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Determinants of quinolone resistance in *Escherichia coli* causing community-acquired urinary tract infection in Bejaia, Algeria

Yanat Betitra[1,2], Vinuesa Teresa[2], Viñas Miguel[2], Touati Abdelaziz[1*]

[1]*Laboratoire d'écologie Microbiologie. Université A/Mira de Bejaia, Algérie*

[2]*Laboratory of Molecular Microbiology and Antibiotics, Dept. Pathology and Experimental therapeutics. Medical School, University of Barcelona, Spain*

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ABSTRACT

Objective: To investigate the mechanisms of quinolone resistance and the association with other resistance markers among *Esherichia coli* (*E. coli*) strains isolated from outpatient with urinary tract infection in north of Algeria. **Methods:** A total of 30 nalidixic acid-resistant *E. coli* isolates from outpatient with urinary tract infections from January 2010 to April 2011 in north of Algeria (Bejaia) were studied. Antimicrobial susceptibility was determined by disc diffusion assay, minimal inhibitory concentrations (MIC) of quinolone were determined by microdilution. Mutations in the Quinolone Resistance-Determining Region (QRDR) of *gyr*A and *par*C genes and screening for qnr (A, B and S) and bla genes were done by PCR and DNA sequencing. **Results:** Most of the *E. coli* isolates (56.66%) were shown to carry mutations in *gyr*A and *par*C (*gyr*A: Ser83Leu + Asp87Asn and *par*C:Ser80Ile). While, 16.66% had only an alteration in *gyr*A: Ser83Leu. One isolate produced *qnr*B-like and two qnrS-like. Four isolates were CTX-M-15 producers associated with TEM-1 producing in one case. Co-expression of $bla_{CTX-M-15}$ and *qnr*B was determined in one *E. coli* isolate. **Conclusions:** Our findings suggested the community emergence of *gyr*A and *par*C alterations and Qnr determinants that contributed to the development and spread of fluoroquinolone resistance in Algerian *E. coli* isolates.

1. Introduction

Escherichia coli (*E. coli*) is a major cause of urinary tract infections (UTI). Quinolones are commonly used to treat UTI due to *E. coli*[1]. These synthetic antimicrobial agents include nalidixic acid and ciprofloxacin which is a fluoroquinolone (FQ) with a wide spectrum of antibacterial activity in vitro, particularly against gram negative bacteria[2]. The extensive use of FQ has led to an increasing resistance in *E. coli*[3]. Resistance to quinolone occurs as a result of chromosomic and plasmidic mechanisms. Chromosomic-mediated quinolone resistance concerns an accumulation of mutations in the Quinolone Resistance Determining Region (QRDR) primarily in DNA gyrase (*gyr*A), then in topoisomerase IV (*par*C). It can be associated with decreased outer membrane permeability and/or with an overexpression of the efflux pump systems[4]. Plasmid-mediated quinolone resistance (PMQR) was first described in 1998[5]. Since then, five major groups of *qnr* determinants (*qnr*A, *qnr*S, *qnr*B, *qnr*C and *qnr*D) have been identified[5-9]. Two additional PMQR determinants, the AAC(6′)Ib-cr enzyme, which acetylates not only aminoglycosides but also ciprofloxacin and norfloxacin[10] and quinolone extrusion by QepA or OqxAB[11, 12] have been also described. The association between PMQR and multidrug resistant isolates producing Extended Spectrum Beta-Lactamas (ESBL) has been reported in urinary *Enterobacteriaceae* isolates in Algeria[13, 14] and in different countries[15].

Fluoroquinolone resistance in *E. coli* is an important issue all over the world. To date there is no publication from Algeria evaluating the genetic determinants of resistance to this class of antimicrobials in *E. coli* causing community acquired urinary tract infections (no results on PubMed). The aim of our study was to investigate the mechanisms

*Corresponding author: Abdelaziz Touati, Département de Microbiologie, FSNV, Université A/MIRA de Béjaia 06000, Algeria.
Tel/Fax : 213.34214762
E-mail: ziz1999@yahoo.fr

of quinolone resistance and the association with other resistance markers among *E. coli* strains isolated from outpatient with UTI in north of Algeria.

2. Materials and methods

2.1. Bacterial strains

Thirty non-repetitive nalidixic acid-resistant *E. coli* isolates from community-acquired UTI in private laboratories of medical analysis in the region of Bejaia (North Algeria) from January 2010 to April 2011 were included in this study. These isolates were identified by API 20E identification system (BioMérieux, France). *E. coli* ATCC25922 was used for susceptibility testing control. The following β-lactamase-producing isolates were used as control isolates: *Enterobacter aerogenes* CF 2403 (*E. aerogenes*) for TEM, *Klebsiella pneumoniae (K. pneumoniae)* KpS12 for SHV; and *K. pneumoniae* Bhe CD13 for CTX-M.

Qnr-positive strains provided by Pr. J Vila and Dr. A. Fàbrega were used as positive controls: *Enterobacter cloacae (E. cloacae)* for *qnr*A1, *K. pneumoniae* positive for qnrB1 and *Salmonella enterica (S. enterica)* serovar Saintpaul positive for *qnr*S1.

2.2. Antibiotic susceptibility testing

Susceptibility to different antimicrobials was performed by disc diffusion method on Mueller-Hinton agar and was interpreted according to Clinical and Laboratory Standards Institute recommendations[16]. Tested antibiotics included norfloxacin, ofloxacin, ciprofloxacin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime, cefoxitin, aztreonam, imipenem, gentamycin, kanamycin, tobramycin, amikacin, and cotrimoxazole (Oxoid Ltd., Basingstoke, UK).

The nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin and levofloxacin minimum inhibitory concentration (MIC) were determined by broth microdilution method with cation-adjusted Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) according to Clinical and Laboratory Standards Institute recommendations[16].

2.3. Phenotypic ESBL detection

Extended-Spectrum beta-Lactamase production was detected by a double-disk synergy test (DDST) and was performed by placing disks of ceftazidime, cefotaxime and aztreonam at a distance of 20 mm (centre to centre) from a disk with amoxicillin/clavulanic acid (20/10 μg). Enhancement of the inhibition zone between the disks containing clavulanic acid and cefotaxime, ceftazidime or aztreonam indicated the ESBL production[17].

2.4. Analysis of quinolone resistance-determining regions (QRDRs) of gyrA and parC genes

The QRDR of *gyr*A and *par*C were amplified using the primers showed in Table 1. PCR conditions were as follows: initial denaturation step of 5 min at 94 ℃, 1 min at 94 ℃, 1 min at the annealing temperature (54 ℃ for *gyr*A and 55 ℃ for *par*C) and 1 min at 72 ℃ for 30 cycles, final extension step was 10 min at 72 ℃[18]. Reaction mixes without a DNA template served as negative controls. Amplified fragments were purified (Qiagen kit, Hilden, Germany).

Table 1
Primers used in this study.

Gene		Primers
*gyr*A	*gyr*A1	ACGTACTAGGCAATGACTGG
	*gyr*A2	AGAAGTCGCCGTCGATAGAAC
*par*C	*par*C1	AGTATGCGATGTCTGAACTG
	*par*C2	CTCAATAGCAGCTCGGAATA
*qnr*A	*qnr*A1	ATTTCTCACGCCAGGATTTG
	*qnr*A2	GATCGGCAAAGGTTAGGTCA
*qnr*B	*qnr*B1	GATCGTGAAAGCCAGAAAGG
	*qnr*B2	ACGATGCCTGGTAGTTGTCC
*qnr*S	*qnr*S1	ACGACATTCGTCAACTGCAA
	*qnr*S2	TAAATTGGCACCCTGTAGGC
bla_{CTX-M}	$bla_{CTX-M-A2}$	CTTCCAGAATAAGGAATC
	628R	CCTTTCATCCATGTCACCA
	405F	GTGGCGATGAATAAGCTGA
	$bla_{CTX-M-B2}$	CCGTTTCCGCTATTACAA
bla_{TEM}	bla_{TEM-A}	TAAAATTCTTGAAGACG
	bla_{TEM-B}	TTACCAATGCTTAATCA

Nucleotide sequences were determined using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The gyrA and parC nucleotide sequences and the deduced amino acids were compared with that of *E. coli* K12 using ClustalW alignment program.

2.5. Multiplex PCR detection of the qnr genes

Screening for the *qnr*A, *qnr*B and *qnr*S genes was carried out by a multiplex PCR amplification using specific primers (Table 1) according to Robicsek *et al* 2006[19].

2.6. Detection and characterization of bla genes

Isolates positive for the DDST were screened for the presence of bla_{CTX-M}, bla_{TEM} and bla_{SHV} by PCR as previously described[20]. PCR products were sequenced and the DNA alignments and the deduced amino acid sequences were examined using the BLAST program.