Discrepancies in fluoroquinolone clinical categories between the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI for Escherichia coli harbouring qnr genes and mutations in gyrA and parC

Jose M. Rodríguez-Martínez1*, Alejandra Briales1, Carmen Velasco1, Paula Díaz de Alba1, Luis Martínez-Martínez2,3 and Alvaro Pascual1,4

1Department of Microbiology, University of Seville, Seville, Spain; 2Service of Microbiology, University Hospital Marqués de Valdecilla-IFIMAV, Santander, Spain; 3Department of Molecular Biology, University of Cantabria, Santander, Spain; 4University Hospital Virgen Macarena, Seville, Spain

*Corresponding author. Tel: +34-954-55-28-63; Fax: +34-954-37-74-13; E-mail: jmrodriguez@us.es

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Sir,

Fluoroquinolones are broad-spectrum antibacterial agents commonly used in clinical practice. When quinolones became widely used, bacterial resistance to them emerged rapidly and, over the past three decades, resistance has continued to increase. In Gram-negative bacteria, quinoline resistance is due primarily to mutations in chromosomal genes encoding quinolone targets DNA gyrase and topoisomerase IV.1 More recently, plasmid-mediated mechanisms have been reported, such as those mediated by qnr genes encoding pentapeptide repeat proteins, aac(6′)-Ib-cr, encoding an acetyltransferase, and qepA or qexAB, encoding active efflux pumps.3

The CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria to define clinical breakpoints for fluoroquinolones in Enterobacteriaceae5,6 are indicated in Table 1. The EUCAST/European Medicines Agency has set somewhat lower breakpoints and those values have been accepted by the BSAC Resistance Surveillance Project.5 Although the epidemiological cut-off of ciprofloxacin for Escherichia coli has been established at 0.032 mg/L (www.eucast.org), the definition of wild-type strains is difficult due to the possible expression of unknown low-level quinoline resistance mechanisms.5

Plasmid-mediated quinoline resistance (PMQR) genes confer low levels of quinoline resistance, and their precise effect on selection for quinolone resistance in association with other mechanisms is not well known. Recently we have reported the influence of qnrA, qnrB and qnrS genes on the development of quinoline resistance in E. coli wild-type strains, and isogenic E. coli harbouring a Ser83Leu substitution in GyrA and/or a Ser80Arg substitution in ParC.7 Strains containing the combined substitutions—Ser83Leu in GyrA and Ser80Arg in ParC—in E. coli ATCC 25922 remained susceptible to fluoroquinolones, according to CLSI breakpoints. In contrast, the presence and expression of qnr genes increased the MIC of ciprofloxacin up to 2 mg/L, the intermediate susceptibility value according to CLSI guidelines (Table 1). However, in all cases the clinical category of every isogenic combination was susceptible or intermediately susceptible.

When we analysed these results using the EUCAST breakpoints we observed significant differences in terms of clinical category. E. coli containing qnr genes as the only mechanism of resistance were always susceptible to fluoroquinolones as was observed using CLSI breakpoints. Strains containing the substitution Ser83Leu in GyrA in E. coli ATCC 25922 remained susceptible to fluoroquinolones according to EUCAST breakpoints, while the derived strains expressing the qnrS1 gene were categorized as resistant to ciprofloxacin, moxifloxacin and norfloxacin, and those expressing qnrA1 or qnrB1 were categorized as resistant to norfloxacin, in contrast to what was observed using CLSI breakpoints (Table 1). Strains containing the combined substitutions—Ser83Leu in GyrA and Ser80Arg in ParC—in E. coli ATCC 25922 remained susceptible to fluoroquinolones, except norfloxacin, according to EUCAST breakpoints. In contrast, the presence and expression of qnr genes increased the MIC of fluoroquinolones to 1–8 mg/L, making most of these strains resistant to fluoroquinolones according to EUCAST breakpoints.6

It has been observed that Qnr proteins facilitate selection of higher-level quinoline-resistant mutants, despite which the therapeutic relevance of the acquisition of qnr genes to the bactericidal activity of fluoroquinolones remains unclear.8 In this context previous in vivo studies have shown that the presence of qnr genes in association with additional quinolone resistance mechanisms seems to be relevant for the in vivo activity of these antimicrobial agents.6 According to our results, 10 major errors (susceptible to resistant) and seven minor errors (intermediate to resistant) could be observed when we compare CLSI criteria against EUCAST criteria and these differences could be related to higher quinoline-resistant mutant frequency in strains harbouring PMQR genes.

In a recent study,8 the combined effect of topoisomerase mutations on fluoroquinolone resistance in isogenic E. coli C600 strains showed that at least three mutations—two of which had to be in gyrA—were necessary to exceed CLSI resistance.
Table 1. Comparison of fluoroquinolone MIC values (in mg/L) and clinical categories of bacterial strains according to CLSI and EUCAST guidelines

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant features</th>
<th>PMQR genes</th>
<th>CIP</th>
<th>LVX</th>
<th>MXF</th>
<th>NOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli ATCC 25922</td>
<td>wild-type</td>
<td>none</td>
<td>0.002/S/S</td>
<td>0.008/S/S</td>
<td>0.008/S/S</td>
<td>0.015/S/S</td>
</tr>
<tr>
<td>E. coli ATCC/qnrA</td>
<td>wild-type</td>
<td>pBK-QnrA1</td>
<td>0.125/S/S</td>
<td>0.5/S/S</td>
<td>0.25/S/S</td>
<td>0.5/S/S</td>
</tr>
<tr>
<td>E. coli ATCC/qnrB</td>
<td>wild-type</td>
<td>pBK-QnrB1</td>
<td>0.125/S/S</td>
<td>0.125/S/S</td>
<td>0.25/S/S</td>
<td>0.25/S/S</td>
</tr>
<tr>
<td>E. coli ATCC/qnrS</td>
<td>wild-type</td>
<td>pBK-QnrS1</td>
<td>0.125/S/S</td>
<td>0.5/S/S</td>
<td>0.25/S/S</td>
<td>0.25/S/S</td>
</tr>
<tr>
<td>E. coli ATCC 25922-S83L</td>
<td>GyrA Ser83Leu</td>
<td>none</td>
<td>0.125/S/S</td>
<td>0.125/S/S</td>
<td>0.06/S/S</td>
<td>0.125/S/S</td>
</tr>
<tr>
<td>E. coli ATCC-S83L/qnrA</td>
<td>GyrA Ser83Leu</td>
<td>pBK-QnrA1</td>
<td>0.5/S/S</td>
<td>0.5/S/S</td>
<td>0.5/S/S</td>
<td>2/S/R</td>
</tr>
<tr>
<td>E. coli ATCC-S83L/qnrB</td>
<td>GyrA Ser83Leu</td>
<td>pBK-QnrB1</td>
<td>0.5/S/S</td>
<td>0.25/S/S</td>
<td>0.5/S/S</td>
<td>1/S/R</td>
</tr>
<tr>
<td>E. coli ATCC-S83L/qnrS</td>
<td>GyrA Ser83Leu</td>
<td>pBK-QnrS1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli ATCC 25922-S83L-S80R</td>
<td>GyrA Ser83Leu, ParC Ser80Arg</td>
<td>none</td>
<td>0.25/S/S</td>
<td>0.25/S/S</td>
<td>0.25/S/S</td>
<td>2/S/R</td>
</tr>
<tr>
<td>E. coli ATCC-S83L-S80R/qnrB</td>
<td>GyrA Ser83Leu, ParC Ser80Arg</td>
<td>pBK-QnrB1</td>
<td>1/S/R</td>
<td>1/S/R</td>
<td>1/S/R</td>
<td>4/S/R</td>
</tr>
<tr>
<td>E. coli ATCC-S83L-S80R/qnrS</td>
<td>GyrA Ser83Leu, ParC Ser80Arg</td>
<td>pBK-QnrS1</td>
<td>2/I/R</td>
<td>4/I/R</td>
<td></td>
<td>2/I/R</td>
</tr>
</tbody>
</table>

*S*, susceptible; I, intermediately susceptible; R, resistant.

Comparison of MIC values and CLSI and EUCAST clinical categories, respectively. MICs (in mg/L) of ciprofloxacin (CIP), levofloxacin (LVX), moxifloxacin (MXF) and norfloxacin (NOR) were determined by microdilution. Since moxifloxacin breakpoints for Enterobacteriaceae are not indicated in CLSI guidelines, ciprofloxacin breakpoints were used. Category differences are indicated in bold.

Clinical breakpoints (mg/L) of fluoroquinolones for Enterobacteriaceae as proposed by CLSI and EUCAST are as follows. CLSI: CIP, susceptible ≤1 mg/L, intermediate = 2 mg/L and resistant ≥4 mg/L; LVX, susceptible ≤2 mg/L, intermediate = 4 mg/L and resistant ≥8 mg/L; and NOR, susceptible ≤0.5 mg/L, intermediate = 8 mg/L and resistant ≥16 mg/L (no breakpoints are defined for MXF). EUCAST: CIP, susceptible ≤0.5 mg/L and resistant ≥1 mg/L; LVX, susceptible ≤1 mg/L and resistant ≥2 mg/L; MXF, susceptible ≤0.5 mg/mL and resistant ≥1 mg/L; and NOR, susceptible ≤0.5 mg/L and resistant ≥1 mg/L.

breakpoints. Strains with two substitutions, one in GyrA and one in ParC, were susceptible to fluoroquinolones with MICs of ciprofloxacin of ≤0.5 mg/L. These data agree with our results and support that at least two mutations—one in gyrA and one in parC—in combination with qnr genes are necessary to exceed EUCAST resistance breakpoints.

Finally, we evaluated a set of isogenic *E. coli* strains harbouring different modifications at GyrA and/or ParC and expressing or not expressing qnr genes and we found significant differences in terms of clinical category when we compared CLSI and EUCAST breakpoints overall, in isolates that are on the borderline of resistance according to CLSI or EUCAST breakpoints and expressing qnr genes. Taking into account that the pharmacokinetic parameter AUC/MIC is the best predictor of quinolone efficacy *in vivo*, small changes in MICs due to qnr gene expression may play a significant role in therapeutic failure. Animal models are necessary to confirm the *in vitro* results.

Transparency declarations

None to declare.

References


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Nitazoxanide is active against *Clostridium difficile* strains with reduced susceptibility to metronidazole

Jane Freeman, Simon D. Baines, Sharie L. Todhunter, Grace S. Huscroft and Mark H. Wilcox*

Microbiology, University of Leeds and Leeds Teaching Hospitals, Old Medical School, Leeds General Infirmary, Leeds, LS1 3EX, UK

*Corresponding author. Microbiology, The General Infirmary, Old Medical School, Leeds LS1 3EX, UK. Tel: +44-113-3926818; Fax: +44-113-3435649; E-mail: mark.wilcox@leedsth.nhs.uk

**Keywords:** epidemic PCR ribotypes, new treatments, *C. difficile*

Sir,

Treatments strategies for *Clostridium difficile* infection (CDI) have changed little over the past two decades. Metronidazole and vancomycin continue to be the first-line antimicrobial treatments for CDI. While the drugs were thought to be similar in terms of response and recurrence rates, recent reports suggest that metronidazole efficacy may be reducing. A study of 52 patients by Al-Nassir et al. showed that while diarrhoea resolved in a majority of patients (>90%) treated with either metronidazole or vancomycin, the former was associated with a slower and less consistent microbiological response. In addition, poor gut levels of metronidazole and emerging evidence of reduced susceptibility in epidemic *C. difficile* strains, particularly among epidemic *C. difficile* PCR ribotypes, may play a role in reduced antibiotic efficacy. These issues highlight the need for effective new treatments for CDI.

Nitazoxanide is a 5-nitrothiazole compound showing good antimicrobial activity against a range of helminthic and protozoal parasites, as well as anaerobic bacteria. We evaluated nitazoxanide efficacy against a panel of 127 *C. difficile* strains isolated from patients in the Leeds Teaching Hospitals and CDI cases referred to the national *C. difficile* Ribotyping Network (CDRN). These included epidemic strains (PCR ribotypes 001, 027 and 106) with and without reduced susceptibility to metronidazole. The strains comprised 39 epidemic isolates (2005 and 2008) with reduced susceptibility to metronidazole (MIC 4–8 mg/L) (CD-RM), 57 epidemic isolates (2005 and 2008) susceptible to metronidazole (MIC ≤2 mg/L) and 31 isolates from the remainder of the top 10 most common *C. difficile* PCR ribotypes, including PCR ribotype 010 (non-toxigenic), which shows reduced metronidazole susceptibility. MICs were determined by agar incorporated method. Briefly, following culture in pre-reduced Schaedler’s anaerobic broth at 37°C for 48 h, strains were multipoint inoculated (10⁶ cfu/spot) onto Wilkins–Chalgren agar containing nitazoxanide (a gift from Romark) and cultured anaerobically for 48 h. *Bacteroides fragilis* NCTC 6343 and *Staphylococcus aureus* NCTC 6571 were used as control organisms.

In agreement with previous reports, we found nitazoxanide to be very active against all *C. difficile* isolates tested (MIC range 0.03–0.5 mg/L) (Table 1). Geometric mean MICs of nitazoxanide appeared to be unaffected by metronidazole susceptibility and there was little variation among CD-RM epidemic PCR ribotypes or between isolation dates. Nitazoxanide is moderately well absorbed, with 67% of the dose eliminated in faeces, mainly as the desacetyl-metabolite tizoxanide. Tizoxanide activity was not investigated in the present study, but previous studies have shown good activity against *C. difficile*. The promising in vitro activity of nitazoxanide has been borne out by recent reports of its clinical use in CDI treatment. A recent prospective, double-blind study of nitazoxanide and vancomycin in hospitalized patients with CDI found no difference in outcome, although patient numbers were small (27 received vancomycin and 23 received nitazoxanide).

In light of the recent emergence of reduced metronidazole susceptibility among epidemic *C. difficile* PCR ribotypes, the good activity (range 0.03–0.5 mg/L) of nitazoxanide against epidemic CD-RM is encouraging, and indicates that the mechanism underlying reduced metronidazole susceptibility does not affect the activity of nitazoxanide. The mechanism underlying reduced metronidazole susceptibility among *C. difficile* has not yet been elucidated. Both metronidazole and nitazoxanide are thought to affect the pyruvate ferredoxin oxidoreductase (PFOR)

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Table 1. Nitazoxanide susceptibility (mg/L) of *C. difficile* PCR ribotypes, including those with reduced metronidazole susceptibility (CD-RM)

<table>
<thead>
<tr>
<th>PCR ribotypes</th>
<th>2008 (n=24)</th>
<th>2005 (n=33)</th>
<th>CD-RM, 2005 and 2008 (n=39)</th>
<th>Remainder of the top 10 most common <em>C. difficile</em> PCR ribotypes (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC₅₀ (metronidazole MIC₅₀)</td>
<td>0.03 (2)</td>
<td>0.03 (2)</td>
<td>0.03 (4)</td>
<td>0.03 (0.5)</td>
</tr>
<tr>
<td>MIC₉₀</td>
<td>0.06</td>
<td>0.125</td>
<td>0.125</td>
<td>0.06</td>
</tr>
<tr>
<td>Range</td>
<td>0.03–0.125</td>
<td>0.03–0.25</td>
<td>0.03–0.25</td>
<td>0.03–0.5³</td>
</tr>
<tr>
<td>Geometric mean MIC</td>
<td>0.036</td>
<td>0.038</td>
<td>0.037</td>
<td>0.034</td>
</tr>
</tbody>
</table>

³Includes toxin A–B– C. difficile PCR ribotype 010 (metronidazole MIC=8–16 mg/L).—nitazoxanide MIC=0.5 mg/L.