Designing Fluoroquinolone Breakpoints for *Streptococcus pneumoniae* by Using Genetics instead of Pharmacokinetics-Pharmacodynamics

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We determined fluoroquinolone microbiological resistance breakpoints for *Streptococcus pneumoniae* by using genetic instead of pharmacokinetic-pharmacodynamic parameters. The proposed microbiological breakpoints define resistance as the MIC at which >50% of the isolates carry quinolone resistance-determining region mutations and/or, if data are available, when Monte Carlo simulations demonstrate a <90% chance of bacteriological eradication. The proposed microbiological resistant breakpoints are as follows (in micrograms per milliliter): gatifloxacin, >0.25; gemifloxacin, >0.03; levofloxacin, >1; and moxifloxacin, >0.12. Monte Carlo simulations of the once daily 400-mg doses of gatifloxacin and 750-mg doses levofloxacin demonstrated a high level of target attainment (free-drug area under the concentration-time curve from 0 to 24 h/MIC ratio of 30) by using these new genetically derived breakpoints.

Respiratory fluoroquinolones such as gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin are increasingly used in the empirical therapy of community-acquired respiratory infections likely caused by *Streptococcus pneumoniae*. The respiratory fluoroquinolones are an important advance in the treatment of these infections, as clinical trials have reported excellent bacteriological and clinical cures rates, including cures against penicillin-resistant, macrolide-resistant, and multiply antibiotic-resistant *S. pneumoniae* (18). However, it has recently been observed that many *S. pneumoniae* isolates that are defined as fluoroquinolone “susceptible” according to the National Committee for Clinical Laboratory Standards (NCCLS) breakpoints actually have resistance-causing mutations (9; H. Smith, K. Schurek, K. Nichol, A. Noreddin, D. J. Hoban, and G. G. Zhanel, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-157, 2003).

Fluoroquinolones inhibit DNA synthesis through interactions with the type II topoisomerases DNA gyrase and topoisomerase IV (5, 18). DNA gyrase and topoisomerase IV are heterotetramers composed of two A and two B subunits, encoded by *gyrA* *parC* and *gyrB* *parE*, respectively (5, 9, 17, 18). Fluoroquinolone resistance in *S. pneumoniae* is primarily mediated by spontaneous point mutations in the quinolone resistance-determining regions (QRDRs) of *gyrA* and/or *parC* (5, 9, 17, 18). Fluoroquinolone efflux-mediated resistance has also been documented, although the role of efflux in resistance remains unknown (9, 14, 18, 19).

The NCCLS designs fluoroquinolone breakpoints utilizing various factors including frequency distributions, clinical data,

and pharmacokinetic-pharmacodynamic properties, which incorporate the MIC, to determine the probability of bacteriological and clinical success, the detection of resistant populations, or both (9, 11, 12). Breakpoints may be subdivided into clinical breakpoints and microbiological breakpoints. Currently, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) defines clinical breakpoints and epidemiological cutoff values, whereas the NCCLS does not (7). Rather, the NCCLS focuses on clinical evidence as well as frequency distributions for setting clinical breakpoints. Clinical breakpoints are dependent on antimicrobial activity (MIC) as well as antimicrobial pharmacokinetics (i.e., pharmacodynamics). These breakpoints are derived in order to predict the probability of achieving bacteriological eradication from an infection site and ultimately achieving clinical success. Microbiologic breakpoints, on the other hand, are established to identify isolates that may be categorized as susceptible when applying clinical breakpoints but that harbor resistance mutations that have been associated with reduced susceptibility to that antimicrobial agent or antimicrobial class. Microbiologic breakpoints may thus be useful in monitoring the emergence of resistance, especially over time. Like the EUCAST epidemiology cutoff values, the microbiological breakpoints separate wild-type organisms, isolates with no acquired or mutational resistance mechanisms to the particular antimicrobial, and non-wild-type organisms, isolates with acquired or mutational resistance mechanism for the evaluated antimicrobial (7).

The aim of this study was to evaluate the use of genetic parameters to determine fluoroquinolone (gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin) microbiological breakpoints for *S. pneumoniae*. These microbiological breakpoints may serve to minimize mutant generation with the fluoroquinolones and reduce bacteriologic failures. We compared these microbiological breakpoints with the current NCCLS clinical breakpoints for gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin versus *S. pneumoniae*. The probability of bacterial eradication of gatifloxacin and levofloxacin was tested

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in parallel to minimizing mutant generation by the Monte Carlo simulation technique. At this time, Monte Carlo simulations cannot be conducted with gemifloxacin and moxifloxacin, as there are no established models and no population pharmacokinetic studies.

The *S. pneumoniae* clinical isolates investigated in this study were collected as part of an ongoing national respiratory organism surveillance program (the Canadian Respiratory Organism Susceptibility Study [CROSS]) (19). The isolates were obtained from 24 medical centers in 9 of the 10 Canadian provinces between 1997 and 2003 (19). Isolates were identified by using conventional methodology and were deemed to be significant respiratory pathogens by each laboratory's existing protocols.

MICs were determined by the NCCLS broth microdilution technique (13) after the isolates were subcultured twice from frozen stock, grown on blood agar, and incubated at 37°C in 5% CO₂ for 24 h (19). The antibiotics tested included ciprofloxacin, gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin. The susceptibility interpretive criteria for gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin were as described in NCCLS document M100-S12 (13), and ciprofloxacin non-susceptibility was defined as an MIC of ≥ 4 $\mu\text{g/ml}$. Strains *S. pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 29213 were used as controls for all MIC determinations. All MICs were determined a minimum of three times on separate days to ensure precision. These MICs are included in Table 1.

As part of CROSS, the QRDRs of *gyrA* and *parC* are sequenced for all ciprofloxacin-resistant *S. pneumoniae*. All ciprofloxacin-resistant isolates that are susceptible to the other fluoroquinolones studied were included in this study ($n = 40$). Additionally, 116 fluoroquinolone-susceptible *S. pneumoniae* isolates were randomly selected to encompass all years of CROSS (1997 to 2003) and all Canadian geographic regions. More isolates were selected from 2003 than other years, as it was hypothesized that mutations have likely become increasingly prevalent in recent years.

Primers previously described by Morrissey and George were used to generate PCR products of the QRDRs of *gyrA* and *parC* (10). Sequencing of the QRDRs was carried out with primers described by Morrissey and George in the forward and reverse directions (10). An ABI PRISM Big Dye Terminator kit and an ABI PRISM 310 genetic analyzer (PE Applied Biosystems, Mississauga, Ontario, Canada) were used to conduct the sequencing (20). A total of 156 isolates were selected based on the presence of mutations in the QRDRs of *GyrA* and *ParC*. Three groups of isolates were chosen to determine microbiological breakpoints: group 1, with no mutations in *ParC* or *GyrA*; group 2, with *ParC* mutations alone; and group 3, with mutations in both *ParC* and *GyrA*.

Monte Carlo simulation (3, 6, 16) was employed to estimate the probability of the once daily (OD) 400-mg doses of gatifloxacin and 500- and 750-mg doses of levofloxacin achieving free-drug area under the concentration-time curve from 0 to 24 h (AUC_{0-24})/MIC ratios with both clinical breakpoints and microbiological breakpoints for *S. pneumoniae*. Gatifloxacin and levofloxacin exposure (free-drug AUC_{0-24} /MIC) was derived from previously validated population pharmacokinetic models (1, 15). Variables from hospitalized patients with community-acquired pneumonia and MICs from a previous CROSS study (19) as well as the full variability of encountered

drug exposure were integrated via Monte Carlo simulation by using the Professional Crystal Ball 2000 program (Decisioneering UK, Ltd.). A 10,000-patient Monte Carlo simulation was performed to determine the percentage of patients achieving free-drug AUC_{0-24} /MIC ratios of 30, 40, 60, and 100 for levofloxacin as well as gatifloxacin dosing schemes evaluated against Canadian respiratory isolates of *S. pneumoniae* from the CROSS study.

The percentages of isolates with QRDR mutations at MICs considered susceptible by current NCCLS standards are presented in Table 1. For gatifloxacin-susceptible isolates ($\text{MIC} \leq 1$ $\mu\text{g/ml}$), 25% of the isolates ($n = 143$) had *ParC* mutations, 2% had *GyrA* mutations, 1% had mutations in both *ParC* and *GyrA*, and 72% had no QRDR mutations. For gemifloxacin-susceptible isolates ($\text{MIC} \leq 0.12$ $\mu\text{g/ml}$) ($n = 142$), 25% had *ParC* mutations, 2% had *GyrA* mutations, 1% had mutations in both *ParC* and *GyrA*, and 72% had no QRDR substitutions. According to the current NCCLS susceptibility category for levofloxacin ($\text{MIC} \leq 2$ $\mu\text{g/ml}$), 24% of the isolates ($n = 156$) had *ParC* mutations, 2% had *GyrA* mutations, 2% had both *ParC* and *GyrA* mutations, and 72% of the isolates had no QRDR mutations. For moxifloxacin-susceptible isolates ($\text{MIC} \leq 1$ $\mu\text{g/ml}$), 24% of the isolates ($n = 155$) had *ParC* mutations, 2% had *GyrA* mutations, 1% had mutations in both *ParC* and *GyrA*, and 73% had no QRDR mutations.

Interestingly, at a gatifloxacin MIC of ≤ 1 $\mu\text{g/ml}$ ($n = 105$), a gemifloxacin MIC of ≤ 0.12 $\mu\text{g/ml}$ ($n = 105$), a levofloxacin MIC of ≤ 2 $\mu\text{g/ml}$ ($n = 116$), and a moxifloxacin MIC of ≤ 1 $\mu\text{g/ml}$ ($n = 116$) (all susceptible by NCCLS breakpoints), as well as a ciprofloxacin MIC of ≤ 2 $\mu\text{g/ml}$ (susceptible), 9% of isolates had *ParC* QRDR mutations and 2% had QRDR mutations in *GyrA*. However, at a gatifloxacin MIC of ≤ 1 $\mu\text{g/ml}$ ($n = 38$), a gemifloxacin MIC of ≤ 0.12 $\mu\text{g/ml}$ ($n = 37$), a levofloxacin MIC of ≤ 2 $\mu\text{g/ml}$ ($n = 40$), and a moxifloxacin MIC of ≤ 1 $\mu\text{g/ml}$ ($n = 39$) (all susceptible by NCCLS breakpoints), as well as a ciprofloxacin MIC of ≥ 4 $\mu\text{g/ml}$ (resistant), 71, 70, 68, and 70% of isolates, respectively, have mutations in the QRDR of *ParC*, 3, 3, 3, and 3%, respectively, have mutations in the QRDR of *GyrA*, and 3, 5, 8, and 5%, respectively, have mutations in both *ParC* and *GyrA*.

Based on the high prevalence of QRDR mutations in isolates considered susceptible by current clinical breakpoints, we evaluated isolates with lower MICs and separated them into categories of few QRDR mutations ($<15\%$ of isolates), likely QRDR mutations, and very likely QRDR mutations ($>60\%$ of isolates) in order to establish microbiological breakpoints. These categories are presented in Table 2.

A total of 156 isolates were sequenced to evaluate the presence of QRDR mutations in the proposed microbiological breakpoint categories. The sequencing results are presented in Table 1. Based on the proposed few QRDR mutations category, 90, 94, 86, and 91% of the isolates had no QRDR mutations; 9, 6, 14, and 8% had *ParC* mutations; and 1, 0, 0, and 1% had *GyrA* mutations for gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin, respectively. In the proposed likely QRDR mutation category, 48, 61, 96, and 47% had no QRDR mutations; 48, 33, 3, and 49% had *ParC* mutations; 2, 4, 1, and 2% had *GyrA* mutations; and 2, 2, 0, and 2% had double mutations for gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin, respectively. In the proposed very likely QRDR mutations category, 20, 27, 36, and 25% had no

TABLE 1. Target site alterations and MIC in *S. pneumoniae* isolates

Isolate	Yr(s) isolated ^b	Geographic origin ^c	MIC (μg/ml) of ^d :					Mutation(s) present in:	
			Ciprofloxacin	Gatifloxacin	Gemifloxacin	Levofloxacin	Moxifloxacin	GyrA	ParC
ATCC 49619 ^a			1	0.25	0.03	1	0.12	None	None
ATCC 29213 (<i>Staphylococcus aureus</i>) ^a			0.5	≤0.06	0.03	≤0.25	≤0.06	None	None
3447	97–98	Victoria, BC	1	ND	ND	0.5	0.06	None	None
3979	97–98	Sherbrooke, QC	0.25	ND	ND	0.5	0.06	None	None
11438	98–99	Montreal, QC	0.5	ND	ND	0.5	0.06	None	None
11059	98–99	Hamilton, ON	1	ND	ND	0.5	0.06	None	None
18720	99–00	Ottawa, ON	0.25	0.06	0.015	0.5	0.06	None	Asn91Asp
19839	99–00	Montreal, QC	0.25	0.06	0.015	0.5	0.06	None	None
19840	99–00	Montreal, QC	0.5	0.12	0.015	0.5	0.06	None	None
21473	99–00	Calgary, AB	0.25	0.12	0.015	0.5	0.06	None	None
22623	99–00	Montreal, QC	0.5	0.12	0.015	0.5	0.06	None	None
23536	00–01	Winnipeg, MB	0.5	0.12	0.008	0.5	0.06	None	None
24091	00–01	Montreal, QC	1	0.12	0.015	0.5	0.06	None	None
26393	00–01	Sherbrooke, QC	0.5	0.12	0.008	0.5	0.06	None	None
28102	00–01	Toronto, ON	0.25	0.12	0.008	0.25	0.06	None	None
31003	01–02	Halifax, NS	0.5	0.12	0.008	0.5	0.06	None	None
31173	01–02	Charlottetown, PEI	0.5	0.12	0.008	0.5	0.06	None	Asp78Ala
31831	01–02	Ottawa, ON	0.5	0.12	0.008	0.5	0.06	None	None
32382	01–02	Regina, SK	0.5	0.12	0.008	0.5	0.06	None	None
32393	01–02	Regina, SK	0.5	0.12	0.008	0.5	0.06	None	None
32541	01–02	Montreal, QC	0.5	0.12	0.008	0.5	0.06	None	None
35181	01–02	Toronto, ON	0.5	0.12	0.008	0.5	0.06	None	Ser52Gly
43780	01–02	Hamilton, ON	0.5	0.12	0.008	0.5	0.06	None	None
45777	03	Vancouver, BC	0.5	0.25	0.008	0.5	0.12	None	None
3492	97–98	Regina, SK	1	ND	ND	1	0.12	None	None
3873	97–98	Halifax, NS	1	ND	ND	1	0.12	None	None
10158	98–99	London, ON	1	ND	ND	1	0.12	None	None
16539	99–00	Halifax, NS	1	0.25	0.015	1	0.12	None	None
17011	99–00	Victoria, BC	1	0.25	0.015	1	0.12	None	None
17723	99–00	Montreal, QC	2	0.25	0.03	1	0.12	None	None
18922	99–00	Moncton, NB	1	0.25	0.03	1	0.12	None	None
19519	99–00	Hamilton, ON	1	0.25	0.03	1	0.12	None	None
23063	00–01	Winnipeg, MB	1	0.25	0.015	1	0.12	None	None
23493	00–01	Winnipeg, MB	1	0.25	0.015	1	0.12	None	None
25506	00–01	Winnipeg, MB	1	0.25	0.03	1	0.12	None	None
27406	00–01	Edmonton, AB	1	0.25	0.03	1	0.12	None	None
27991	00–01	Calgary, AB	0.5	0.25	0.03	1	0.12	None	None
28364	00–01	Toronto, ON	1	0.25	0.03	1	0.12	None	None
29929	01–02	Montreal, QC	0.5	0.25	0.015	1	0.12	None	None
30478	01–02	Ottawa, ON	1	0.25	0.015	1	0.12	None	None
31318	01–02	Ottawa, ON	2	0.25	0.03	1	0.12	None	None
39727	01–02	London, ON	1	0.25	0.03	1	0.12	None	None
42745	01–02	Halifax, NS	1	0.25	0.03	1	0.12	Asp58Tyr	None
43805	01–02	Edmonton, ON	0.5	0.25	0.03	1	0.12	None	None
44443	03	Winnipeg, MB	1	0.25	0.015	1	0.12	None	None
44889	03	London, ON	2	0.5	0.03	1	0.12	None	None
45783	03	Vancouver, BC	1	0.5	0.03	1	0.25	None	None
45864	03	Moncton, NB	0.5	1	0.015	1	0.5	None	None
45685	03	Moncton, NB	0.5	1	0.015	1	0.5	None	None
45966	03	London, ON	1	0.25	0.015	1	0.12	None	None
46039	03	Hamilton, ON	1	0.25	0.015	1	0.25	None	None
46194	03	St. John, NB	1	0.25	0.015	1	0.12	None	None
46196	03	St. John, NB	1	0.25	0.015	1	0.12	None	None
46312	03	Montreal, QC	1	0.25	0.015	1	0.12	None	None
46658	03	Winnipeg, MB	1	0.25	0.015	1	0.12	None	None
46676	03	Calgary, AB	1	0.25	0.008	1	0.12	None	None
46679	03	Calgary, AB	0.5	0.25	0.015	1	0.12	None	None
46710	03	Calgary, AB	1	0.25	0.008	1	0.12	None	None
46899	03	Winnipeg, MB	1	0.25	0.015	1	0.12	None	None
47071	03	Edmonton, AB	0.5	0.25	0.008	1	0.12	None	None
47077	03	Edmonton, AB	1	0.25	0.008	1	0.12	None	None
47087	03	Edmonton, AB	1	0.25	0.008	1	0.12	None	None
47092	03	Sydney, NS	1	0.25	0.015	1	0.12	None	None
47130	03	Ottawa, ON	1	0.25	0.015	1	0.12	None	None
47131	03	Ottawa, ON	1	0.25	0.015	1	0.12	None	None
47137	03	Ottawa, ON	1	0.25	0.015	1	0.12	None	None
47292	03	Montreal, QC	0.5	0.25	0.015	1	0.12	None	None
47303	03	Montreal, QC	1	0.25	0.015	1	0.12	None	None
47380	03	Hamilton, ON	1	0.25	0.015	1	0.12	None	None
47528	03	Halifax, NS	1	0.25	0.015	1	0.12	None	None

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TABLE 1—*Continued*

Isolate	Yr(s) isolated ^b	Geographic origin ^c	MIC (µg/ml) of ^d :					Mutation(s) present in:	
			Ciprofloxacin	Gatifloxacin	Gemifloxacin	Levofloxacin	Moxifloxacin	GyrA	ParC
47531	03	Halifax, NS	1	0.25	0.015	1	0.12	None	None
47532	03	Halifax, NS	1	0.25	0.015	1	0.12	None	None
47533	03	Halifax, NS	1	0.25	0.015	1	0.12	None	None
47760	03	Hamilton, ON	1	0.25	0.03	1	0.12	None	None
47823	03	Montreal, QC	1	0.25	0.03	1	0.12	None	None
47824	03	Montreal, QC	1	0.25	0.015	1	0.12	None	None
47924	03	Victoria, BC	1	0.25	0.015	1	0.12	None	None
47925	03	Victoria, BC	1	0.25	0.015	1	0.12	None	None
47935	03	Victoria, BC	1	0.25	0.015	1	0.12	None	None
48107	03	Winnipeg, MB	1	0.25	0.015	1	0.12	None	None
48160	03	Saskatoon, SK	1	0.25	0.03	1	0.12	None	None
48163	03	Saskatoon, SK	1	0.25	0.03	1	0.12	None	None
48348	03	Toronto, ON	1	0.5	0.015	1	0.12	None	None
48350	03	Toronto, ON	1	0.25	0.03	1	0.12	None	None
48351	03	Toronto, ON	1	0.25	0.008	1	0.12	None	None
48353	03	Toronto, ON	1	0.25	0.015	1	0.12	None	None
48355	03	Toronto, ON	1	0.25	0.015	1	0.12	None	None
48356	03	Toronto, ON	1	0.25	0.03	1	0.25	None	None
48426	03	Vancouver, BC	2	0.25	0.03	1	0.25	None	None
48427	03	Vancouver, BC	1	0.25	0.015	1	0.12	None	None
48430	03	Vancouver, BC	1	0.5	0.03	1	0.25	None	None
48631	03	London, ON	1	0.25	0.015	1	0.12	None	None
48362	03	London, ON	0.5	0.25	0.03	1	0.12	None	None
48873	03	Montreal, QC	1	0.5	0.03	1	0.25	None	None
48944	03	Regina, SK	1	0.25	0.03	1	0.12	None	None
48949	03	Regina, SK	1	0.25	0.008	1	0.12	None	None
19103	99–00	Vancouver, BC	4	0.25	ND	1	0.12	None	None
27396	00–01	Edmonton, AB	4	0.5	0.12	1	0.5	None	Ser79Phe
28397	00–01	Toronto, ON	4	0.5	0.015	1	0.25	None	None
28669	00–01	Vancouver, BC	4	0.5	0.03	1	0.25	None	Ser107Tyr
801	97–98	Victoria, BC	2	ND	ND	2	0.25	None	Asp83Asn
4455	97–98	Montreal, QB	2	ND	ND	2	0.25	None	None
10250	98–99	Winnipeg, MB	2	ND	ND	2	0.25	None	None
12208	98–99	Calgary, AB	2	ND	ND	2	0.25	None	None
17484	99–00	Ottawa, ON	2	0.5	0.03	2	0.25	None	None
22784	99–00	Saskatoon, SK	2	0.5	0.015	2	0.25	None	None
24120	00–01	Winnipeg, MB	2	0.5	0.06	2	0.25	None	Ser79Phe
29098	00–01	Montreal, QC	2	0.5	0.03	2	0.25	None	Ser79Phe
29248	00–01	Halifax, NS	2	0.5	0.03	2	0.25	None	None
29317	00–01	Ottawa, ON	2	0.5	0.03	2	0.25	None	None
29377	00–01	London, ON	1	0.5	0.03	2	0.25	None	None
29403	00–01	Ottawa, ON	2	0.5	0.03	2	0.25	None	None
29453	00–01	Regina, SK	2	0.5	0.06	2	0.25	None	Leu30Phe, Tyr46Asp
29460	00–01	Regina, SK	2	0.5	0.06	2	0.25	None	None
29496	00–01	Hamilton, ON	1	0.5	0.03	2	0.25	None	None
29523	01–02	Winnipeg, MB	1	0.5	0.03	2	0.25	None	None
29644	01–02	Winnipeg, MB	2	0.5	0.03	2	0.25	None	None
30115	01–02	Winnipeg, MB	2	0.5	0.03	2	0.25	Gly54Cys	None
30462	01–02	Saskatoon, SK	2	0.5	0.03	2	0.25	None	Ser79Phe
32480	01–02	Montreal, QC	2	0.5	0.03	2	0.25	None	Glu135Asp
34860	01–02	St. John, NB	2	0.5	0.03	2	0.25	None	None
35599	01–02	Vancouver, BC	2	0.5	0.03	2	0.25	None	Ser52Gly, Asn91Asp
3104	97–98	Winnipeg, MB	4	0.5	0.03	2	0.25	None	Ser79Phe
4610	97–98	Montreal, QC	4	0.5	0.06	2	0.25	None	Ser79Phe Ser79Arg,
9286	97–98	Vancouver, BC	4	0.5	0.03	2	0.25	Ala17Thr,	Asn91Asp, Glu125Gln,
								Ser114Gly	Glu135Asp
18705	99–00	Hamilton, ON	4	0.5	0.06	2	0.25	None	Ser79Tyr
10277	98–99	Montreal, QC	4	1	0.12	2	0.5	None	Ser79Phe
11434	98–99	Montreal, QC	4	0.5	0.03	2	0.06	None	Ser79Phe
12070	98–99	Winnipeg, MB	4	1	0.06	2	0.25	None	Ser79Tyr
12547	98–99	Montreal, QC	4	0.5	0.03	2	0.12	None	Ser79Phe
12883	98–99	Moncton, NB	4	0.5	0.03	2	0.25	None	Ser79Phe
13817	98–99	Montreal, QC	4	0.5	0.06	2	0.25	None	Ser52Gly, Ser79Tyr,
									Asp83Ala, Asn91Asp
14744	98–99	Montreal, QC	4	0.5	0.06	2	0.25	None	Ser79Phe
12291	98–99	Montreal, QC	4	1	0.06	2	0.25	None	Ser79Tyr
12292	98–99	Montreal, QC	4	0.5	0.03	2	0.12	None	Ser79Phe
15017	98–99	Hamilton, ON	4	0.25	0.06	2	0.12	None	None
1282	97–98	Calgary, AB	4	0.5	0.06	2	0.25	None	None
12873	98–99	Moncton, NB	4	1	0.06	2	0.25	None	Ser79Phe
16072	99–00	Winnipeg, MB	4	0.5	0.06	2	0.12	None	None

Continued on following page

TABLE 1—Continued

Isolate	Yr(s) isolated ^b	Geographic origin ^c	MIC (μ g/ml) of ^d :					Mutation(s) present in:	
			Ciprofloxacin	Gatifloxacin	Gemifloxacin	Levofloxacin	Moxifloxacin	GyrA	ParC
22203	99–00	Vancouver, BC	4	0.5	0.03	2	0.25	None	None
22627	99–00	Montreal, QC	4	0.25	0.015	2	0.25	None	Asn53Asp, Asp56His, Lys57Gln, Asp83Gly
22668	99–00	Hamilton, ON	4	0.5	0.03	2	0.25	None	Ser79Tyr
23448	00–01	London, ON	4	0.5	0.06	2	0.25	None	Ser79Phe
25074	00–01	Edmonton, AB	4	0.5	0.03	2	0.25	None	Ser79Phe
27908	00–01	St. John, NB	4	0.5	0.03	2	0.25	None	Asp83Asn
27917	00–01	St. John, NB	4	0.5	0.03	2	0.25	None	Asp83Asn
28368	00–01	Toronto, ON	4	0.5	0.015	2	0.25	None	None
29012	00–01	Moncton, NB	4	0.5	0.06	2	0.25	None	None
29245	00–01	Halifax, NS	4	1	0.06	2	0.5	Glu85Lys	None
29262	00–01	Halifax, NS	4	0.5	0.06	2	0.25	None	None
14769	98–99	Montreal, QC	8	0.25	0.03	2	0.25	None	Ser79Phe
17913	99–00	Hamilton, ON	8	0.5	0.06	2	0.25	None	Asp83Gly
20336	99–00	Regina, SK	8	0.5	0.03	2	0.25	None	Asp83Ala
22360	99–00	Regina, SK	8	0.25	0.03	2	0.25	None	Asp83Ala
24086	00–01	Montreal, QC	8	1	0.03	2	0.5	None	Tyr59Asp
27224	00–01	Sherbrooke, QC	8	2	ND	2	2	Ser81Tyr	Ser79Phe
19120	99–00	Vancouver, BC	16	0.25	0.25	2	0.12	None	Asp83Asn
26608	00–01	Saskatoon, SK	16	4	0.12	2	0.5	Ser81Phe	Ser79Phe

^a Control isolates not incorporated in percent resistance calculations.^b Last two numbers of each year are shown.^c BC, British Columbia; QC, Quebec; ON, Ontario; AB, Alberta; MB, Manitoba; NS, Nova Scotia; PEI, Prince Edward Island; SK, Saskatchewan; NB, New Brunswick.^d ND, not determined.

QRDR mutations; 50, 64, 55, and 38% had ParC mutations; 10, 5, 3, and 13% had GyrA mutations; and 20, 5, 5, and 25% had double mutations for gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin, respectively.

The probabilities of 500- and 750-mg levofloxacin and 400-mg OD achieving free-drug AUC_{24}/MIC ratios by using current NCCLS and new microbiological breakpoint categories for *S. pneumoniae* are shown in Table 3. Levofloxacin 500- and 750-mg-dose OD probabilities of achieving a free-drug AUC_{24}/MIC ratio of 30 when using current NCCLS breakpoint categories were as follows: for susceptible or few QRDR mutations, 93.0 and 97.9%; for intermediate or likely QRDR mutations, 1.9 and 11.0%; and for resistant or very likely QRDR mutations, 0.0 and 0.0%, respectively. The levofloxacin 500- and 750-mg-dose OD probabilities of achieving a free-drug AUC_{24}/MIC ratio of 30 with new microbiological breakpoint categories were as follows: susceptible or few QRDR mutations, 99.6 and 97.9%; intermediate or likely QRDR mutations, 92.5 and 97.5%; resistant or very likely QRDR mutations, 40.0 and 71.5%. For gatifloxacin 400-mg-dose OD, the probabilities of achieving a free-drug AUC_{24}/MIC ratio of 30 using current NCCLS breakpoint categories were as follows: susceptible or few QRDR mutations, 98.8%; intermediate or

likely QRDR mutations, 12.1%; and resistant or very likely QRDR mutations, 0.0%. The probabilities of achieving a free-drug AUC_{24}/MIC ratio of 30 with new microbiological breakpoint categories were as follows: susceptible or few QRDR mutations, 99.6%; intermediate or likely QRDR mutations, 98.9%; resistant or very likely QRDR mutations, 67.9%.

Monte Carlo simulations showed that OD 500- and 750-mg doses of levofloxacin and a 400-mg dose of gatifloxacin have high probabilities of eradicating *S. pneumoniae* isolates that are classified as few QRDR mutations or likely QRDR mutations by using our new microbiological breakpoints. Although the probability of gatifloxacin achieving a free-drug AUC_{24}/MIC ratio of 30 for isolates with a gatifloxacin MIC of 0.5 μ g/ml was 98.9%, we chose to classify isolates with gatifloxacin MICs of 0.5 μ g/ml as resistant because the frequency of QRDR mutations in these isolates was 52% (Table 2). For isolates that are very likely to harbor QRDR mutations in ParC and/or are likely to have mutations in both ParC and GyrA, none of the OD 400-mg dose of gatifloxacin and 500- and 750-mg doses of levofloxacin demonstrated acceptable probability for bacterial eradication (71.5, 40.0, and 67.9%, respectively).

TABLE 2. Current pharmacokinetic-pharmacodynamic breakpoints and proposed microbiological resistance breakpoint for fluoroquinolones and *S. pneumoniae*^a

Fluoroquinolone	Current PK-PD breakpoints	MIC ₉₀ (μ g/ml) for isolates separated into categories of (% of isolates with MT)			Microbiological resistant breakpoint
		Few QRDR MT	Likely QRDR MT	Very likely QRDR MT	
Gatifloxacin	1, 2, 4	≤ 0.25 (10)	0.5 (52)	≥ 1 (80)	> 0.25
Gemifloxacin	0.12, 0.25, 0.5	≤ 0.015 (6)	0.03 (39)	≥ 0.06 (73)	> 0.03
Levofloxacin	2, 4, 8	≤ 0.5 (14)	1 (4)	≥ 2 (64)	> 1
Moxifloxacin	1, 2, 4	≤ 0.12 (9)	0.25 (53)	≥ 0.5 (75)	> 0.12

^a PK-PD, pharmacokinetic-pharmacodynamic; MT, mutations; MIC₉₀, MIC at which 90% of the isolates are inhibited.

TABLE 3. Probability of OD 500- and 750-mg doses of levofloxacin and a 400-mg dose of gatifloxacin of achieving free-drug AUC₀₋₂₄/MIC ratios using current NCCLS and microbiological categories for *S. pneumoniae*

Fluoroquinolone and free-drug AUC ₀₋₂₄ /MIC ratio (dose in mg)	% of isolates for which current NCCLS breakpoint MICs (μg/ml) were:			% of isolates for which new microbiological breakpoint category MICs (μg/ml) were:		
	≤2	4	≥8	≤0.5	1	≥2
Levofloxacin						
30 (500)	93.0	1.9	0.0	97.9	92.5	40.0
30 (750)	97.9	11.0	0.0	99.6	97.5	71.5
40 (500)	82.3	0.0	0.0	98.7	83.2	16.9
40 (750)	95.0	5.5	0.0	99.4	94.7	48.9
60 (500)	57.3	0.0	0.0	92.8	57.4	2.7
60 (750)	83.5	0.0	0.0	99.0	83.4	8.1
100 (500)	24.6	0.0	0.0	55.1	7.6	0.0
100 (750)	49.4	0.0	0.0	75.7	35.8	0.0
Gatifloxacin						
30 (400)	98.8	12.1	0.0	99.6	98.9	67.9
40 (400)	96.8	0.0	0.0	99.5	97.8	52.3
60 (400)	89.2	0.0	0.0	98.9	91.3	8.6
100 (400)	52.5	0.0	0.0	95.8	37.0	0.0

The currently used NCCLS breakpoints for fluoroquinolones and *S. pneumoniae* define many isolates as susceptible even though they harbor QRDR mutations. Based on the likelihood of QRDR mutations and, for gatifloxacin and levofloxacin, the probability of bacteriological eradication as determined by Monte Carlo analysis, we propose a microbiological resistance breakpoint. Our proposed microbiological resistance breakpoint is the MIC at which >50% of the isolates carry QRDR mutations and/or, when data are available, when Monte Carlo simulations demonstrate a <90% chance of bacteriological eradication. The proposed microbiological resistance breakpoints are as follows (in micrograms per milliliter): gatifloxacin, >0.25; gemifloxacin, >0.03; levofloxacin, >1, and moxifloxacin, >0.12.

The recent occurrence of treatment failures resulting from the use of levofloxacin in the treatment of community-acquired pneumonia caused by susceptible *S. pneumoniae* isolates that harbored QRDR mutations (4, 8) has led to the need to re-evaluate current breakpoints. It has previously been demonstrated that secondary mutations are acquired much more rapidly than first-step mutations, resulting in highly resistant isolates (2) which have led to the observed treatment failures. As Lim et al. have recently suggested, emerging resistance patterns cannot be detected based on clinical breakpoints that are unable to identify first-step mutations (9). Thus, it is clinically important that we develop rapid identification methods for QRDR mutations to avoid treating an *S. pneumoniae* isolate carrying a first-step mutation with a fluoroquinolone in order to limit the development and propagation of highly resistant isolates. The MICs of numerous quinolones should be considered prior to fluoroquinolone treatment, as we see a much larger percentage of gatifloxacin-, gemifloxacin-, levofloxacin-, and moxifloxacin-susceptible isolates harboring mutations when they are ciprofloxacin resistant. We do not expect or recommend that the microbiological resistance breakpoint be used in clinical practice. The intent of this research is to create awareness of the potential for fluoroquinolone resistance propagation in *S. pneumoniae*, as many reportedly sus-

ceptible isolates carry resistance mutations and high-level resistance results from the sequential acquisition of mutations.

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