Mechanisms of Resistance to Fluoroquinolone Antibiotic in Uropathogenic Escherichia coli

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Abstract

Urinary tract infections (UTI) is a bacterial infection of the urogenitals and mostly caused by uropathogenic *Escherichia coli* (UPECs). Antibiotics such as fluoroquinolones (FQ) were used in the treatment of UPECs. Misuse of FQ in the treatment of UTI may have resulted in the prevalence of FQ-resistant UPECs. The occurrence and prevalence of antibiotic-resistant pathogenic UPECs in clinical ecosystem is considered as a threat to the efficacy of the antibiotic and public health by the USFDA and many other regulatory agencies. A study was undertaken to characterize FQ-resistant UPEC isolates and the mutations in the quinolone-resistant-determining regions (QRDR) of the isolates that confer resistance to FQ antibiotics. Twenty three of the 104 UPEC isolated from patients with UTI were resistant to FQ antibiotics. Purified PCR amplicons of *gyr*A and *par*C genes from the total DNA of the isolates were partially sequenced and analyzed for point mutations that confer resistance to FQ antibiotics. Sequence analysis indicated that simultaneous point mutations in the QRDR of *gyr*A (at position 83Ser→Leu and 87Asp→Asn) accompanied by point mutations in the QRDR of *par*C (80Ser→Ile or 84Glu→Val) were responsible for conferring resistance to FQ antibiotics in these isolates. Efflux pumps (EPs) that dilute the accumulation of the antibiotic inside the cell was determined by the ethidium bromide accumulation and measurement of the relative fluorescence units (RFUs). All FQ-resistant UPECs had EP activity. The EP activities were higher in FQ-resistant UPECs than antibiotic sensitive strains of UPECs. Our results indicate that point mutations in the QRDR of *gyr*A and *par*C coupled with EP mechanisms may contribute to FQ resistance in UPECs.

Introduction

Urinary tract infection (UTI) is a chronic, recurrent bacterial infection of the urogenitals. It is broadly defined as an infection of the urinary system and may involve the lower urinary tract or the lower and upper urinary tracts combines. Infections of the urethra or the bladder can cause symptoms such as painful frequent urination; cloudy, foul-smelling urine; and mild abdominal pain. Currently, more than a billion women suffer from UTI infections worldwide. The infection affects about 8 million American women every year resulting in approximately 100,000 hospitalizations. Untreated UTI contributes to preterm labor, reduced kidney function or failure, agitation, delirium, and behavior instability in the elderly. Additionally, recurrent urinary tract infections (rUTI) are extremely common, with 25.0% of all women experiencing a recurrence within one year of original infection. The incidence of UTI increases with age and sexual activity. A majority (80%) of community acquired UTI are caused by uropathogenic *Escherichia coli* (UPEC); antibiotics such as tetracycline, ampicillin and ciprofloxacin were widely used in the treatment of UTI. UPEC strains belong to *E. coli* phylogenetic group B2 or D and are often clonal, with the most common sequence type (STs) isolated worldwide being ST69, ST73, ST95 and ST131. These clones have become a major contributor to hospital and community acquired UTI and blood stream infections and are strongly associated with multidrug resistance (MDR), including resistance to fluoroquinolones. Dissemination of antibiotic resistance determinants among pathogenic bacteria has been a concern for public health because the prevalence of such bacteria can make the antimicrobial regimen in the treatment of the disease ineffective. Besides, the overuse and/ or misuse of antimicrobials facilitates the spread of antibiotic resistance determinants associated with mobile genetic elements.

Materials and Methods

Bacterial Isolates: UPEC isolates were obtained from different medical centers in the US.

Determination of antibiotic susceptibility and the Minimum Inhibitory Concentration (MIC) of the isolates: Antibiotic susceptibility of each *E. coli* isolate was determined using a disk diffusion assay. The susceptibility of each isolate was determined as per the criteria specified by the Clinical and Laboratory Standards Institute. A sensitive strain of *E. coli* (in house) was used as control. The MICs for the antibiotics ciprofloxacin and nalidixic acid were determined by the broth dilution method using Mueller-Hinton broth. The concentration ranges for ciprofloxacin were 0.125 to 128 µg/mL and for nalidixic acid were 4 to 512 µg/mL.

Genomic DNA Extraction: Genomic DNA was extracted from cells grown overnight at 37oC with the QIAamp DNA Mini Prep kit (Qiagen, Valencia, CA).

Primer Design and Detection of Quinolone-Resistance Genes by PCR: The presence of quinolone-resistance genes (*gyr*A,B and *par*C-E) was investigated in the template DNA by PCR. The detection and amplification of the quinolone-resistance genes were carried out as detailed elsewhere.

Detection of Mutations in the Quinolone Resistance Determining Region (QRDR): The target genes were amplified by PCR and purified by the QIAquick PCR purification kit (Qiagen). Both strands of the purified PCR amplicons were sequenced by the primers used for the amplification of QRDR.

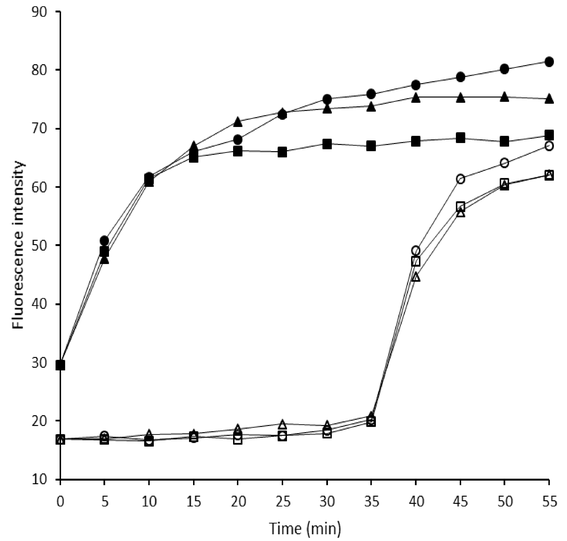
Accumulation of Ethidium Bromide in Fluoroquinolone-Sensitive and Resistant Strains of *E. coli*: Accumulation of ethidium bromide was monitored as described elsewhere. Fluorescence was read in a Synergy 2 Multi-Mode Microplate Reader (BIOTEK Instruments, Winooski, VT) every 1 min every minute for the next 30 mins. at excitation 530 nm and emission 600 nm. Carbonyl cyanide m-chlorophenylhydrazone (CCCP), an inhibitor of the efflux pump, was added to the assay mixture at a final concentration of 100 µM. The natural fluorescence of the cells was subtracted and the fluorescence intensity was expressed in relative fluorescence units (RFU). All experiments were performed in triplicate.

Results

Table 1. Mutations in the quinolone resistance determining region of *gyrA* and *parC* in UTI *E. coli*

Isolate	*gyr*A	*par*C
1	S83L, D87N, D147N	S80I
2	V146F	S80I, E84V
3	S83L, D87N	S80I
4	S83L, D87N	S80I, E84V, Q103R
6	S83L, D87N, N165S	S80I, E84V

Figure 1. Accumulation of ethidium bromide by fluoroquinolone-resistant isolates (#21: circle, #36: triangle, #63: square). CCCP was added before the plate reading (filled) or 35 min after reading (open) at a final concentration of 100 µM.



Conclusion

- **Mutations in the quinolone resistance determining regions of *gyr*A (at positions 83Ser→Leu; 87Asp→Asn) and *par*C (at positions 80Ser→Ile) were responsible for conferring resistance to FQ antibiotics.**
- **Efflux pump activity was higher in FQ-resistant UPEC isolates than FQ-sensitive UPEC strains.**
- **Our results demonstrate that point mutations in *gyr*A and *par*C along with higher efflux pump activities may contribute to FQ resistance in these isolates.**

Disclaimer

We thank Drs. Carl Cerniglia and John Sutherland for their critical comments. The views expressed herein do not necessarily reflect those of the Food and Drug Administration. The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the FDA. The findings and conclusions in this Perspective have not been formally disseminated by the FDA and should not be construed to represent any agency determination or policy.