nine bacterial isolates were obtained from an equal number of UTI cases (132 and 137 isolates from outpatients and inpatients, respectively), throughout the study period. Among all urinary tract pathogens, *E. coli* was the most commonly isolated (n=199) accounting for 73.9% isolation rate from both groups 86.4% from outpatients and 62.04% from n patients.

Resistance to NA was detected in 154 out of 199 (77.38%) *E. coli* isolates; 71% of outpatient isolates and 85.8% of inpatient isolates. Out of 199 *E. coli* isolates 52 (26.13%) were resistant to ciprofloxacin, 17.5% of outpatient isolates and 37.6% of inpatient isolates).

Inpatient isolates exhibited significantly higher resistance ratio(85.8%) to NA compared to outpatient counterparts(71%) (*P*=0.01). The same result was found regarding ciprofloxacin where significantly higher resistance ratio in inpatient isolates (37.6%) compared to that of outpatient isolates (17.5%),was detected (*P* = 0.001). The results are shown in table 1.

Table1. Distribution of NA and ciprofloxacin resistance in urinary tract isolates of *E. coli* from outpatients and inpatients (n=199).

Antibiotic	Resistant isolates		*P* value
	Outpatient isolates (n= 114) No. (%)	Inpatient isolates (n= 85) No. (%)	
NA	81(71)	73 (85.8)	0.01*
Ciprofloxacin	20 (17.5)	32 (37.6)	0.001**

*significant, **highly significant

When NA-resistant *E. coli* isolates, obtained in this study (n= 154), were compared to NA -susceptible one (n=45), a significantly fewer resistant isolates were found to express beta-hemolysis (16.2% versus 91.1%, $P < 0.0001$) and to harbor a *papEF*genotype (33.1% versus 86.7%, $P < 0.0001$). The results are shown in table 2 and figure 1.

Table 2. Distribution of beta-hemolysis and *papEF*genotype in urinary tract isolates of *E. coli* obtained throughout the study (n =199)

Antibiotic susceptibility pattern	No. (%) of isolates with hemolysis	*P* value	No. (%) of isolates with *papEF* genotype	*P* value
NA				
Resistant (n=154)	25 (16.2)		51 (33.1)	
Susceptible (n=45)	41 (91.1)	<0.0001**	39 (86.7)	<0.0001**
Ciprofloxain				
Resistant (n=52)	4 (7.7)		15 (28.8)	
Susceptible (n=147)	124 (84.4)	<0.0001**	107 (72.8)	<0.0001**

**highly significant

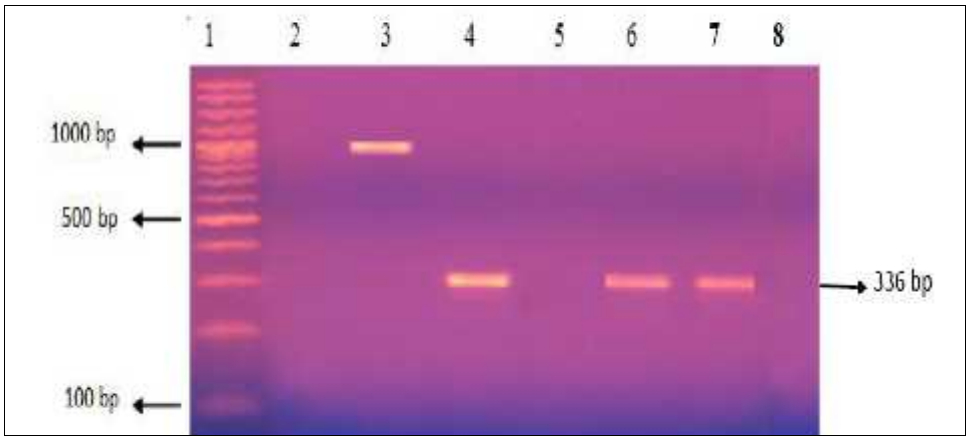

Fig. 1: Lane 1: MW marker. Lane 2: Reagent control. Lane 3: positive control. Lanes 4,6 and 7 positive samples with 336-bp portion of the *papEF genotype*. Lanes 5 and 8: negative samples.

Similarly, a significantly lower ratio of ciprofloxacin-resistant isolates (n=52) expressed beta-hemolysis (7.7% versus 84.4%, $P < 0.0001$) and had a *papEF* genotype (28.8% versus 72.8%, $P < 0.0001$), compared to their susceptible counterparts (n=147) (Table2).

DISCUSSION

Owing to their extensive use in the treatment of bacterial UTI, resistance to quinolone (Q) and fluoroquinolone (FQ) antimicrobials has emerged among strains of UPEC, posing an increased risk of treatment failure.

This study aimed to determine the current levels of NA and ciprofloxacin resistance in urinary tract isolates of *E. coli* in Doha, Qatar and to assess the occurrence of the gene coding for pilus associated with pyelonephritis (*papEF*) among Q and FQ resistant isolates, compared to their susceptible counterparts, from inpatients and outpatients.

In this study, *E. coli* was the most common organism causing UTI, isolated with a frequency of 73.9% (n = 199). It accounted for 86.36% from outpatient isolates and 62.04% from inpatient isolates. Similar results were documented by Drews et al. [14], who isolated *E. coli* with a frequency of 77% from outpatient isolates and 65% from inpatient isolates in Canada. *E. coli* was the causative agent of 90% of uncomplicated UTIs and 78% of complicated UTIs as reported by Arslan et al.[15] in Turkey when community-acquired UTIs were studied. In Qatar, Afifiet al.[16] documented that *E. coli* accounted for 92.3% of UTI isolates.

In the current work, resistance to NA was detected in 154 out of 199 (77.38%) *E. coli* isolates; 71% of outpatient isolates and 85.8% of inpatient isolates. Whereas, 26.13% of *E.coli* isolates were resistant to ciprofloxacin; 17.5% of outpatient isolates and 37.6% of inpatient isolates. In a previous local study in Qatar, Alshahat[17] found that 46.2% of *E. coli* isolates were resistant to ciprofloxacin. Variable results were reported from different countries. Sanchez et al. [5] examined *in vitro* antimicrobial resistance data of *E. coli* isolates obtained from urine samples of U.S. outpatients between 2000 and 2010. Their study demonstrateda great increase in *E. coli* resistance to ciprofloxacin (from 3% to 17.1%). In a study carried out by Jeon et al. [18]in Korea, they documented 15% resistance ratio of *E. coli* isolates obtained from community acquired UTI, to ciprofloxacin. Sahuquillo-Arce et al.[19] compared the resistance ratios of urinary *E. coli* isolates obtained from hospital and community-acquired UTI in Spain; they revealed a greater proportion of resistant organisms to ciprofloxacin in hospital isolates (37%) compared to their community counterparts (31.6%). This comes consistent with the current study where significantly higher (P=0.001) ciprofloxacin resistance ratio was recorded in inpatient isolates (37.6%) compared to outpatients (17.5%). In Denmark, Olesen et al.[20] documented a higher ratio of ciprofloxacin resistance (56%). Drews et al.[14] stated that resistance to ciprofloxacin and NA acid was detected in 6% and 11%, respectively, of outpatient UTI *E. coli* isolates and 18% and 21%, respectively, of inpatient UTI *E. coli* isolates in Canada.

It is possible that the geographic source of isolates represents an important element to be taken into consideration [21]. The analysis of a collection of UPEC strains from a particular region may therefore be useful in order to correlate the patterns of antibacterial resistance with local trends in the human usage of antibiotics and/or consumption of animal products.

In the current study, a significantly lower ratio of NA-resistant isolates (n=154) were found to express beta-hemolysis (16.2%% versus 91.1%, $P < 0.0001$) and to harbor a *papEF* genotype (33.1% versus 86.7%, $P<0.0001$), when compared to NA -susceptible isolates (n=45).Similarly, a significantly lower ratio of ciprofloxacin-resistant isolates (n=52) expressed beta-hemolysis (7.7% versus 84.4%, $P < 0.0001$) and had a *papEF*genotype (28.8% versus 72.8%, $P <0.0001$), compared to their susceptible counterparts (n=147).

Vila et al.[6] studied *papC* and *hlyA* genes and hemolysin production in NA-susceptible and-resistant *E. coli* strains causing cystitis, in Spain. Only 10.3% in NA-susceptible group and 10.3% in NA-resistant group carried the *papC* gene. Hemolysin production was significantly (P=0045) associated with NA susceptibility, occurring in 27.6% of NA susceptible strains but in none of the NA resistant strains. Overall, the results obtained in their study revealed that NA resistant UPEC strains carried less-frequent urovirulence factors, such as *hlyA* and *cnf*. Besides, a decrease in the expression of type 1 fimbriae also was observed. In another study, Piatti et al. [3]documented that in susceptible *E. coli* strains, the incidences of *papC*, *hlyA*, and *cnf1* were, respectively, 68%, 62%, and 61%, whereas in resistant isolates, the incidence was 9% for *papC* and 7% for both *hlyA* and *cnf1* (P <0.001).

Moreno et al.[22] found that FQ-resistant UPEC strains did not harbor VFs such as *hlyA* (3%), *cnf1* (3%), and *papC* (11%). This was explained by the loss of the corresponding PAI, probably because of the mutation that causes resistance, as already assessed by other investigators [23].

It remains undecided whether drug-refractory *E. coli* strains are intrinsically less virulent bacteria or if they become less virulent following acquisition of the *gyrA* mutation[24]. There is clear evidence that a complex relationship exists between virulence properties of *E. coli*, phylogenetic background, and antibiotic resistance. It is also possible that the geographic source of isolates represents an important additional element to be taken into consideration [3].

How FQ resistance is linked to loss of beta-hemolysis and *papEF*is not obvious. It has been proposed that FQ-resistant bacteria may be less fit than susceptible isolates due to decreased efficiency of gyrase and topoisomerase [25]. Loss of beta-hemolysis may result

from decreases in beta-hemolysin mRNA due to transcription-coupled DNA supercoiling. Loss of PAIs in face of possible inhibition of gyrase or topoisomerase has been described previously; the driving force behind PAI loss may be a signal to escape a genome, which is less fit to replicate [26].

CONCLUSION

This study demonstrated that ciprofloxacin-and NA-resistant urinary *E. coli* isolates, in Doha, Qatar, showed less virulence factors than their susceptible counterpart. Yet, resistance itself may be a virulence factor that allows for the survival of a bacterium within the urinary tract of treated patients. Further studies may be necessary to reveal the association between ciprofloxacin/NA resistance and the loss of other virulence factors, including other toxins and other adhesion mechanisms in *E. coli*. Given the frequency with which UTIs are treated empirically, compounded with the speed that *E. coli* acquires resistance, prudent use of antimicrobial agents remains crucial.

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