

Comparison of minimal inhibitory and mutant prevention drug concentrations of 4 fluoroquinolones against clinical isolates of methicillin-susceptible and -resistant *Staphylococcus aureus*

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Abstract

Staphylococcus aureus remains an important human pathogen affecting both outpatients and those hospitalized. Increasing antimicrobial resistance is global but prevalence rates are variable for different geographical areas. Fluoroquinolones have been used to treat *S. aureus* infections and the newer quinolones have enhanced in vitro activity against this organism. The mutant prevention concentration (MPC) defines the antimicrobial drug concentration threshold that would require an organism to simultaneously possess two mutations for growth in the presence of the drug. We tested clinical isolates of methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) *S. aureus* by minimum inhibitory concentration (MIC) and MPC against gatifloxacin, gemifloxacin, levofloxacin and moxifloxacin. For MSSA strains, the rank order of potency based on MIC₉₀ values were gemifloxacin (0.063 mg/l) = moxifloxacin (0.063 mg/l) > gatifloxacin (0.05 mg/l) = levofloxacin (0.25 mg/l) and by MPC values moxifloxacin (0.25 mg/l) > gemifloxacin (0.5 mg/l) > gatifloxacin (1 mg/l) = levofloxacin (1 mg/l). For 87% of the isolates the MPC value was 0.5 mg/l for gatifloxacin. The rank order of potency based on the time the serum drug concentration exceeded the MPC₉₀, was as follows: moxifloxacin (>24 h) > levofloxacin (>18 h) > gatifloxacin (12 h) > gemifloxacin (9 h). Serum drug concentration remained in excess of the MPC₃₇ for 24 h for gatifloxacin. Both MIC₉₀ and MPC₉₀ values were higher against MRSA strains and the time above the MPC₉₀ was significantly shorter for all agents.

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1. Introduction

Staphylococcus aureus ranks among the most commonly recovered human pathogens from both community and hospital acquired infections. β -lactams have been the major therapeutic agents for decades. In β -lactam allergic patients, macrolides, tetracycline or vancomycin have been used depending on the site of infection and disease severity. The introduction of the fluoroquinolones in the latter part of the 1980s provided yet another class of agents that was poten-

tially useful for treating *S. aureus* infections. Ciprofloxacin was the first fluoroquinolone approved for treating systemic infections including those caused by *S. aureus*. Unfortunately, *S. aureus* resistance to ciprofloxacin readily occurred [1–3] and in some surveys of hospital-acquired infections, 90% or more of *S. aureus* isolates were ciprofloxacin resistant [1,4]. Nevertheless, many community-acquired isolates recovered from various infections remained susceptible to the compound [5–8]. Methicillin-resistance has compromised the use of the β -lactam class [9] and the recent descriptions of isolates with reduced susceptibility to vancomycin [10,11] or resistance [12] is frightening. The impact of these resistance trends on compounds such as teicoplanin, quinupristin/dalfopristin [13] and linezolid [14] must be monitored carefully so as to document emerg-

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ing resistance and the potential impact on the use of these various compounds for patient therapy. Coresistance between methicillin-resistance strains and fluoroquinolones (i.e. ciprofloxacin) is well known [15], however, in many countries and/or geographical areas, the prevalence of MRSA remains low or less than 20%. For example, in the USA, prevalence rates for MRSA have been reported to be >50% in intensive care units, 30–35% on the general hospital ward and 14–15% from outpatient isolates [16,17]. In contrast, MRSA rates in Canada remain lower than those from the USA [9,18]. From European hospitals (1–4 participating hospitals per country) participating in the Sentry surveillance program (1997–1998), the prevalence of MRSA strains ranged from 2 to 9% in the Netherlands, Switzerland, Germany and Austria, 19 to 28% in Spain, France, Poland, Belgium and the United Kingdom and 34 to 54% in Greece, Turkey, Italy and Portugal [15,19]. Of the European isolates of MRSA, 89.5% were also ciprofloxacin resistant whereas only 8.4% of MSSA strains were resistant to ciprofloxacin [20]. The new fluoroquinolones (gatifloxacin, gemifloxacin, moxifloxacin) have been reported to have enhanced activity against Gram-positive pathogens when compared with older fluoroquinolones [21,22]. We were interested in determining whether differences in MIC values between the compounds parallel differences in mutant prevention concentrations (MPC) [23–25]—a novel measurement for comparing antimicrobial potency based on the likelihood of selecting for resistance when the compounds are exposed to a larger inoculum of bacteria. By definition, MPC can be defined as the antimicrobial drug concentration threshold that would require an organism simultaneously to possess two resistance mutations to grow in the presence of the drug. The MPC may also be defined as the drug concentration required to block the growth of the most resistant first-step resistant mutant(s) present in a heterogeneous bacterial population. The measurement of MPC applies to organisms deemed to be susceptible to the antimicrobial agent by standardized susceptibility testing utilizing an inoculum of 10^5 cfu/ml—such as testing recommended by the National Committee for Clinical Laboratory Standards [26]. For MPC testing, approximately 10^{10} cells are applied to agar plates containing drug and following incubation are screened for growth. The high inoculum is needed to detect first-step resistant mutants that occur at frequencies of 1×10^{-7} to 1×10^{-8} or lower [27].

2. Method

The clinical isolates tested in this study were collected through the Department of Clinical Microbiology, Royal University Hospital, Saskatoon, Saskatchewan, Canada. No pre-selection criteria was used to favour the MIC distribution to any of the fluoroquinolones tested. Duplicate isolates from the same patient were excluded. Organisms were identified by reference methodologies [28].

2.1. Fluoroquinolones

Sources of fluoroquinolones were: Bayer AG (moxifloxacin), Bristol-Myers Squibb (gatifloxacin), Glaxo-Smith Kline (gemifloxacin), Janssen-Ortho (levofloxacin). All compounds were prepared in accordance with manufacturer's specifications/instructions.

2.2. Susceptibility determination

Minimal inhibitory concentrations were determined by microbroth dilution with interpretation in accordance with NCCLS guidelines [26]. Briefly, Mueller–Hinton broth containing two-fold concentration increments of antimicrobial agents was added to 96-well microdilution trays. Test organism suspension equal to a 0.5 McFarland standard was further diluted and added to the trays to achieve a final inoculum of 5×10^5 cfu/ml. Inoculated plates were incubated for 18–24 h at 35 °C in ambient air. For microbroth dilution, the minimal inhibitory concentration was recorded as the lowest dilution showing no growth.

Staphylococcus aureus isolates were screened for susceptibility to methicillin by plating on to Mueller–Hinton agar plates containing 6 µg/ml of oxacillin.

2.3. Nucleotide sequence analysis

Recovered colonies were subsequently screened for the *mecA* gene using an in house polymerase chain reaction assay utilizing the following primers: *mec-1*: 5'-GGG ATC ATA GCG TCA TTA TTC-3' and *mec-2*: 5'-AAC GAT TGT GAC ACG ATA GCC-3'.

2.4. MPC measurements

The method for measuring MPC is a modification of that previously described [24]. Briefly, 2–3 tryptic soy agar with 5% sheep blood plates per organism were inoculated to produce confluent growth and then incubated overnight (18–24 h at 35–37 °C in ambient air). The next day, the contents of the plates were transferred to 100 ml of Mueller–Hinton broth and incubated overnight as described. The following day a total of 100 µl containing 10^{10} cfu was inoculated to tryptic soy agar plates containing antimicrobial agent. Drug containing plates were prepared in-house and used within seven days of preparation. A range of seven drug concentrations were inoculated with the lowest drug concentration being the MIC. Inoculated plates were incubated for 24 h as described and then examined for growth. Plates were re-incubated as described for an additional 24 h and then re-examined. Colonies growing on plates containing drug at concentrations exceeding the susceptibility breakpoint or ≥ 3 doubling dilutions above the MIC were subcultured to tryptic soy agar plates containing the same drug concentration as the plate they were recovered from. Plates that had a film (making it difficult to identify indi-

vidual colonies) were re-streaked onto a fresh drug plate, re-incubated overnight and screen for colonies the following morning. This confirmed the presence or absence of organism and the MPC value. The MPC was recorded as the lowest concentration that prevented the growth of any colonies.

3. Results

A total of 122 methicillin-susceptible and 22 of methicillin-resistant clinical isolates of *S. aureus* were tested against gatifloxacin, gemifloxacin, levofloxacin and moxifloxacin. The distribution of MIC and MPC values for the methicillin-susceptible strains are summarized in Table 1. Table 2 shows the MIC and corresponding MPC values for 22 MRSA strains against the four fluoroquinolones tested. The calculation of MIC_{50/90}, MPC_{50/90}, time above MPC measurements and other pharmacological related calculations are summarised in Table 3.

By microbroth dilution testing of methicillin-susceptible strains, MIC₉₀ values for gemifloxacin and moxifloxacin were 0.063 mg/l (mode = 0.031 mg/l) compared with 0.25 mg/l for gatifloxacin (mode = 0.063 mg/l) and levofloxacin (mode = 0.25 mg/l). A total of four isolates had MICs >2 mg/l for gatifloxacin as compared with two for gemifloxacin, two for levofloxacin and one for moxifloxacin. By MPC (Table 1), the rank order of potency was moxifloxacin (mode 0.125 mg/l; MPC₉₀ 0.25 mg/l) > gemifloxacin (mode = 0.125 mg/l; MPC₉₀ 0.5 mg/l) > gatifloxacin (mode = 0.25 mg/l; MPC₉₀ 1 mg/l) = levofloxacin (mode 0.5 mg/l; MPC₉₀ 1 mg/l). Eight isolates had MPCs >2 mg/l against gatifloxacin compared with five for gemifloxacin, nine for levofloxacin and three for moxifloxacin.

The calculation of the MPC₉₀ or MIC₉₀ values is additive starting from the lowest MIC or MPC value to the highest. As such, a single or a few strains may change the MIC or MPC₉₀ percent value by one doubling drug dilution. Such was the case with this study. For gatifloxacin, the drug concentration 0.25 mg/l inhibited 86–94% of the isolates tested such that the MIC₈₅ was 0.125 mg/l. For gemifloxacin, 0.063 inhibited 70–93% of isolates and the MIC₆₉ would be 0.031 mg/l.

For levofloxacin 0.25 mg/l inhibited 45–93% of isolates and the MIC₄₄ would be 0.125 mg/l. Finally, for moxifloxacin 0.063 mg/l inhibited 65–93% of isolates such that the MIC₆₄ would be 0.03 mg/l.

By MPC testing, a drug concentration of 1 mg/l inhibited 89–92% of isolates and the MPC₈₈ was 0.5 mg/l for gatifloxacin. Similarly, the drug concentration of 0.5 mg/l inhibited 90–93% of isolates tested to gemifloxacin and the MPC₈₉ was 0.125 mg/l. For levofloxacin, 1 mg/l inhibited 79–91% of isolates and the MPC₇₉ was 0.5 mg/l. Finally, for moxifloxacin, 0.25 mg/l inhibited 75–91% of isolates and the MPC₇₄ was 0.125 mg/l.

A summary of the MIC and MPC values for the 22 MRSA strains is shown in Table 2. Also, the calculations of MIC_{50/90}, MPC_{50/90} and various pharmacological calculations with the MRSA strains are shown in Table 3. A total of seven isolates had MICs to gatifloxacin ≥8 mg/l, 7 at 4 mg/l and the remainder (eight isolates) at ≤0.25 mg/l. For gemifloxacin, three isolates had MICs ≥8 mg/l, five at 4 mg/l, six at 2 mg/l and the remainder (eight) at ≤0.016 mg/l. Finally, 13 isolates had MICs to levofloxacin ≥8 mg/l and the remainder (nine) at 0.5 mg/l. Only one isolate had an MIC to moxifloxacin at 8 mg/l, two at 4 mg/l and the remainder were at 2 mg/l (7 isolates) or less (12 isolates)—nine with MICs ≤0.25 mg/l.

The MPC values against MRSA strains were considerably higher than MIC values against all four agents: 13 isolates had MPCs ≥16 mg/l to gatifloxacin and nine were ≤0.25 mg/l; for gemifloxacin, 14 isolates had MPCs ≥16 mg/l (three at ≥256 mg/l) and the remainder were at ≤0.125 mg/l; for levofloxacin 15 isolates had MPCs ≥8 mg/l (14 at ≥64 mg/l) and the remainder (seven) at ≤0.5 mg/l; for moxifloxacin, 14 isolates had MPCs ≥8 mg/l (three at 16 mg/l) and the remainder (eight) were at ≤1 mg/l (seven at ≤0.25 mg/l). Organisms with elevated MPCs to one agent had elevated MPCs to all four compounds and vice versa.

We have previously argued that the time the drug concentration remains in excess of the MPC may be important for restricting the development of resistance [25,29]. Based on published data for the various fluoroquinolones showing maximum serum concentration and subsequent declining values over the duration of conventional dosing (Table 3),

Table 1

The MIC and MPC distribution of 122 clinical isolates of methicillin-susceptible *S. aureus* against four fluoroquinolones

Fluoroquinolone	MIC (mg/l) values									
	<0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	≥8
Gatifloxacin		18	56	30	11	3		2	2	
Gemifloxacin	37	47	29	5	1	1			2	
Levofloxacin			10	44	59	4	3		2	
Moxifloxacin	34	44	36	4	2		1	1		
Fluoroquinolone	MPC (mg/l) values									
				24	61	22	5	5	1	4
			24	66	16	4	4	1	4	
					3	93	16	2	7	1
			2	89	20	3	5	1	2	

Table 2
Comparative MIC and MPC values of 22 methicillin-resistant strains of *Staphylococcus aureus* against four fluoroquinolones

Organism no.	Gatifloxacin		Gemifloxacin		Levofloxacin		Moxifloxacin	
	MIC	MPC	MIC	MPC	MIC	MPC	MIC	MPC
11	0.031	0.25	<0.008	0.063	0.125	0.5	0.016	0.125
12	4	16	2	16	8	64	1	8
13	4	16	2	16	8	64	1	8
14	4	16	2	32	8	64	2	8
15	0.25	0.25	<0.008	0.125	0.125	0.5	0.031	0.125
16	>8	32	4	32	16	64	2	8
17	0.063	0.25	<0.008	0.125	0.5	0.5	0.016	0.25
18	4	16	4	16	16	64	0.016	8
19	8	32	8	512	>16	128	0.016	16
36	4	16	2	32	8	64	2	8
37	0.063	0.25	<0.008	0.125	0.063	0.5	0.016	0.125
38	0.063	0.25	0.016	0.125	0.125	8	0.016	1
40	8	16	4	32	8	64	2	8
41	0.063	0.25	<0.008	0.125	0.125	0.5	0.125	0.25
42	8	32	>8	512	>16	128	4	16
43	8	16	2	16	8	64	2	8
44	0.063	0.25	<0.008	0.063	0.125	0.5	0.25	0.125
45	0.25	0.25	<0.008	0.063	0.25	0.5	0.031	0.031
46	8	0.25	>8	256	>16	128	8	16
47	8	32	4	32	8	64	4	8
49	4	16	2	16	8	64	2	8
50	4	16	4	16	0.16	64	2	8

Table 3
Fluoroquinolone activity with clinical isolates of *Staphylococcus aureus*

	Gatifloxacin ^a		Gemifloxacin ^b		Levofloxacin ^c		Moxifloxacin ^d									
	MSSA		MRSA		MSSA		MRSA		MSSA		MRSA					
MIC ₅₀ ^e (mg/l)	0.063		4		0.031		2		0.25		8		0.031		1	
MIC ₉₀ ^e (mg/l)	0.25		8		0.063		8		0.25		>16		0.063		4	
MPC ₅₀ ^f (mg/l)	0.25		16		0.125		16		0.5		8		0.125		8	
MPC ₉₀ ^f (mg/l)	1		32		0.5		256		1		128		0.25		16	
C _{max} ^g (mg/l)	4.2		4.2		1.6		1.6		5.7		5.7		4.5		4.5	
Half-life (h) ^g	8–10		8–10		7–8		7–8		5–7		5–7		12–14		12–14	
Time (h) >MIC ₅₀ ^g	>24		~2		>24		0		>24		0		>24		>24	
Time (h) >MIC ₉₀ ^g	>24		0		~24		0		>24		0		>24		~3	
Time (h) >MPC ₅₀ ^g	>24		0		~18		0		~24		0		>24		0	
Time (h) >MPC ₉₀ ^g	~12		0		~9		0		~18		0		>24		0	
AUC _{0–24} (mg/l)	51.3		51.3		8.4		8.4		48		48		47.5		47.5	
	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free
AUC _{0–24} /MIC ₅₀ ^g	814.3	651.4	12.8	10.2	270.9	108.4	4.2	1.7	192	142.1	6	4.4	1532.3	965.4	47.5	29.9
AUC _{0–24} /MIC ₉₀ ^g	205.2	164.2	6.4	5.1	133.3	53.3	1.1	0.4	192	142.1	3	2.2	753.9	474.9	11.9	7.5
AUC _{0–24} /MPC ₅₀ ^g	205.2	164.2	3.2	2.6	67.2	26.9	0.5	0.2	96	71	0.8	0.6	380	239.4	5.9	3.7
AUC _{0–24} /MPC ₉₀ ^g	51.3	41	1.6	1.3	16.8	6.7	0.03	0.01	48	35.5	0.4	0.3	190	119.7	2.9	1.8
C _{max} /MIC ₅₀ ^g	66.7	53.4	1.1	0.9	51.6	20.6	0.8	0.3	22.8	16.9	0.7	0.5	145.2	91.5	4.5	2.8
C _{max} /MIC ₉₀ ^g	16.8	13.4	0.5	0.4	25.4	10.2	0.2	0.08	22.8	16.9	0.4	0.3	71.4	44.9	1.1	0.69
C _{max} /MPC ₅₀ ^g	16.8	13.4	0.3	0.2	12.8	5.1	0.1	0.04	11.4	8.4	0.09	0.07	36	22.7	0.6	0.38
C _{max} /MPC ₉₀ ^g	4.2	3.4	0.1	0.08	3.2	1.3	0.006	0.002	5.7	4.2	0.04	0.03	18	11.3	0.3	0.19

^a 20% protein binding [42].

^b 60% protein binding [43].

^c 26% protein binding [44].

^d 37% protein binding [45].

^e MIC₅₀;MIC₉₀ drug concentration (μg/ml) at which 50 and 90% respectively of isolates are inhibited (mg/l).

^f MPC₅₀;MPC₉₀ drug concentration (μg/ml) at which no mutants were recovered from 50 and 90% of isolates respectively. Centration (mg/l).

^g Table from Hansen et al. [25]

we estimated that the time (T) the serum drug concentration remains above the MIC_{90} and MPC_{90} for the various quinolones against MSSA strains resulted in a rank order of potency as follows respectively: moxifloxacin $T > MIC_{90} > 24$ h and $T > MPC_{90} > 24 >$ gatifloxacin (>24 and ~ 12 h respectively) $>$ levofloxacin (>24 and ~ 18 h, respectively) $>$ gemifloxacin (~ 24 and ~ 9 h respectively). The MPC_{88} for gatifloxacin was calculated to be 0.5 mg/l and as such $T > MPC_{88}$ would be approximately 24 h. The MPC_{89} for gemifloxacin was calculated to be 0.125 mg/l and $T > MPC_{89}$ would be approximately 14 h. For levofloxacin, extended time above MPC would be possible for 78% of the isolates when $T > MPC_{78}$ would be approximately 24 h.

4. Discussion

The MPC measurement has previously been applied to clinical isolates of *Streptococcus pneumoniae* [25,30] and *Pseudomonas aeruginosa* [31,32] and we were interested in determining the MPC of four fluoroquinolones against methicillin-susceptible and methicillin-resistant strains of *S. aureus*. The newer fluoroquinolones (gatifloxacin, gemifloxacin, moxifloxacin) have been reported [21,22] to have enhanced (lower MIC values) activity against Gram-positive pathogens than do the other agents (ciprofloxacin, levofloxacin) and numerous investigations [33,34] have confirmed these observations reporting MIC_{90} values of 0.063–0.5 mg/l for the newer agents (gatifloxacin, gemifloxacin, moxifloxacin) and 0.25–2 mg/l for the older compounds (ciprofloxacin, levofloxacin) against methicillin-susceptible strains.

It remains uncertain as to how much or what can be done to affect pathogenic bacteria once they have become resistant to a particular antimicrobial agent. For example, Gillespie et al. [35] recently reported on the relative fitness of drug resistant mutants of *S. pneumoniae* when compared with the wild-type susceptible parental strain. They found that the mutants did not appear to be disadvantageous relative to wild-type, based on growth and suggested their ability to survive in a heterogeneous nutritional sufficient environment. How similar experiments would relate to *S. aureus* is unknown, however, the emergence and dissemination of methicillin and quinolone resistant strains clearly suggests an ability of these mutant strains to survive. Hence, the best approach of dealing with resistant pathogens appears dependent on strategies that prevent the emergence of resistance. The mutant prevention concentration is one such approach [36]. In essence, the MPC defines the drug concentration threshold that prevents first-step resistant cells from growing out of what appears to be a susceptible bacterial population. Clearly, preventing the selective amplification of resistant cells is a desirable goal. For MPC to be useful, the drug concentration required to prevent the growth of first-step resistant mutants must be achievable and sustainable at dosages that do not involve intolerable side effects that would either

harm the patient and/or result in premature discontinuation of therapy with that drug. In some instances, combination therapy may be essential—not necessarily to affect clinical outcome but to prevent resistance emergence. In other instances, combination therapy may be necessary to affect both clinical outcome and prevent resistance. The mutant selection window has been defined as the drug concentration between the MIC and the MPC drug concentrations and it appears to be central to the selective amplification of resistant bacterial subpopulations [37]. For drug concentrations that fall below the MIC value, selective amplification of resistant cells is unlikely to occur as both susceptible and resistant cells can replicate as the drug concentration is insufficient to prevent growth of the bacterial population. When the drug concentration is at or in excess of the MPC value, both wild-type susceptible and first-step resistant cells are inhibited from growth. However, when the drug concentration falls within the mutant selection window—between the MIC and MPC drug concentrations—wild-type susceptible cells are inhibited while first-step resistant cells may proliferate. Dosing to achieve, exceed and maintain drug concentration in excess of the MPC value restricts the selective amplification of resistant bacterial subpopulations present in what appears to be a susceptible bacterial population.

The existence of the mutant selective window for *S. aureus* and quinolones was recently reported by Firsov et al. [38]. They reported data using an in vitro pharmacological model. From studies with ciprofloxacin, gatifloxacin, levofloxacin and moxifloxacin, significant increases in MICs were observed with three-day treatments at drug concentrations that fell inside the mutant selection window. Losses in susceptibility were not observed when drug concentrations were below the MIC or above the MPC values. The observations of Firsov et al. [38] appear to provide clear data that dosing to exceed the MPC is essential to prevent the selective amplification of resistance in the presence of fluoroquinolones. As quinolones are concentration dependent bacteriocidal agents, how much time does the drug concentration need to exceed the MPC value?

To our knowledge, kill studies based on MPC parameters and *S. aureus* have yet to be published. However, such studies have been published for *S. pneumoniae* [29]. Blondeau et al. compared the killing of *S. pneumoniae* by gemifloxacin, levofloxacin and moxifloxacin using the MIC, MPC and C_{max} drug concentrations against bacterial inoculum ranging from 10^6 to 10^9 cfu/ml. When 10^9 cfu/ml was exposed to the MIC drug concentration, there was a 68% reduction in viable cells (by 4 h) following exposure to gemifloxacin, 39% for levofloxacin and 76% for moxifloxacin. A $\geq 99.99\%$ reduction in viable cells was seen for all agents by 24 h following drug exposure. At the MPC drug concentration, reduction in viable cells (by 4 h) was as follows: 78, 97, 94 and $\geq 99.99\%$ respectively by 24 h for all three drugs. Finally, following exposure to the C_{max} drug concentration, reduction in viable cells (by 4 h) was as follows: 72, 76, 94 and $\geq 99.78\%$ respectively by 24 h for all three drugs. These

in vitro kill experiments suggest that maintaining drug concentration above the MPC values for extended durations is necessary to cause a high reduction (kill) in viable cells. The limitation of these and any kill experiments relate to the fact that drug elimination over time does not occur in a test tube as it does in humans and that these measurements are in the absence of immune interactions. These in vitro experiments suggest some interesting observations that require in vivo confirmation. Similar kill studies involving *S. aureus* and based on the MPC principles may show similar observations to those for *S. pneumoniae*.

Schentag et al. [39] summarized data showing that certain pharmacological measurements have been associated with clinical response and prevention of resistance. These values included the AUC (area under the drug concentration curve)/MIC ratio or AUIC (area under the inhibitory curve) and the C_{\max}/mic ratio. For AUIC, a value of >125 was desirable as was a C_{\max}/mic of 8–10. Others have argued that for Gram-positive organisms, an AUIC of 30–50 was sufficient [40,41]. The MPC data suggests that minimization of drug concentrations based on MIC measurements predisposes for the selective amplification of first-step resistant mutants in the presence of fluoroquinolones. If, indeed, the higher AUIC and C_{\max}/mic values are desirable, then the rank order of potency for the four agents tested in this study would be as follows for MPC₉₀ calculations: AUIC moxifloxacin > gatifloxacin > levofloxacin > gemifloxacin. For the MPC₉₀ calculation based on C_{\max}/MPC_{90} is moxifloxacin > levofloxacin > gatifloxacin > gemifloxacin. While studies have been completed based on these calculations in reference to MIC values, no such research has yet to be completed for the MPC values. It does, however, appear reasonable that similar calculations will extend to MPC measurements. Until such time, it remains speculative as to the significance of these calculations with the agents reported in this study. Perhaps a more thought provoking question might be that should such calculations be considered now and in the absence of completed studies to try and minimize the further selection of antimicrobial resistance. It would be unfortunate to have such data ignored, resistance increase and the calculation subsequently shown to be valuable predictors. Other studies have reported either C_{\max} or AUC data to be higher on lower than some of the values used in this report. Clearly, use of other values will give different AUIC or C_{\max}/MIC ratios than calculated in this study (summarized in Dalhoff and Schmitz) [46].

The MPC is a novel concept for comparing various fluoroquinolone compounds for their likelihood of selecting for antimicrobial resistance based on conventional dosing and clinically achievable drug concentrations. Despite the observation of clinical equivalency between these compounds for clinical indications that have been studied, pharmacological and microbiological measurements indicate that these compounds are not all equivalent. The limitation of the data to date relates to the fact that the data has been generated in vitro. The subsequent addition of in vivo data will help

clarify the in vitro observations. Despite these limitations, the data presented in this report suggests that the fluoroquinolone compounds can be differentiated based on MIC, MPC and various pharmacological calculations against clinical isolates of *S. aureus*. Preferential use of the most active compounds based on the above parameters (i.e. best in class – reference) may prolong the rate at which resistance emerges [47].

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