

# Mechanisms of reduced susceptibility to ciprofloxacin in *Escherichia coli* isolates from Canadian hospitals

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**OBJECTIVE:** To determine whether plasmid-mediated quinolone resistance (PMQR) determinants play a role in the increasing resistance to fluoroquinolones among *Escherichia coli* isolates in Canadian hospitals, and to determine the mechanisms of reduced susceptibility to ciprofloxacin in a recent collection of 190 clinical *E coli* isolates.

**METHODS:** *E coli* isolates (n=1702) were collected as part of the 2007 Canadian Hospital Ward Antibiotic Resistance Surveillance (CANWARD) study. Antimicrobial susceptibility testing was performed by Clinical and Laboratory Standards Institute (CLSI) broth microdilution. Using a representative subset of isolates (n=190), the mechanisms of reduced susceptibility to ciprofloxacin were detected by polymerase chain reaction and sequencing of the quinolone resistance-determining regions (QRDR) of chromosomal *gyrA* and *parC* genes, and by polymerase chain reaction for the PMQR genes: *qnr*, *aac(6')Ib-cr* and *qepA*.

**RESULTS:** 2.1% and 1.1% of *E coli* harboured *aac(6')Ib-cr* and *qnrB*, respectively. Single amino acid substitutions in the QRDR of *gyrA* were observed among isolates with ciprofloxacin minimum inhibitory concentrations as low as 0.12 µg/mL. As the ciprofloxacin minimum inhibitory concentration increased to 1 µg/mL (which is still considered to be susceptible by the CLSI), the vast majority of isolates demonstrated both *gyrA* and *parC* mutations.

**CONCLUSION:** PMQR determinants and QRDR mutants among clinical *E coli* isolates with reduced susceptibility to ciprofloxacin demonstrates the need for increased surveillance and the need to re-evaluate the current CLSI breakpoints to prevent further development of fluoroquinolone resistance.

**Key Words:** Ciprofloxacin; *Escherichia coli*; Fluoroquinolone; Plasmid-mediated quinolone resistance; Quinolone resistance-determining region

In 2007, the Canadian Hospital Ward Antibiotic Resistance Surveillance (CANWARD) study – an annual national surveillance study assessing antimicrobial resistance – identified *Escherichia coli* as the most commonly encountered pathogen within Canadian hospitals (1). In that study, 25.4% of *E coli* isolates were fluoroquinolone resistant (www.can-r.ca) (1).

Until recently, resistance to fluoroquinolones was considered to be only chromosomally encoded and most commonly involved amino acid substitutions in the quinolone resistance-determining regions (QRDR) of DNA gyrase (*gyrA*) and/or topoisomerase IV (*parC*), which are the main targets of fluoroquinolones (2). Reduced uptake by decreased expression of outer membrane porins and overexpression of efflux pumps also contributes to chromosomal fluoroquinolone resistance (3). Although originally considered to be improbable due to the

## Les mécanismes de susceptibilité réduite à la ciprofloxacine dans les isolats d'*Escherichia coli* des hôpitaux canadiens

**OBJECTIF :** Établir si les déterminants de la résistance aux quinolones à médiation plasmidique (RQMP) contribuent à la résistance croissante aux fluoroquinolones dans les isolats d'*Escherichia coli* des hôpitaux canadiens et déterminer les mécanismes de susceptibilité réduite à la ciprofloxacine dans un récent échantillonnage de 190 isolats cliniques d'*E coli*.

**MÉTHODOLOGIE :** Les chercheurs ont colligé les isolats d'*E coli* (n=1 702) dans le cadre de l'étude de surveillance canadienne CANWARD de 2007 sur la résistance antibiotique dans les unités hospitalières. Le *Clinical and Laboratory Standards Institute* (CLSI) a effectué les tests de susceptibilité antimicrobienne au moyen de la technique de microdilution en milieu liquide. Au moyen d'un sous-groupe représentatif d'isolats (n=190), les chercheurs ont décelé les mécanismes de susceptibilité réduite à la ciprofloxacine à l'aide de la réaction en chaîne de la polymérase et du séquençage des régions responsables de la résistance aux quinolones (RRRQ) pour les gènes chromosomiques *gyrA* et *parC*, et à l'aide de la réaction en chaîne de la polymérase pour les gènes de RQMP, soit *qnr*, *aac(6')Ib-cr* et *qepA*.

**RÉSULTATS :** Les chercheurs ont constaté que 2,1 % et 1,1 % des *E coli* hébergent les gènes *aac(6')Ib-cr* et *qnrB*, respectivement. Les chercheurs ont observé des substitutions d'acides aminés simples dans les RRRQ du gène *gyrA* d'isolats aux concentrations minimales inhibitrices de ciprofloxacine aussi basses que 0,12 µg/mL. Lorsque la concentration minimale inhibitrice de ciprofloxacine passait à 1 µg/mL (que le CLSI considère toujours comme susceptible), la majorité des isolats démontrait à la fois des mutations des gènes *gyrA* et *parC*.

**CONCLUSION :** Les déterminants de la RQMP et les mutants des RRRQ d'isolats cliniques d'*E coli* ayant une susceptibilité réduite à la ciprofloxacine démontrent qu'il est nécessaire d'accroître la surveillance et de réévaluer les points de rupture du CLSI pour prévenir l'apparition d'une résistance aux fluoroquinolones.

plasmid curing effect of quinolones, plasmid-mediated quinolone resistance (PMQR) was first reported in 1998 and has become an emerging concern (4,5). Three different types of PMQR determinants have recently been reported. The first PMQR determinant includes the following quinolone resistance genes: *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qnrS*. *Qnr* determinants are believed to bind to and protect DNA gyrase and/or topoisomerase IV from fluoroquinolone inhibition. The second type of PMQR determinant, *aac(6')Ib-cr*, is an aminoglycoside-modifying enzyme that acetylates several fluoroquinolones including ciprofloxacin (3). Although both the *qnr* and *aac(6')Ib-cr* genes only confer reduced susceptibility (increased minimum inhibitory concentration [MIC] but not elevated past the susceptible breakpoint) to the fluoroquinolones, they do provide a background for in vivo selection of chromosomal-borne mechanisms of resistance and result in the

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recovery of resistant mutants with higher levels of resistance than chromosomal changes alone (6). The third and most recently described PMQR determinant, *qepA*, is an efflux pump that extrudes hydrophilic fluoroquinolones such as ciprofloxacin (7). The purpose of the present study was to determine whether PMQR determinants play a role in increasing resistance to fluoroquinolones among *E coli* isolates in Canadian hospitals and to determine the mechanisms of reduced susceptibility to ciprofloxacin in a recent collection of *E coli* clinical isolates.

## PATIENTS AND METHODS

*E coli* isolates (n=1702) were collected as part of the CANWARD surveillance study. CANWARD is a laboratory-based surveillance study coordinated at the Health Sciences Centre in Winnipeg, Manitoba. From January 1 to December 31, 2007, inclusive, 12 sentinel hospital centres across Canada submitted pathogens from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units, as previously described (1).

Following two subcultures from frozen stock, the in vitro activity of ciprofloxacin was determined by microbroth dilution in accordance with Clinical Laboratory and Standards Institute guidelines (8). Of the 1702 *E coli* isolates, a representative cohort of 190 *E coli* isolates with ciprofloxacin MICs ranging from  $\leq 0.06$   $\mu\text{g/mL}$  to 8  $\mu\text{g/mL}$  were further analyzed, including 20 of 1158 isolates with a ciprofloxacin MIC of  $\leq 0.06$   $\mu\text{g/mL}$  (susceptible/wildtype; control group). These 20 isolates were selected to represent all geographical regions of the country. Thereafter, all 23 isolates with a ciprofloxacin MIC of 0.12  $\mu\text{g/mL}$ , all 46 isolates with an MIC of 0.25  $\mu\text{g/mL}$ , all 33 isolates with an MIC of 0.5  $\mu\text{g/mL}$ , all 20 isolates with an MIC of 1  $\mu\text{g/mL}$ , all five isolates with an MIC of 2  $\mu\text{g/mL}$ , all nine isolates with an MIC of 4  $\mu\text{g/mL}$  and all 34 isolates with an MIC of 8  $\mu\text{g/mL}$  were studied. Reduced susceptibility to ciprofloxacin was defined as an MIC ranging from 0.12  $\mu\text{g/mL}$  to 1  $\mu\text{g/mL}$  (CLSI MIC breakpoints for ciprofloxacin are  $\leq 1.0$   $\mu\text{g/mL}$  [susceptible], 2.0  $\mu\text{g/mL}$  [intermediate], and  $\geq 4.0$   $\mu\text{g/mL}$  [resistant]).

The molecular mechanisms of reduced susceptibility to ciprofloxacin were determined by polymerase chain reaction and sequencing of the QRDR of chromosomal *gyrA* and *parC* genes and the PMQR genes – *qnrA*, *qnrB*, *qnrS*, *aac(6')Ib-cr* and *qepA* – using appropriate controls as previously described (9).

## RESULTS

The distribution of ciprofloxacin MICs against the 1702 *E coli* isolates collected as part of CANWARD 2007 is as follows: 1158 (67.9%) with an MIC of  $\leq 0.06$   $\mu\text{g/mL}$ , 23 (1.4%) with an MIC of 0.12  $\mu\text{g/mL}$ , 46 (2.7%) with an MIC of 0.25  $\mu\text{g/mL}$ , 33 (1.9%) with an MIC of 0.5  $\mu\text{g/mL}$  and 20 (1.2%) with an MIC of 1  $\mu\text{g/mL}$ . Thus, 8.4% of *E coli* demonstrated reduced susceptibility to ciprofloxacin (MIC 0.12  $\mu\text{g/mL}$  to 1  $\mu\text{g/mL}$ ). Five (0.3%) isolates had a ciprofloxacin MIC of 2  $\mu\text{g/mL}$ , nine (0.6%) with an MIC of 4  $\mu\text{g/mL}$ , 34 (2.0%) with an MIC of 8  $\mu\text{g/mL}$  and 374 (22.0%) with an MIC of  $> 8$   $\mu\text{g/mL}$ .

The mechanisms of reduced susceptibility to ciprofloxacin in a cohort of 190 clinical *E coli* isolates are summarized in Table 1.

### QRDR mutations in *gyrA* and *parC*

None of the 20 wildtype *E coli* contained mutations within the QRDR of *gyrA* and *parC*. However, single amino acid substitutions in the QRDR of *gyrA* were present in *E coli* with MICs as low as 0.12  $\mu\text{g/mL}$ , in which 10 of 23 (43.5%) *E coli* had mutations in *gyrA* resulting in amino acid changes – S83L (40%), S83A (10%), D87G (30%) and D87N (20%). One isolate had a *parC* mutation outside of the QRDR, resulting in D111Y. At MICs of 0.25  $\mu\text{g/mL}$  and 0.5  $\mu\text{g/mL}$ , nearly all isolates had *gyrA* mutations, with 42 (91.3%) of the 46 *E coli* with a MIC of 0.25  $\mu\text{g/mL}$  demonstrating amino acid changes – S83L (80.4%), D87G (6.5%), D87Y (2.2%) and D87N (2.2%); 29 (87.9%) of the 33 *E coli* with an MIC of 0.5  $\mu\text{g/mL}$  had an S83L amino acid change. At an MIC of 1  $\mu\text{g/mL}$ , mutations within the QRDR of both *gyrA* and *parC* were observed. *gyrA* mutations were present in all *E coli*

with an MIC of 1  $\mu\text{g/mL}$ , resulting in S83L (95%) and D87N (5%). *parC* single- and double-step mutations were observed in 16 of 19 (84.2%) isolates with *gyrA* mutations, and resulted in S80I (45%), E84G (20%), S80I and E84G (10%), and S80I and E84V (5%). Double-step *gyrA* mutations were observed in all but one (S83L; 2.1%) *E coli* with MICs of  $\geq 2$   $\mu\text{g/mL}$ , resulting in S83L and D87N (91.7%), S83L and D87Y (2.1%), S83L and D87G (2.1%), and S83A and D87Y (2.1%). Both single-step and double-step *parC* mutations were observed among the same cohort of *E coli* with MICs of  $\geq 2$   $\mu\text{g/mL}$ , resulting in S80I (37.5%), E84V (2.1%), S57T and S80I (8.3%), S80I and E84V (45.8%), S80I and E84G (4.2%), and S80I and D111Y (2.1%). All mutations observed within the QRDR of *gyrA* and *parC* have been previously observed for clinical isolates and are known to increase fluoroquinolone MICs (10). Figure 1 demonstrates the step-wise accumulation of mutations in the QRDR of *gyrA*, followed by *parC* QRDR changes as the ciprofloxacin MIC increases in *E coli*.

**PMQR genes:** Four (2.1%) of the 190 isolates were found to carry the *aac(6')Ib-cr* gene. Two of the four isolates had reduced susceptibilities (0.5  $\mu\text{g/mL}$  and 1  $\mu\text{g/mL}$ ) to ciprofloxacin and had mutations within the QRDR of *gyrA* (S83L) and one also had a *parC* mutation (S80I). The remaining two isolates harbouring *aac(6')Ib-cr* gene had resistant MICs (4  $\mu\text{g/mL}$  and 8  $\mu\text{g/mL}$ ) and had mutations within the QRDR of *gyrA* and *parC*.

Two (1.1%) of the 190 isolates were found to carry the *qnrB* gene. Of the two isolates harbouring *qnrB*, one isolate had reduced susceptibility to ciprofloxacin (MIC 0.5  $\mu\text{g/mL}$ ) with no QRDR mutations, and the other isolate was resistant to ciprofloxacin and contained double mutations in both *gyrA* (S83L, D87N) and *parC* (S80I, E84V). No *qnrA*, *qnrS* or *qepA* PMQR genes were observed among the cohort. Demographic data for PMQR positive isolates are summarized in Table 2.

## DISCUSSION

Increasing ciprofloxacin resistance among clinical isolates of *E coli* in Canadian hospitals is worrisome because ciprofloxacin is often used empirically in Canada and around the world to treat various types of infections. Although it is well known that resistance to the quinolones in *E coli* most commonly results from the accumulation of mutations primarily occurring in *gyrA* followed by *parC*, we tried to determine the prevalence of PMQR determinants and mechanisms of resistance among clinical isolates of *E coli* with reduced ciprofloxacin MICs. Because the most common PMQR determinants, *qnr* and *aac(6')Ib-cr*, only provide low levels of resistance (or reduced susceptibility) to the fluoroquinolones, we decided to use a cohort of *E coli* ranging in ciprofloxacin MICs from susceptible to resistant ( $\leq 0.12$   $\mu\text{g/mL}$  to 8  $\mu\text{g/mL}$ ). All available *E coli* isolates with reduced susceptibility to ciprofloxacin were studied. PMQR determinants are clinically important because they increase the mutant prevention concentration of ciprofloxacin, thus facilitating the recovery of mutants with higher levels of resistance to quinolones (5,11). Therefore, it has been suggested that such isolates provide a background for in vivo selection of additional chromosomal mechanisms of resistance to occur during or after treatment with fluoroquinolones (3,12). As a consequence, *E coli* isolates with reduced susceptibility to ciprofloxacin exhibit the potential for developing complete resistance and effectively eliminating the possibility of ciprofloxacin-based treatment of *E coli* infections. Thus, we set out to test the hypothesis that increasing fluoroquinolone resistance observed in *E coli* in Canada may be due to the presence of PMQR determinants among clinically significant *E coli* isolates with reduced ciprofloxacin susceptibilities.

Many PMQR prevalence studies in *E coli* focus on populations of ciprofloxacin intermediate/resistant *E coli* or populations of extended spectrum beta-lactamase (ESBL)-producing *E coli* (9,13,14). There are very few studies that have focused on an *E coli* population with ciprofloxacin MICs ranging from susceptible to resistant (15-17). We demonstrate that the prevalence of PMQR determinants among this cohort remains low in Canada. Only 2.1% of isolates harboured the *aac(6')Ib-cr* gene and 1.1% carried a *qnrB* gene, in which equal

**TABLE 1**  
**Mechanisms of reduced susceptibility to ciprofloxacin (CIP) in a cohort of 190 clinical *Escherichia coli* isolates from Canadian hospitals**

CIP MIC	Isolates, n (%)	<i>gyrA</i> AA variants	<i>parC</i> AA variants	<i>qnrA</i>	<i>qnrB</i>	<i>qnrS</i>	<i>aac(6')Ib</i>	<i>qepA</i>
<b>CIP MIC ≤0.06 µg/mL; n=20; susceptible/wildtype</b>								
0.015	9 (45)	No AA variants	No AA variants	Negative	Negative	Negative	Negative	–
0.03	10 (50)	No AA variants	No AA variants	Negative	Negative	Negative	Negative	–
0.06	1 (5)	No AA variants	No AA variants	Negative	Negative	Negative	Negative	–
<b>CIP MIC 0.12 µg/mL; n=23; reduced susceptible</b>								
0.12	12 (52.2)	No AA variants	No AA variants	Negative	Negative	Negative	Negative	–
0.12	4 (17.4)	S83L	No AA variants	Negative	Negative	Negative	Negative	–
0.12	3 (13.0)	D87G	No AA variants	Negative	Negative	Negative	Negative	–
0.12	2 (8.7)	D87N	No AA variants	Negative	Negative	Negative	Negative	–
0.12	1	S83A	No AA variants	Negative	Negative	Negative	Negative	–
0.12	1	No AA variants	D111Y	Negative	Negative	Negative	Negative	–
<b>CIP MIC 0.25 µg/mL; n=46; reduced susceptible</b>								
0.25	1 (2.2)	D87Y	No AA variants	Negative	Negative	Negative	Negative	–
0.25	1 (2.2)	D87N	No AA variants	Negative	Negative	Negative	Negative	–
0.25	3 (6.5)	D87G	No AA variants	Negative	Negative	Negative	Negative	–
0.25	4 (8.7)	No AA variants	No AA variants	Negative	Negative	Negative	Negative	–
0.25	37 (80.4)	S83L	No AA variants	Negative	Negative	Negative	Negative	–
<b>CIP MIC 0.5 µg/mL; n=33; reduced susceptible</b>								
0.5	1 (3.0)	No AA variants	No AA variants	Negative	<i>qnrB</i>	Negative	Negative	–
0.5	1 (3.0)	S83L	No AA variants	Negative	Negative	Negative	<i>aac(6')Ib-cr</i>	–
0.5	3 (12.1)	No AA variants	No AA variants	Negative	Negative	Negative	Negative	–
0.5	28 (84.8)	S83L	No AA variants	Negative	Negative	Negative	Negative	–
<b>CIP MIC 1 µg/mL; n=20; reduced susceptible</b>								
1	1 (5.0)	S83L	S80I, E84V	Negative	Negative	Negative	Negative	–
1	1 (5.0%)	D87N	No AA variants	Negative	Negative	Negative	Negative	–
1	1 (5.0%)	S83L	S80I	Negative	Negative	Negative	<i>aac(6')Ib-cr</i>	–
1	2 (10.0)	S83L	S80I, E84G	Negative	Negative	Negative	Negative	–
1	3 (15.0)	S83L	No AA variants	Negative	Negative	Negative	Negative	–
11	4 (20.0)	S83L	E84G	Negative	Negative	Negative	Negative	–
	8 (40.0)	S83L	S80I	Negative	Negative	Negative	Negative	–
<b>CIP MIC 2 µg/mL; n=5; intermediate</b>								
2	3 (60.0)	S83L, D87N	S80I	Negative	Negative	Negative	Negative	Negative
2	1 (20.0)	S83L, D87N	S80I, E84V	Negative	Negative	Negative	Negative	Negative
2	1 (20.0)	S83L, D87N	S80I, D111Y	Negative	Negative	Negative	Negative	Negative
<b>CIP MIC 4 µg/mL; n=9; resistant</b>								
4	1 (11.1)	S83A, D87Y	S80I	Negative	Negative	Negative	Negative	Negative
4	1 (11.1)	S83L, D87N	S80I	Negative	Negative	Negative	Negative	Negative
4	1 (11.1)	S83L	S80I	Negative	Negative	Negative	<i>aac(6')Ib-cr</i>	Negative
4	1 (1.11)	S83L, D87N	S80I	Negative	Negative	Negative	Negative	Negative
4	2 (22.2)	S83L, D87N	S57T, S80I	Negative	Negative	Negative	Negative	Negative
4	3 (33.3)	S83L, D87N	S80I, E84V	Negative	Negative	Negative	Negative	Negative
<b>CIP MIC 8 µg/mL; n=34; resistant</b>								
8	1 (2.9)	S83L, D87N	S80I, E84V	Negative	Negative	Negative	<i>aac(6')Ib-cr</i>	Negative
8	1 (2.9)	S83L, D87N	S80I, E84V	Negative	Negative	Negative	Negative	Negative
8	1 (2.9)	S83L, D87N	E84V	Negative	Negative	Negative	Negative	Negative
8	1 (2.9)	S83L, D87N	S80I, E84V	Negative	<i>qnrB</i>	Negative	Negative	Negative
8	1 (2.9)	S83L, D87Y	S80I	Negative	Negative	Negative	Negative	Negative
8	2 (5.9)	S83L, D87N	S57T, S80I	Negative	Negative	Negative	Negative	Negative
8	2 (5.9)	S83L, D87N	S80I, E84G	Negative	Negative	Negative	Negative	Negative
8	10 (29.4)	S83L, D87N	S80I	Negative	Negative	Negative	Negative	Negative
8	15 (44.1)	S83L, D87N	S80I, E84V	Negative	Negative	Negative	Negative	Negative
	n=190	149 (78.4%)	65 (34.2%)	0	2 (1.1%)	0	4 (2.1%)	0

*qepA* polymerase chain reaction was only performed on resistant isolates (minimum inhibitory concentration [MIC] ≥2 µg/mL). – Not applicable; AA Amino acid

proportions were observed among *E coli* with reduced susceptible and resistant MICs. No other PMQR determinants were identified. Similar rates of *qnr* (1.1%) and *aac(3')Ib-cr* (3.2%) were detected among *E coli* and *Klebsiella* species from two sets of consecutive isolates collected

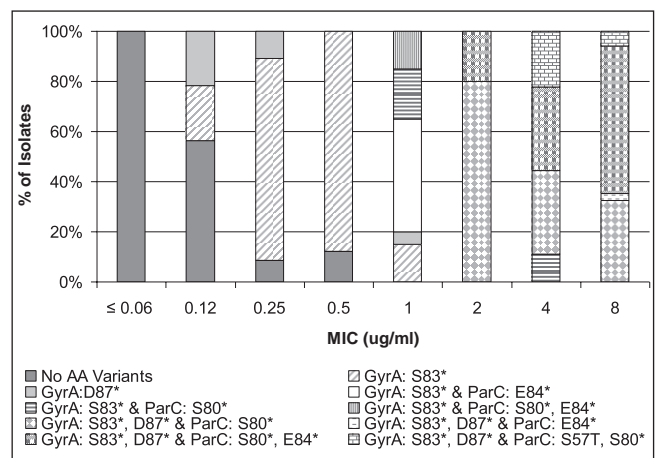
from 2004 to 2005 in Norway and Sweden, with resistance to nalidixic acid and/or reduced susceptibility/resistance to ciprofloxacin (17). It is not surprising that *aac(6')Ib-cr* was the most common PMQR determinant because two of the four isolates were ESBL producers,

and many studies have shown its predominance among other populations of *E coli*, especially among ESBL producers (9,14,17-19). With only a limited number of isolates harbouring PMQR determinants, it is difficult to link demographic data that may predict their presence (Table 2). However, because both *qnrB*-positive isolates were collected from the province of Ontario and isolated from blood, this provides increased awareness of the presence and potential spread among Ontario hospitals. The *aac(6')Ib-cr* harbouring isolates from the present study were obtained only from female patients, but were present across Canada. Thus, it appears that PMQR determinants do not have much of an impact on the increasing ciprofloxacin resistance among *E coli* in Canada because their prevalence remains low. Their presence, however, should be concerning because dissemination may occur and potentially fuel rapid and further increases in fluoroquinolone resistance among *E coli* (14). This was observed in the clinical setting when a fluoroquinolone-susceptible, *qnrA*-producing *E coli* developed chromosomal mutations in the QRDR of *gyrA* and *parC*, resulting in ensuing resistance after treatment with norfloxacin (12).

The primary mechanism of reduced susceptibility to ciprofloxacin in *E coli* was due to the stepwise accumulation of mutations in the QRDR of *gyrA*, followed by *parC* QRDR changes as previously reported (2,3,10,20). Disturbingly, 43.5% and 91.3% of *E coli* with ciprofloxacin MICs of 0.12 µg/mL and 0.25 µg/mL, respectively, demonstrated QRDR changes in *gyrA*, which is well below the CLSI ciprofloxacin intermediate and resistant breakpoints of 2 µg/mL and 4 µg/mL, respectively. In addition, the vast majority of *E coli* with ciprofloxacin MICs of 1 µg/mL and defined as susceptible by CLSI breakpoints demonstrated QRDR changes in both *gyrA* and *parC*. Consequently, the risk for development of high-level resistance to the fluoroquinolones is greatly underestimated by the current CLSI breakpoints, and they do not adequately detect these mutants.

There were a few limitations regarding the present study. We did not assess for reduced uptake of ciprofloxacin by measuring decreased expression of outer membrane porins, nor did we assess the presence of overexpression of efflux pumps (other than *qepA*). In particular, reduced uptake may play a role in the decreased susceptibility to ciprofloxacin in the one isolate harbouring *qnrB* with a ciprofloxacin MIC of 0.5 µg/mL, with no amino acid variants within the QRDR of *gyrA* and *parC*. However, from a clinical perspective, it is clear that the most common and important mechanisms conferring reduced susceptibility and resistance to ciprofloxacin among these isolates involved amino acid substitutions in the QRDR of *gyrA* and *parC*.

Clinically significant *E coli* isolates from the present study were isolated from blood, urine, wound and respiratory specimens. Isolates obtained from the urinary tract had ciprofloxacin MICs ranging from susceptible to resistant (≤0.06 µg/mL to 8 µg/mL). For the treatment of lower urinary tract infections, because the fluoroquinolones ciprofloxacin and levofloxacin attain very high concentrations in the urine (eg, approximately 300 µg/mL), these high concentrations may theoretically overcome *E coli* with reduced susceptibility or even resistance to fluoroquinolones (21). However, because fluoroquinolone MICs are known to be elevated in acidic urine (pH approximately 5.5 versus broth at approximately 7.2), and the concern that strains with a first-step QRDR mutation or PMQR determinants may rapidly develop resistance on therapy, we would not recommend fluoroquinolone therapy for urinary infections or any other infections due to *E coli* with



**Figure 1** *gyrA* and *parC* quinolone resistance-determining regions amino acid (AA) variants among *Escherichia coli* with increasing ciprofloxacin minimum inhibitory concentrations (MIC). \*Any AA variant

known fluoroquinolone-resistance determinants. In addition, no data are available describing whether once-daily dosing or twice-daily dosing of fluoroquinolones for treatment of such strains is the preferred dosage. Thus, treatment of these strains with fluoroquinolones is not recommended for fear of microbiological failure.

**CONCLUSION**

Reduced susceptibilities to ciprofloxacin in Canadian clinical isolates of *E coli* were primarily due to the accumulation of mutations in the QRDR of *gyrA* followed by *parC*. Although the prevalence of PMQR determinants among this cohort was low, their presence and the presence of *gyrA* and *parC* QRDR mutants among *E coli* with reduced susceptibility to ciprofloxacin has clinical implications and may fuel the development of high-level fluoroquinolone resistance, effectively eliminating the possibility of ciprofloxacin-based treatment of *E coli* infections. The present study demonstrates the need for increased monitoring of PMQR determinants among clinical isolates of *E coli* demonstrating susceptibility and resistance to fluoroquinolones to determine whether these determinants will continue to emerge among these isolates. There appears to be a need to reassess the current CLSI breakpoints because susceptible isolates with MICs that approach the susceptible breakpoint possess *gyrA* and *parC* resistance mechanisms, and continued use of fluoroquinolones to treat infections caused by these isolates may accelerate the development of resistance.

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**TABLE 2**  
**Demographic data of plasmid-mediated quinolone resistance (PMQR)-positive isolates**

Stock number	PMQR determinant	City, province	Location	Age, years	Sex	Source
74886	<i>qnrB</i>	Toronto, Ontario	ICU	74	Male	Blood
76265	<i>qnrB</i>	London, Ontario	Medicine general	62	Male	Blood
74912	<i>aac(3)Ib-cr</i>	Toronto, Ontario	Medicine general	63	Female	Urine
77206	<i>aac(3)Ib-cr</i>	Montreal, Quebec	Emergency room	66	Female	Wound
70720	<i>aac(3)Ib-cr</i>	Vancouver, British Columbia	Clinic	30	Female	Urine
75797	<i>aac(3)Ib-cr</i>	Montreal, Quebec	Medicine general	75	Female	Blood

ICU Intensive care unit

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