Activity of and Resistance to Moxifloxacin in *Staphylococcus aureus*

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Moxifloxacin has enhanced potency against *Staphylococcus aureus*, lower propensity to select for resistant mutants, and higher bactericidal activity against highly resistant strains than ciprofloxacin. Despite similar activity against purified *S. aureus* topoisomerase IV and DNA gyrase, it selects for topoisomerase IV mutants, making topoisomerase IV the preferred target in vivo.

8-Methoxyquinolones have been associated with increased bacteriostatic and bactericidal efficacy and the prevention of emergence of mutant strains in several bacteria, including *Staphylococcus aureus* (4, 5, 12, 23, 33). Moxifloxacin is a new 8-methoxyquinolone with enhanced activity against gram-positive cocci, including ciprofloxacin-resistant *S. aureus*, with MICs 4- to 64-fold less than that of ciprofloxacin (2, 9, 15, 16, 21, 22, 30, 31). Although single mutations in topoisomerase IV also contribute to resistance, moxifloxacin preferentially selects for gyrase mutants in *Streptococcus pneumoniae* (10, 14), suggesting dual targeting of DNA gyrase and topoisomerase IV in *S. pneumoniae* (29, 32). In this study, we undertook to evaluate the primary target of moxifloxacin by selection of resistant mutants as well as inhibition of the DNA supercoiling activity of DNA gyrase and the decatenation activity of topoisomerase IV. The list of strains and plasmids used in the study are shown in Table 1.

Activity of moxifloxacin against genetically defined mutants of *S. aureus*. MICs of moxifloxacin and ciprofloxacin against genetically defined mutants of *S. aureus* determined by agar dilution on trypticase soy agar supplemented with twofold-increasing concentrations of the antibiotics are shown in Table 2. Moxifloxacin was four- to eightfold more active against wild-type *S. aureus* ISP794 than ciprofloxacin. A single *gyrA* mutation caused at most a twofold increase in the MICs of each quinolone, whereas a single mutation in either subunit of topoisomerase IV caused a four- to eightfold increase in MICs, suggesting topoisomerase IV to be the primary target of moxifloxacin in *S. aureus*. A double mutation in gyrase and topoisomerase IV caused a 125-fold increase in the MICs of both quinolones, carrying the MIC of moxifloxacin to the proposed breakpoint of resistance of ≥ 4 $\mu\text{g/ml}$ for staphylococci (1), although it remained below the achievable peak serum drug level of 4.73 $\mu\text{g/ml}$ with an 800-mg dose of moxifloxacin (25). Thus, although moxifloxacin is more active than ciprofloxacin against mutants of *S. aureus*, it probably will not be effective against established strains with multiple resistance mutations. Overexpression of the NorA efflux pump caused at most a twofold increase in the MIC of moxifloxacin, consistent with

previously published results (20), probably due to the hydrophobicity of the 8-methoxy group as well as the bulkiness of the C-7 group (19, 27).

Bactericidal effects of moxifloxacin and ciprofloxacin against *S. aureus*. The bactericidal effects of moxifloxacin and ciprofloxacin at 4 times their respective MICs were determined by performance of time-kill curves as described previously against a wild-type *S. aureus* strain, ISP794, and its *grlA* and *grlA gyrA* mutants (18). Colony counts were determined immediately before the addition of the antibiotics and hourly for 5 h. Kill curves with each drug at 4 times the MIC showed similar effects for both the wild-type strain (Fig. 1A) and *grlA* mutant (Fig. 1B). The killing effect of ciprofloxacin appeared more pronounced (1-log difference) for ISP794 at the drug concentrations used (1.0 $\mu\text{g/ml}$ for ciprofloxacin and 0.128 $\mu\text{g/ml}$ for moxifloxacin), perhaps due to the internal variation of MIC results (Fig. 1A). However, a 3-log decrease in colony counts for the *grlA gyrA* mutant was observed with moxifloxacin, whereas ciprofloxacin produced no killing (Fig. 1C). The concentration used for this double mutant (16 $\mu\text{g/ml}$), however, was above the achievable serum drug concentrations, and at simulated peak concentrations (3 $\mu\text{g/ml}$) moxifloxacin has been reported to lack bactericidal activity against highly resistant *S. aureus* isolates (6).

Frequency of selection of resistant mutants. Frequencies of a single-step mutation to resistance were determined by plating *S. aureus* ISP794 cells on brain heart infusion agar containing either moxifloxacin or ciprofloxacin at concentrations of 2, 4, and 8 times the MIC. The experiment was repeated three times, with plating of a maximum of 4.5×10^{11} CFU. Mutation frequencies were calculated as the ratio of the number of resistant colonies at 48 h to the number of CFU inoculated. At 2 times the MIC of each quinolone, frequencies of selection of resistant mutants were similar (Table 3). However, at 4 times the MIC, mutants could rarely be selected with moxifloxacin, and the frequency of selection of resistant mutants was 3 orders of magnitude less than that of ciprofloxacin at 4 times the MIC. Mutants could not be selected at higher concentrations of moxifloxacin.

Characterization of single-step mutants. The MICs of moxifloxacin and ciprofloxacin increased two- to eightfold for three of the four single-step mutants studied (Table 2). MICs of nalidixic acid (used to screen for *gyrA* mutations), novobiocin (used to screen for selected *grlB* or *grlA* mutations [8]), and ethidium bromide (used to screen for NorA overexpression [17])

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype ^a	Source or reference
<i>S. aureus</i> strains		
ISP794	8325 <i>pig-131</i>	24
MT5	8325 <i>nov (gyrB142) hisG15 pig-131</i>	7, 28
SS1	8325 <i>pig-131 gyrB142 (Ile102Ser, Arg144Lys) gyrA (Ser84Leu)</i>	3
MT5224c2	8325 <i>nov (gyrB142) hisG15 pig-131 grlA552</i>	18
MT5224c4	8325 <i>nov (gyrB142) hisG15 pig-131 grlA542 (Ser80Phe)</i>	28
MT5224c9	8325 <i>nov (gyrB142) hisG15 pig-131 grlB543 (Asn470Asp)</i>	28
MT23142	8325 <i>pig-131 flqB</i> Ω (chr::Tn916)1108	18
EN1252a	8325 <i>nov (gyrB142) hisG15 pig-131 grlA542 gyrA</i> Ω 1051 (Erm) Nov ⁺	3
M2	ISP794 <i>grlA559 (Ala176Gly)</i>	This study; spontaneous Mox ^r
M3	ISP794 (unidentified mutation)	This study; spontaneous Mox ^r
M4	ISP794 <i>grlA570 (Arg393Ser)</i>	This study; spontaneous Mox ^r
M6	ISP794 <i>grlA571 (Glu194Gly)</i>	This study; spontaneous Mox ^r
M8	ISP794 <i>grlA (Arg17His) (Glu87Lys) gyrA (Ser84Leu)</i>	This study; serial passage mutant
M11	ISP794 <i>gyrA (Ser84Leu)</i> , additional unidentified mutations	This study; serial passage mutant
<i>E. coli</i> strains		
DH5 α	F ⁻ ϕ 80dlacZ Δ M15 Δ (lacZYA-argF)U169 <i>deoR recA1 endA1 phoA hsdR17 (r_K⁻ m_K⁻) supE44λ⁻ thi-1 gyrA96 relA1</i>	GIBCO-BRL
Plasmid		
pTrcHisA, -B, and -C	4.4-kb expression vector, Ap ^r	Invitrogen

^a Abbreviations: Mox^r, moxifloxacin resistance; Ap^r, ampicillin resistance.

showed at most a twofold change (data not shown). The similar increase in the MICs of moxifloxacin and ciprofloxacin suggested that a mutation in topoisomerase IV was responsible for resistance, and direct sequencing of PCR products for the entirety of *grlBA* and the quinolone resistance-determining regions (QRDRs) of *gyrBA* performed using automated ABI 3100 DNA sequencers (Tufts Core Facility, Boston, Mass.) revealed two novel mutations outside the QRDR of *grlB* (Glu194Gly and Arg393Ser). Although genetic experiments were not performed to prove the role of these mutations in resistance, the absence of any other mutation in the entirety of *grlBA* suggested that these

mutations were responsible for the resistance phenotype. The third mutant had a mutation in *grlA* (Ala176Gly), which we have previously selected with gatifloxacin (12). We have also previously, by allelic exchange experiments, demonstrated that another amino acid substitution at the same position in GrlA (Ala176Thr) is responsible for resistance (11). A fourth single-step mutant (M3) with a two- to fourfold increase in the MIC of moxifloxacin and at most a twofold increase in that of ciprofloxacin did not have any mutation in the entirety of *grlBA* and *gyrBA*. The MIC of ethidium bromide was also not increased for this strain, suggesting no increase in the expression of the efflux pump NorA. Se-

TABLE 2. Activity of moxifloxacin and ciprofloxacin against *S. aureus*

Strain	Mutation ^a	MIC (μg/ml)	
		Moxifloxacin	Ciprofloxacin
Genetically defined mutants of <i>S. aureus</i>			
ISP794	Wild type (parent)	0.032	0.125–0.25
MT5	<i>gyrB142</i> (Ile102Ser, Arg144Ile)	0.064	0.25–0.5
SS1	<i>gyrA</i> (Ser84Leu)	0.064	0.25
MT5224c2	<i>grlA</i> (Ala116Pro)	0.125–0.25	1.0
MT5224c4	<i>grlA</i> (Ser80Phe)	0.125–0.25	1.0
MT5224c9	<i>grlB</i> (Asn470Asp)	0.125–0.25	1.0
MT23142	<i>flqB</i> (NorA overexpression)	0.064	0.5–1.0
EN1252a	<i>grlA</i> (Ser80Phe) <i>gyrA</i> (Ser84Leu)	4.0	16–32
Single-step mutants selected in this study			
M2	<i>grlA</i> (Ala176Gly)	0.25	1.0
M3	Unidentified mutation	0.125	0.25
M4	<i>grlB</i> (Arg393Ser)	0.25	1.0
M6	<i>grlB</i> (Glu194Gly)	0.125–0.25	0.5
Serial-passage mutants selected in this study			
M8	<i>grlA</i> (Arg17His, Glu87Lys) <i>gyrA</i> (Ser84Leu)	1.0	2.0
M11	<i>gyrA</i> (Ser84Leu) and unidentified mutation(s)	4.0	32

^a The indicated mutations in *grlA* in single-step or serial-passage mutants resulted from the following nucleotide changes: Arg17His, CGC→CAC; Glu84Lys, GAA→AAA, Ala176Gly, GCG→GGG. The indicated mutations in *grlB* or *gyrB* in single-step or serial-passage mutants resulted from the following nucleotide changes: Glu194Gly in *grlB*, GAG→GGG Arg393Ser in *grlB*, CGT→AGT, and Ser84Leu in *gyrA*, TCA→TTA.

FIG. 1. Bactericidal activities of moxifloxacin and ciprofloxacin. Normalized viable counts of *S. aureus* strains in the presence of the indicated concentrations of moxifloxacin ($\triangle$) and ciprofloxacin ($\square$) or with no drug ($\diamond$) are shown. (A) ISP794, wild-type *S. aureus* (*grlA*⁺ *gyrA*⁺), at a ciprofloxacin concentration of 1.0 μ g/ml and moxifloxacin concentration of 0.128 μ g/ml; (B) MT5224c4, *grlA* (Ser80Phe), at a ciprofloxacin concentration of 4.0 μ g/ml and moxifloxacin concentration of 0.5 μ g/ml; (C) EN1252a, *grlA* (Ser80Phe) *gyrA* (Ser84Leu), at a ciprofloxacin concentration of 64 μ g/ml and moxifloxacin concentration of 16 μ g/ml. $\diamond$, no antibiotic; $\triangle$, 4 times the MIC of moxifloxacin; $\square$, 4 times the MIC of ciprofloxacin.

FIG. 1—Continued.

lection of mutants without topoisomerase mutations and without evidence of increased efflux at low selecting concentrations of moxifloxacin has also been reported for *S. pneumoniae* (10, 14).

Selection and characterization of serial passage mutants. Serial passage of ISP794 on brain heart infusion agar containing twofold-increasing concentrations of moxifloxacin yielded mutants with up to a 64- to 128-fold increase in the MIC (4.0 µg/ml). The MICs of moxifloxacin and ciprofloxacin were increased 16- to 32-fold and 8- to 16-fold, respectively, for mutant M8, the most resistant mutant in one series, and 64- to 128-fold and 128- to 256-fold for mutant M11, the most resistant mutant in a second independent series (Table 2). The MICs of nalidixic acid, novobiocin, and ethidium bromide showed at most a twofold change (data not shown). Direct sequencing of the PCR products for the QRDRs of *grlBA* revealed a novel mutation (Arg17His) and a commonly encountered mutation (Glu87Lys) in *grlA*, and sequencing of the QRDRs of *gyrBA* revealed a common mutation in *gyrA* (Ser84Leu) (Table 2).

Activities of moxifloxacin and ciprofloxacin against purified

topoisomerase IV and gyrase. The cloning of *grlA*, *grlB*, *gyrA*, and *gyrB* into expression vectors pTrcHisA, -B, or -C, purification to >90% homogeneity, and reconstitution of histidine-tagged topoisomerase IV and DNA gyrase from wild-type *S. aureus* ISP794 and topoisomerase IV from the *GrlA* mutant (Ser80Phe) as well as the specific activities of the recombinant proteins and the assay conditions for kinetoplast (kDNA) decatenation and DNA supercoiling assays have been reported previously (13). The drug concentrations of moxifloxacin and ciprofloxacin causing 50% inhibition of kDNA decatenation by topoisomerase IV (IC₅₀), estimated visually, were similar or twofold lower for moxifloxacin (1.25 to 2.5 µg/ml and 2.5 to 5.0 µg/ml, respectively) (Table 4; Fig. 2A), and the IC₅₀ for the *GrlA* mutant increased similarly for both quinolones (up to 100 to 200 µg/ml and 250 µg/ml, respectively) (Fig. 2B). The drug concentration causing 50% inhibition of DNA supercoiling by DNA gyrase (IC₅₀) was two- to fourfold lower for moxifloxacin than for ciprofloxacin (2.5 to 5.0 and 10 µg/ml, respectively), suggesting a higher activity of moxifloxacin

TABLE 3. Frequency of selection of resistant ISP794 mutants

Selecting drug concn (µg/ml)	Factor of MIC		Frequency of selection of mutants	
	Moxi-floxacin	Cipro-floxacin	Moxifloxacin	Ciprofloxacin
0.064	2		4.2×10^{-6}	
0.125	4		4.5×10^{-11}	
0.25	8		$<4.5 \times 10^{-11}$	
0.5		2		2.8×10^{-6} – 1.5×10^{-5}
1.0		4		3.0×10^{-8} – 6.1×10^{-8}
2.0		8		2.8×10^{-9} – $<4.5 \times 10^{-11}$

TABLE 4. Inhibitory effects of moxifloxacin and ciprofloxacin on wild-type and mutant (*GrlA*) topoisomerase IV and wild-type gyrase

Enzyme	Subunit				IC ₅₀ (µg/ml)	
	GrlA	GrlB	GyrA	GyrB	Moxi-floxacin	Cipro-floxacin
Topoisomerase IV	Wild type	Wild type			1.25–2.5	2.5–5.0
Topoisomerase IV	Phe80	Wild type			100–200	250
Gyrase			Wild type	Wild type	2.5–5.0	10

FIG. 2. Activity of moxifloxacin and ciprofloxacin against purified topoisomerase IV and DNA gyrase. (A) Decatenation of kDNA by wild-type GrlA and GrlB in the presence of moxifloxacin and ciprofloxacin. Assays were performed as described in the text. M, minicircles. (B) Decatenation of kDNA by GrlA (Ser80Phe) and wild-type GrlB in the presence of moxifloxacin and ciprofloxacin. (C) DNA supercoiling activity of gyrase in the presence of moxifloxacin and ciprofloxacin. R and S indicate relaxed and supercoiled DNA, respectively.

against gyrase than that of ciprofloxacin (Fig. 2C). Under the assay conditions used in this study, which were different for the two assays and possibly different from the conditions in the bacterium, inhibition of topoisomerase IV and DNA gyrase by moxi-

floxacin was similar, if not slightly better, for topoisomerase IV, suggesting similar targeting of both enzymes.

Despite similar inhibition of topoisomerase IV and DNA gyrase by moxifloxacin and ciprofloxacin, moxifloxacin was four- to eightfold more potent against *S. aureus* than ciprofloxacin. This discrepancy might be due to the slightly better targeting of both topoisomerase IV (at most twofold) and DNA gyrase (two- to fourfold) by moxifloxacin in comparison to that of ciprofloxacin, as well as to moxifloxacin not being a good substrate for NorA and possibly other efflux pumps. Moxifloxacin also exerts a greater bactericidal activity against *S. aureus* and its mutants than ciprofloxacin and selects for resistant mutants less frequently. Previously, Takei et al. have also suggested that moxifloxacin is a dual topoisomerase targeting quinolone, based on MIC ratios for topoisomerase IV and DNA gyrase mutants and inhibition of the two target enzymes (26). Our results of in vitro activity of moxifloxacin against reconstituted *S. aureus* topoisomerase IV and DNA gyrase are similar to the data of Takei et al.; however, in our genetically defined mutants, we found a higher increment in the MIC of moxifloxacin in topoisomerase IV mutants than in a gyrase mutant. This finding, together with the initial selection of topoisomerase IV, suggest that moxifloxacin, similar to other 8-methoxyquinolones, preferentially targets topoisomerase IV in vivo in *S. aureus*. Finally, studies with newer quinolones have made localization of target gene mutations to the QRDRs much less consistent, implying more complex interactions among topoisomerases, DNA, and quinolones.

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REFERENCES

- Balfour, J. A., and L. R. Wiseman. 1999. Moxifloxacin. *Drugs* 57:363–373.
- Bauernfeind, A. 1997. Comparison of the antibacterial activities of the quinolones BAY 12–8039, gatifloxacin (AM 1155), trovafloxacin, clinafloxacin, levofloxacin and ciprofloxacin. *J. Antimicrob. Chemother.* 40:639–651.
- Bisognano, C., P. E. Vaudaux, D. P. Lew, E. Y. W. Ng, and D. C. Hooper. 1997. Increased expression of fibronectin-binding proteins by fluoroquinolone-resistant *Staphylococcus aureus* exposed to subinhibitory levels of ciprofloxacin. *Antimicrob. Agents Chemother.* 41:906–913.
- Dong, Y. Z., C. Xu, X. L. Zhao, J. Domagala, and K. Drlica. 1998. Fluoroquinolone action against mycobacteria: effects of C-8 substituents on growth, survival, and resistance. *Antimicrob. Agents Chemother.* 42:2978–2984.
- Dong, Y. Z., X. L. Zhao, J. Domagala, and K. Drlica. 1999. Effect of fluoroquinolone concentration on selection of resistant mutants of *Mycobacterium bovis* BCG and *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 43:1756–1758.
- Entenza, J. M., Y. A. Que, J. Vouillamoz, M. P. Glauser, and P. Moreillon. 2001. Efficacies of moxifloxacin, ciprofloxacin, and vancomycin against experimental endocarditis due to methicillin-resistant *Staphylococcus aureus* expressing various degrees of ciprofloxacin resistance. *Antimicrob. Agents Chemother.* 45:3076–3083.
- Fournier, B., and D. C. Hooper. 1998. Effects of mutations in GrlA of topoisomerase IV from *Staphylococcus aureus* on quinolone and coumarin activity. *Antimicrob. Agents Chemother.* 42:2109–2112.
- Fournier, B., and D. C. Hooper. 1998. Mutations in topoisomerase IV and DNA gyrase of *Staphylococcus aureus*: novel pleiotropic effects on quinolone and coumarin activity. *Antimicrob. Agents Chemother.* 42:121–128.
- Hoogkamp-Korstanje, J. A. A., and J. Roelofs-Willems. 2000. Comparative in vitro activity of moxifloxacin against gram-positive clinical isolates. *J. Antimicrob. Chemother.* 45:31–39.
- Houssaye, S., L. Gutmann, and E. Varon. 2002. Topoisomerase mutations associated with in vitro selection of resistance to moxifloxacin in *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* 46:2712–2715.
- Ince, D., and D. C. Hooper. 2000. Mechanisms and frequency of resistance to premarloxacin in *Staphylococcus aureus*: novel mutations suggest novel drug-target interactions. *Antimicrob. Agents Chemother.* 44:3344–3350.
- Ince, D., and D. C. Hooper. 2001. Mechanisms and frequency of resistance to

- gatifloxacin in comparison to AM-1121 and ciprofloxacin in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **45**:2755–2764.
13. Ince, D., X. Zhang, L. C. Silver, and D. C. Hooper. 2002. Dual targeting of DNA gyrase and topoisomerase IV. Target interactions of garenoxacin (BMS-284756, T-3811ME), a new desfluoroquinolone. Antimicrob. Agents Chemother. **46**:3370–3380.
 14. Li, X. Y., X. L. Zhao, and K. Drlica. 2002. Selection of *Streptococcus pneumoniae* mutants having reduced susceptibility to moxifloxacin and levofloxacin. Antimicrob. Agents Chemother. **46**:522–524.
 15. Malathum, K., K. V. Singh, and B. E. Murray. 1999. In vitro activity of moxifloxacin, a new 8-methoxyquinolone, against gram-positive bacteria. Diagn. Microbiol. Infect. Dis. **35**:127–133.
 16. Milatovic, D., F. J. Schmitz, S. Brisse, J. Verhoef, and A. C. Fluit. 2000. In vitro activities of sitafloxacin (DU-6859a) and six other fluoroquinolones against 8,796 clinical bacterial isolates. Antimicrob. Agents Chemother. **44**:1102–1107.
 17. Ng, E. Y., M. Trucksis, and D. C. Hooper. 1994. Quinolone resistance mediated by *norA*: physiologic characterization and relationship to *flqB*, a quinolone resistance locus on the *Staphylococcus aureus* chromosome. Antimicrob. Agents Chemother. **38**:1345–1355.
 18. Ng, E. Y., M. Trucksis, and D. C. Hooper. 1996. Quinolone resistance mutations in topoisomerase IV: relationship of the *flqA* locus and genetic evidence that topoisomerase IV is the primary target and DNA gyrase the secondary target of fluoroquinolones in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **40**:1881–1888.
 19. Pestova, E., J. J. Millichap, G. A. Noskin, and L. R. Peterson. 2000. Intracellular targets of moxifloxacin: a comparison with other fluoroquinolones. J. Antimicrob. Chemother. **45**:583–590.
 20. Piddock, L. J., and Y. F. Jin. 1999. Antimicrobial activity and accumulation of moxifloxacin in quinolone-susceptible bacteria. J. Antimicrob. Chemother. **43**:39–42.
 21. Pong, A., K. S. Thomson, E. S. Moland, S. A. Chartrand, and C. C. Sanders. 1999. Activity of moxifloxacin against pathogens with decreased susceptibility to ciprofloxacin. J. Antimicrob. Chemother. **44**:621–627.
 22. Schedletzky, H., B. Wiedemann, and P. Heisig. 1999. The effect of moxifloxacin on its target topoisomerases from *Escherichia coli* and *Staphylococcus aureus*. J. Antimicrob. Chemother. **43**:31–37.
 23. Sindelar, G., X. Zhao, A. Liew, Y. Dong, T. Lu, J. Zhou, J. Domagala, and K. Drlica. 2000. Mutant prevention concentration as a measure of fluoroquinolone potency against mycobacteria. Antimicrob. Agents Chemother. **44**:3337–3343.
 24. Stahl, M. L., and P. A. Pattee. 1983. Confirmation of protoplast fusion-derived linkages in *Staphylococcus aureus* by transformation with protoplast DNA. J. Bacteriol. **154**:406–412.
 25. Stass, H., A. Dalhoff, D. Kubitz, and U. Schühly. 1998. Pharmacokinetics, safety, and tolerability of ascending single doses of moxifloxacin, a new 8-methoxy quinolone, administered to healthy subjects. Antimicrob. Agents Chemother. **42**:2060–2065.
 26. Takei, M., H. Fukuda, R. Kishii, and M. Hosaka. 2001. Target preference of 15 quinolones against *Staphylococcus aureus*, based on antibacterial activities and target inhibition. Antimicrob. Agents Chemother. **45**:3544–3547.
 27. Takenouchi, T., F. Tabata, Y. Iwata, H. Hanzawa, M. Sugawara, and S. Ohya. 1996. Hydrophilicity of quinolones is not an exclusive factor for decreased activity in efflux-mediated resistant mutants of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **40**:1835–1842.
 28. Trucksis, M., J. S. Wolfson, and D. C. Hooper. 1991. A novel locus conferring fluoroquinolone resistance in *Staphylococcus aureus*. J. Bacteriol. **173**:5854–5860.
 29. Varon, E., C. Janoir, M. D. Kitzis, and L. Gutmann. 1999. ParC and GyrA may be interchangeable initial targets of some fluoroquinolones in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **43**:302–306.
 30. Von Eiff, C., and G. Peters. 1999. Comparative in-vitro activities of moxifloxacin, trovafloxacin, quinupristin/dalfopristin and linezolid against staphylococci. J. Antimicrob. Chemother. **43**:569–573.
 31. Woodcock, J. M., J. M. Andrews, F. J. Boswell, N. P. Brenwald, and R. Wise. 1997. In vitro activity of BAY 12–8039, a new fluoroquinolone. Antimicrob. Agents Chemother. **41**:101–106.
 32. Yague, G., J. E. Morris, X. S. Pan, K. A. Gould, and L. M. Fisher. 2002. Cleavable-complex formation by wild-type and quinolone-resistant *Streptococcus pneumoniae* type II topoisomerases mediated by gemifloxacin and other fluoroquinolones. Antimicrob. Agents Chemother. **46**:413–419.
 33. Zhao, X. L., J. Y. Wang, C. Xu, Y. Z. Dong, J. F. Zhou, J. Domagala, and K. Drlica. 1998. Killing of *Staphylococcus aureus* by C-8-methoxy fluoroquinolones. Antimicrob. Agents Chemother. **42**:956–958.