In vitro activity of sitafloxacin against *Mycobacterium tuberculosis* with *gyrA/B* mutations isolated in Japan

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Abstract

Purpose. Sitafloxacin (Sfx) is a new fluoroquinolone (FQ) that has shown a strong bactericidal effect against *Mycobacterium tuberculosis* (Mtb) *in vitro*. However, data on Sfx efficacy against Mtb with *gyrA/B* mutations and its epidemiological cut-off (ECOFF) value remain limited. Therefore, we evaluated and compared the *in vitro* activity of Sfx against *gyrA/B*-mutant Mtb to that of moxifloxacin (Mfx), levofloxacin (Lfx), and ciprofloxacin (Cfx), and determined the ECOFF for Sfx.

Methodology. A total of 109 clinical Mtb isolates, including 73 multidrug-resistant (MDR) isolates, were subjected to minimum inhibitory concentration (MIC) analysis in oleic-albumin-dextrose-catalase (OADC)-supplemented Middlebrook 7H9 medium. Our results showed that Sfx had lower cumulative MIC than Mfx, Lfx, and Cfx. Furthermore, we preformed direct DNA sequencing of the quinolone resistance-determining regions (QRDRs).

Results. We identified the following mutations: D94G, D94A, A90V, D94H, D94N, and G88A in *gyrA*; and A543V, A543T, E540D, R485C, D500A, I552S, and D577A in *gyrB*. Based on our results, an ECOFF of 0.125μg/mL was proposed for Sfx. With this ECOFF, 15% of Lfx-resistant isolates with MIC ≥ 2 μg/mL were susceptible to Sfx.

Conclusion. Sfx had the lowest cumulative MIC and a relatively low ECOFF value against Mtb, indicating that Sfx was more effective against not only *gyrA*-mutant isolates but also MDR isolates in Japan.

Keywords: Sitafloxacin; Minimum inhibitory concentrations; Gyrase A gene; Gyrase B gene; Epidemiological cut-off value

Introduction

The World Health Organization (WHO) reported that approximately 500,000 people worldwide developed multidrug-resistant tuberculosis (MDR-TB) in 2015, while additional 100,000 developed rifampicin-resistant tuberculosis (RR-TB) [1]. Better chemotherapeutic interventions are needed to prevent the transmission of drug-resistant TB. Therefore, the WHO has updated the guidelines for the treatment of drug-resistant tuberculosis and currently recommends fluoroquinolones (FQs) as group A drugs to treat MDR-TB and RR-TB [2]. However, FQ-resistant *Mycobacterium tuberculosis* (Mtb) strains and the incidence of FQ-resistant TB have increased [3,4].

Among the group A drugs, levofloxacin (Lfx) is the most frequently used drug to treat MDR-TB in Japan [5]. Lfx resistance was reported in 3.2% and 6.1% of isolates from patients without and with prior treatment, respectively. The relatively high rate of FQ resistance against Mtb may be attributed to the use of these antibiotics before a proper diagnosis for TB was made [5]. Moreover, a previous study reported that half of MDR isolates in Japan were resistant to FQs (such as Lfx, sparfloxacin, and ciprofloxacin [Cfx]) [6]. Thus, it is crucial to determine the most efficacious FQ to treat MDR-TB.

Sitafloxacin (Sfx) is a synthetic broad-spectrum 8-methoxyfluoroquinolone approved for use in Japan [7]. Studies conducted in Japan and Thailand showed good activity for Sfx against Mtb *in vitro* [8,9] A previous study showed that Cfx-resistant clinical isolates were most susceptible to Sfx and gatifloxacin when compared to that to other FQs [10]. However, studies that assess the MICs for Sfx and significance of *gryA*/*B* mutations with larger number of isolates in Japan are lacking. Therefore, we investigated the correlations between the MICs of Sfx and other FQs including moxifloxacin (Mfx), Lfx, and Cfx, and mutations in *gyrA/B* as assessed by direct DNA sequencing of the quinolone resistance-determining regions (QRDRs). We also determined the tentative epidemiological cut-off (ECOFF) value of Sfx against Mtb. To provide a visual comparison of the MICs of different FQs, cumulative percentage distribution was used to compare the MICs of Sfx with those of Mfx, Lfx, and Cfx.

Materials and Methods

Bacterial isolates

A total of 109 *M. tuberculosis* clinical isolates were randomly selected from a collection maintained and preserved by the Tuberculosis Research Committee (Ryoken; Tokyo, Japan). Among the 109 isolates, 73 (67%) were MDR and 36 (33%) were non-MDR isolates. Two of the 36 non-MDR isolates were resistant to isoniazid (INH). (Suppl. Table 1) These isolates were collected from TB patients with Mtb-positive culture results throughout Japan in 2002 and 2007, and each isolate was given a unique identification number.

All isolates were confirmed by the conventional biochemical and/or immuno-chromatography methods (Capilia TB; TAUNS, Numazu, Japan) [11] and tested for drug susceptibility to INH and rifampicin by the conventional proportion method on 1% Ogawa medium (equivalent to the Löwenstein-Jensen [LJ] method) [5]. Furthermore, the susceptibility to Lfx was also evaluated by the proportion method mentioned above. *M. tuberculosis* H37Rv (ATCC 27294; ATCC, Manassas, VA) was used as a control.

Determination of the minimum inhibitory concentrations

The MICs of Sfx (Lot#023WCG; Daiichi Sankyo, Tokyo, Japan), Mfx (#32477; Sigma-Aldrich, St. Louis, MO), Lfx (#28266; Sigma-Aldrich), and Cfx (#17850; Sigma-Aldrich) were determined in Middlebrook 7H9 broth supplemented with 10% oleic-albumin-dextrose-catalase (OADC). A broth microdilution method using 7H9 broth has been described previously [12]. Each drug was dissolved in 0.1 NaOH and a serial two-fold broth microdilution was performed for this study. Sfx, Mfx, Lfx, and Cfx were suspended in 7H9 broth and their final concentrations ranged from 0.008–8, 0.016–16, 0.03–32, and 0.03–32 μg/mL for Sfx, Mfx, Lfx, and Cfx, respectively. Bacterial growth was assessed after 1 week of incubation at 37°C with 5% CO_2. Bacterial suspensions were prepared by adding 5 mL of 7H9 broth medium into 0.05 mL of the original bacterial suspension until an optical density (OD) of 0.15–0.24 was reached. The MIC was defined as the lowest concentration of drug that inhibited visible growth of the bacteria. The

cumulative percentage of MICs was used to compare the MICs for different FQs.

DNA extraction and sequencing

Genomic DNA of the Mtb isolates was extracted according to a previously described method [13], and 5 μg/mL of DNA was used in the PCR mixtures. Primers for *gyrA* were 5′-GAT GAC AGA CAC GAC GTT GC-3′ (forward) and 5′-GGG CTT CGG TGT ACC TCA T-3′ (reverse) [14]. Primers for *gyrB* were 5′-GAG TTG GTG CGG CGT AAG AGC-3′ (forward) and 5′-CAA GAT CGT GCT GAT GGC CG-3′ (reverse) [15]. AmpliTaq Gold® (Roche, Pleasanton, CA) was used to amplify the DNA. After amplification, direct sequencing of the QRDRs was performed. Sequencing primers were 5′-GAT GAC AGA CAC GAC GTT GC-3′ for *gyrA* and 5′-GAG TTG GTG CGG CGT AAG AGC-3′ for *gyrB*. The QRDR in g*yrA* ranged from codon 74 to 113, and the QRDR in *gyrB* ranged from codon 500 to 540 [16,17].

Statistical analysis

The chi-square test was performed to compare the proportions of susceptible and resistant isolates for each FQ. The Mann-Whitney U test was performed to compare MIC values as continuous variables for each FQ. P value of < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA)

Results

Minimum inhibitory concentrations of fluoroquinolones

Fig. 1 shows the cumulative percentage of MICs for Sfx, Mfx, Lfx, and Cfx. The approximated cumulative % (50) values were 0.06, 0.25, 0.5, and 0.5 μg/mL for Sfx, Mfx, Lfx, and Cfx, respectively. The approximated cumulative % (90) values were 1, 4, 8, and 16 μg/mL for Sfx, Mfx, Lfx, and Cfx, respectively. The median (interquartile range; IQR) MIC

of Sfx, Mfx, Lfx, and Cfx against 37 *gyrA* mutants was 0.5 (0.25–1.0 μg/mL), 2 (1.0–4.0 μg/mL), 8 (4–8 μg/mL), and 8 μg/mL (4–16 μg/mL), respectively. Four *gyrB*-mutant isolates showed relatively low MICs; against these four isolates, the highest MICs were 0.125, 0.5, 1, and 1 μg/mL for Sfx, Mfx, Lfx, and Cfx, respectively. The MICs of the reference strain (H37Rv) were 0.5, 0.03, 0.5, and 0.125 μg/mL for the four FQs, respectively. The MICs for all the isolates evaluated in this study are shown in Suppl. Table1.

Correlations between mutations in *gyrA* and *gyrB* and the MIC values

Among the 109 isolates, 37 (34%) isolates had mutations in *gyrA* with mutation patterns of D94G, D94A, A90V, D94H, D94N, and G88A. Among the 73 MDR and 36 non-MDR isolates, 30 (41.1%) and 7 (19.4%) were *gyrA* mutants, respectively. Most mutations (75.7%) in the 37 *gyrA*-mutant isolates were found in codon 94 (Table 1).

Among the 109 isolates, 8 (7%) isolates had mutations in *gyrB* with mutation patterns of A543V, A543T, E540D, R485C, D500A, I552S, and D577A. Half of the *gryB*-mutant isolates also had mutations in *gyrA* and all eight were MDR isolates. Among the four isolates with mutations in *gyrB* only, two had double *gyrB* mutations, while the other two had either the R485C or A543T mutation (Table 2); these four isolates did not considerably increase the MICs of the FQs.

The MICs were significantly higher against the *gyrA* mutants than against wild type isolates ($p < 0.001$). Two isolates showed higher MICs for FQs despite the absence of *gyrA* mutations in the QRDR. The MICs against these two isolates were 0.25, 0.125, 4, and 8 μg/mL, and 0.25, 2, 4, and 8 μg/mL for Sfx, Mfx, Lfx, and Cfx, respectively.

Correlations between different mutation patterns and the MICs

One isolate showed amino-acid substitutions in three different *gyrA* codons (G88A+A90V+D94G). The MICs of Sfx, Mfx, Lfx, and Cfx against this isolate were 0.5, 4,

16, and 32 μg/mL, respectively (Table 1). Two isolates showed substitutions in two different *gyrB* codons (E540D+A543V and I552S+D577A). The MICs of Sfx, Mfx, Lfx, and Cfx against these two isolates were 0.125, 0.5, 1, and 1 μg/mL, and 0.125, 0.25, 0.5, and 0.5 μg/mL, respectively. Isolates with double *gyrB* mutations in the vicinity of the QRDR had higher MICs (Table 2).

Determination of ECOFF value for fluoroquinolones

The distributions of MICs for the four FQs are shown in Fig. 2. Mfx, Lfx, and Cfx, but not Sfx, showed bimodal distribution of MICs, which clearly segregated the phenotypically wild-type isolates from mutants. As previously described, the tentative ECOFF value can be determined by identifying the end of the phenotypically wild-type distribution to distinguish the resistant and susceptible isolates [18]. Therefore, we determined the ECOFF values as 0.125, 0.5, 1, and 1 μg/mL for Sfx, Mfx, Lfx, and Cfx, respectively. We utilized these ECOFFs to screen for Lfx-resistant isolates showing MIC ≥ 2 μg/mL, which is the cut-off value for Lfx against Mtb as described in previous studies [19,20]. A total of 39 isolates were Lfx-resistant showing MIC of ≥ 2 μg/mL. Furthermore, *gyrA* mutations were absence in only two of the isolates. Using these ECOFFs, 15% (6/39), 13% (5/39), and 0% of isolates were susceptible to Sfx, Mfx, and Cfx, respectively. All of the six and five Sfx- and Mfx-susceptible isolates above were MDR isolates. Except for one Mfx-susceptible isolate with no *gyrA* mutation, all six and four isolates were *gyrA* mutants.

Among the 73 MDR isolates, 26 (35.6%), 28 (38.4%), and 29 (39.7%) isolates were resistant to Sfx, Mfx, and Lfx, respectively. Furthermore, *gyrA* mutations were found in 24/26 (92.3%) isolates resistant to Sfx and 6/47 (12.8%) isolates susceptible to Sfx ($p < 0.001$); 26/27 (92.9%) isolates resistant to Mfx and 4/46 (8.7%) isolates susceptible to Mfx ($p < 0.001$); and 30/32 (93.8%) isolates resistant to Lfx and 0/41 (0%) isolate susceptible to Lfx ($p < 0.001$). There were no significant differences in susceptibility or resistance to Sfx, Mfx, and Lfx in isolates with *gyrB* mutations only.

Discussion

Lfx, Mfx, and ofloxacin are currently recommended by the WHO for the treatment of MDR-TB [21]. However, data supporting the efficacy of Sfx against TB are limited because Sfx has not been used worldwide. In this study, we determined the MICs of Lfx, Mfx, Sfx, and Cfx against multiple clinical isolates of Mtb, and performed direct DNA sequencing of the QRDRs in these isolates. We evaluated the activity of Stx *in vitro* against *gyrA*/*B* mutants as well as MDR isolates obtained from Japan. We found that Sfx had the lowest MIC values when compared to other FQs such as Lfx, Mfx and Cfx. Sfx showed the lowest cumulative MIC and had a relatively low ECOFF value against Mtb in our study, indicating that Sfx is potentially a more effective agent against *gyrA*-mutant isolates as well as MDR isolates in Japan.

Using an agar dilution method in 7H11 medium, a previous study showed that Sfx had lower MIC_{50} and MIC_{90} than Lfx against non-MDR and MDR isolates [22]. As noted above, that study utilized solid medium and the proportion of MDR isolates was different from that in our study, which may explain the higher MIC_{50} and MIC_{90} values than those observed in our study. A study conducted in Thailand also showed that Sfx had lower MICs for Sfx than other FQs against FQ-susceptible Mtb and isolates with *gyrA*/*B* mutations in 7H10 medium [9]; thus, Sfx was suggested to be more efficacious for Mtb treatment. In agreement with these findings, our results showed that Sfx had lower cumulative MICs than Mfx, indicating that Sfx may be superior to Mfx for Mtb treatment. To confirm these findings, additional studies using 7H9 medium are needed.

The WHO does not currently recommend the use of Cfx to treat MDR-TB [21]. In our study, Cfx showed the highest MICs among the FQs evaluated; this finding lent further support to the WHO recommendation. However, a recent study showed that the use of Lfx or Mfx did not significantly influence the final treatment outcome in patients with FQ-susceptible MDR-TB [23]. This finding suggested that MIC was not the sole indicator of treatment efficacy. Based on this thought, Sfx may not yield a better treatment outcome when compared to Mfx or Lfx, despite displaying the lowest MIC values.

A previous study using 7H10 agar medium suggested tentative ECOFF values of 1.0 μg/mL for Cfx and 0.5 μg/mL for Lfx and Mfx [24]. On the basis of findings of the present

study, we suggested ECOFF values of 0.125, 0.5, and 1 μg/mL for Sfx, Mfx, and Cfx, respectively. These values were based on a breakpoint at 2 μg/mL for Lfx in 7H9 medium. The proposed breakpoint for Mfx agrees with that recommended by the WHO, which was determined using the mycobacteria growth indicator tube (MGIT) medium [25]. Although the breakpoints for Cfx and Mfx determined in a previous study were comparable to ours [24], additional studies using 7H9 medium should be performed to confirm these breakpoints. Since data on the breakpoint of Sfx are limited, we determined the ECOFF of Sfx in this study. The appropriate breakpoint setting can influence the choice of drug for the most effective treatment; thus, additional information on the breakpoint of FQs, including that of Sfx, is needed. In our study, 15% and 13% of the Lfx-resistant isolates were susceptible to Sfx and Mfx, respectively, indicating that Sfx is a more effective agent against MDR isolates with *gyrA* mutations.

Mtb resistance to FQs can be mainly attributed to mutations in QRDRs [26], specifically that in the *gyrA* gene [27]. The presence of *gyrA* mutation has also been associated with high-level resistance to FQs [28]. The relationship between mutations in *gyrA/B* and the MICs of FQs has been reported in various settings [19,20,29]. Furthermore, double mutations in *gyrA* and *gyrA*/*B*-combined mutations were shown to positively correlate with resistance to FQs [30]. Mutations in certain *gyrB* codons were also associated with a significant increase in MICs of FQs [31], albeit to a lower extent when compared to those observed with *gyrA* mutant isolates [32]. Isolates with *gyrA* mutations showed higher MICs for the four FQs evaluated in our study. Mutations in codon 90 and 94 of *gyrA* were the most frequently observed mutations (34%), with D94G as the most frequently observed mutation pattern. This finding was in agreement with those of previous reports [19,31,33]. In particular, mutations in *gyrA* showed a strong correlation with resistance to FQs among the MDR isolates. In non-MDR isolates in our study, nearly 20% of isolates were *gyrA* mutants; these isolates showed high MICs and resistance to FQs. This finding suggested that the use of FQs in Japan carries a risk of inducing resistance, even when used to treat non-MDR-TB. Since FQs are widely used to treat bacterial infections, the rate of FQ resistance may increase. Therefore, as indicated in a previous study, susceptibility test to

FQs should be considered [6].

Similarly, the association between *gyrB* mutations and FQ resistance has been reported in several studies. These reports showed that E540V and E540D mutations were involved in resistance to FQs [30,31]. Additionally, G512R mutation was reported to correlate with resistance to Mfx and ofloxacin [34]. In our study, E540D and A543V, which were found in the same isolate, and R485C, found in a different isolate, were associated with resistance to Mfx and Sfx. Moreover, I552S and D577A mutations, found in one isolate, raised the MIC of Sfx to its breakpoint of 0.125 μg/mL. However, the A543T mutation in *gyrB* appeared to be less relevant for the efficacy of FQs in our study. Since mutations in *gyrB* are found infrequently, further studies are needed to confirm these findings. Isolates with *gyrB* mutation only did not increase the MICs, indicating that there were no significant differences in susceptibility or resistance to different FQs for the *gyrB* mutants in our study. To our knowledge, the D577A mutation of *gyrB* has not been reported elsewhere.

In conclusion, our study confirmed the importance of mutations in *gyrA* in conferring resistance to Sfx, Mfx, Lfx, and Cfx. Furthermore, despite showing less effects than the *gyrA* mutations, several mutations in *gyrB* were associated with increased MICs for the four FQs evaluated. In our study, Sfx displayed the lowest MIC values and had a relatively low ECOFF against Mtb, suggesting its superior efficacy against not only *gyrA*-mutant Mtb isolates, but also MDR isolates in Japan.

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Competing interests: We declare that we have no conflict of interest.

Ethical approval: The Ryoken General Assembly approved the collection and use of sputum samples from TB patients. All patients provided written informed consents.

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Figure and Table Legends

Figure 1. Cumulative percentage MIC distributions of Lfx, Mfx, Sfx, and Cfx against *M. tuberculosis* isolates.

The horizontal dotted lines indicate the 50%- or 90%- cumulative frequency. MIC, minimum inhibitory concentration; Lfx, levofloxacin; Mfx, moxifloxacin; Sfx, sitafloxacin; Cfx, ciprofloxacin.

Figure 2. Association of *gyrA/B* mutations with increased MIC for different fluoroquinolones and the ECOFF of each fluoroquinolone.

MIC distributions of (a) Lfx, (b) Sfx, (c) Mfx, and (d) Cfx in 7H9 medium for 109 *M. tuberculosis* isolates with or without *gyrA/B* mutations. Lfx, Sfx, and Mfx showed bimodal distributions. MIC, minimum inhibitory concentration; Lfx, levofloxacin; Sfx, sitafloxacin; Mfx, moxifloxacin; Cfx, ciprofloxacin; ECOFF, epidemiological cut-off.

Table 1. Minimum inhibitory concentrations (MICs) of fluoroquinolones against *gyrA*-mutant isolates.

[#]percentage of total *gyrA* mutants.

Lfx, levofloxacin; Mfx, moxifloxacin; Sfx, sitafloxacin; Cfx, ciprofloxacin; MDR, multidrug-resistant.

Table 2. Minimum inhibitory concentrations (MICs) of fluoroquinolones against *gyrB*-mutant isolates.

[#]percentage of total eight *gyrB* mutants.

Lfx, levofloxacin; Mfx, moxifloxacin; Sfx, sitafloxacin; Cfx, ciprofloxacin; MDR, multidrug-resistant.

Figure 1

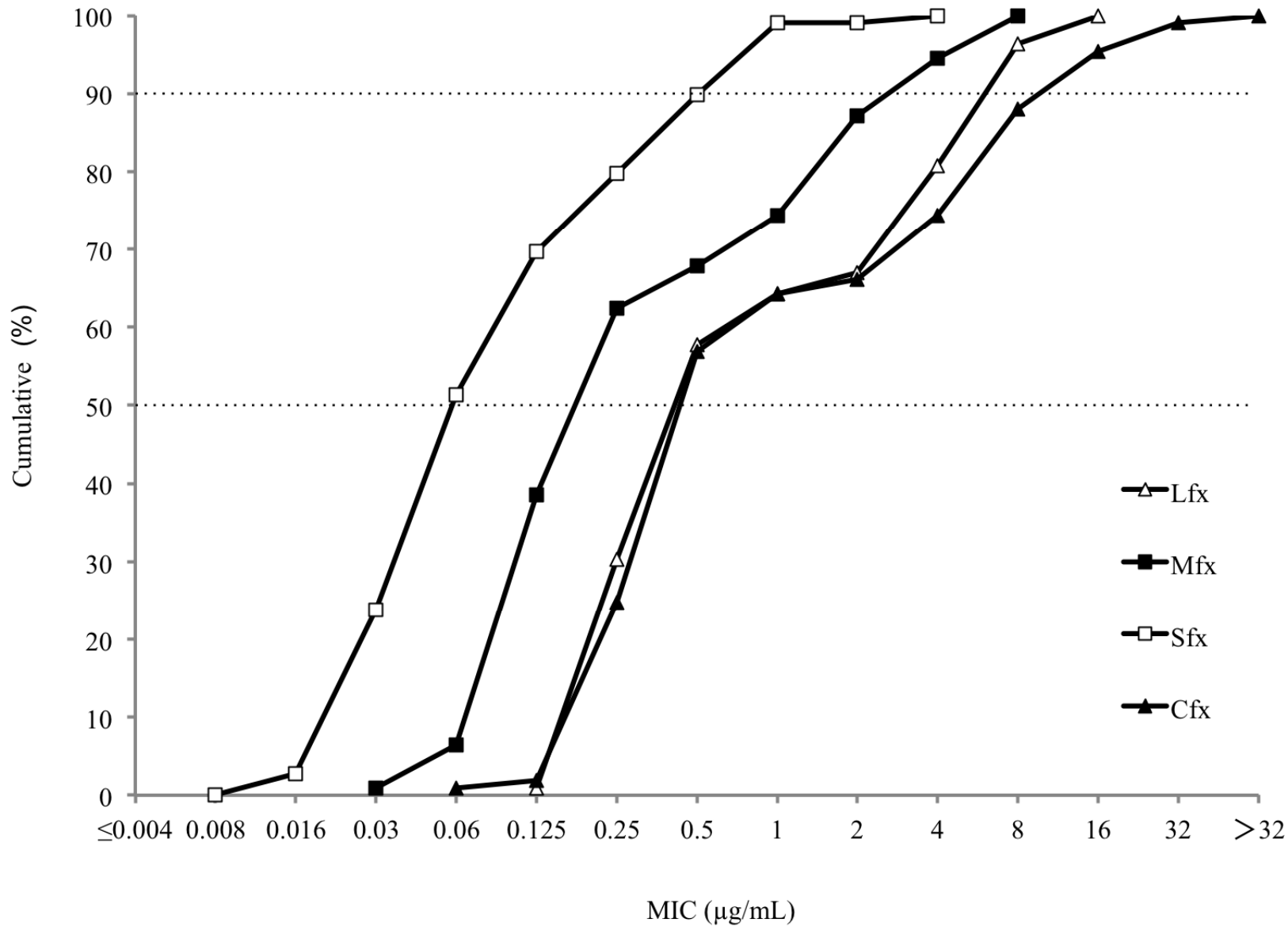
100
90
80
70
60
50
40
30
20
10
0
Cumulative (%)
≤0.004
0.008
0.016
0.03
0.06
0.125
0.25
0.5
1
2
4
8
16
32
>32
MIC (μg/mL)
Lfx
Mfx
Sfx
Cfx

Figure 2

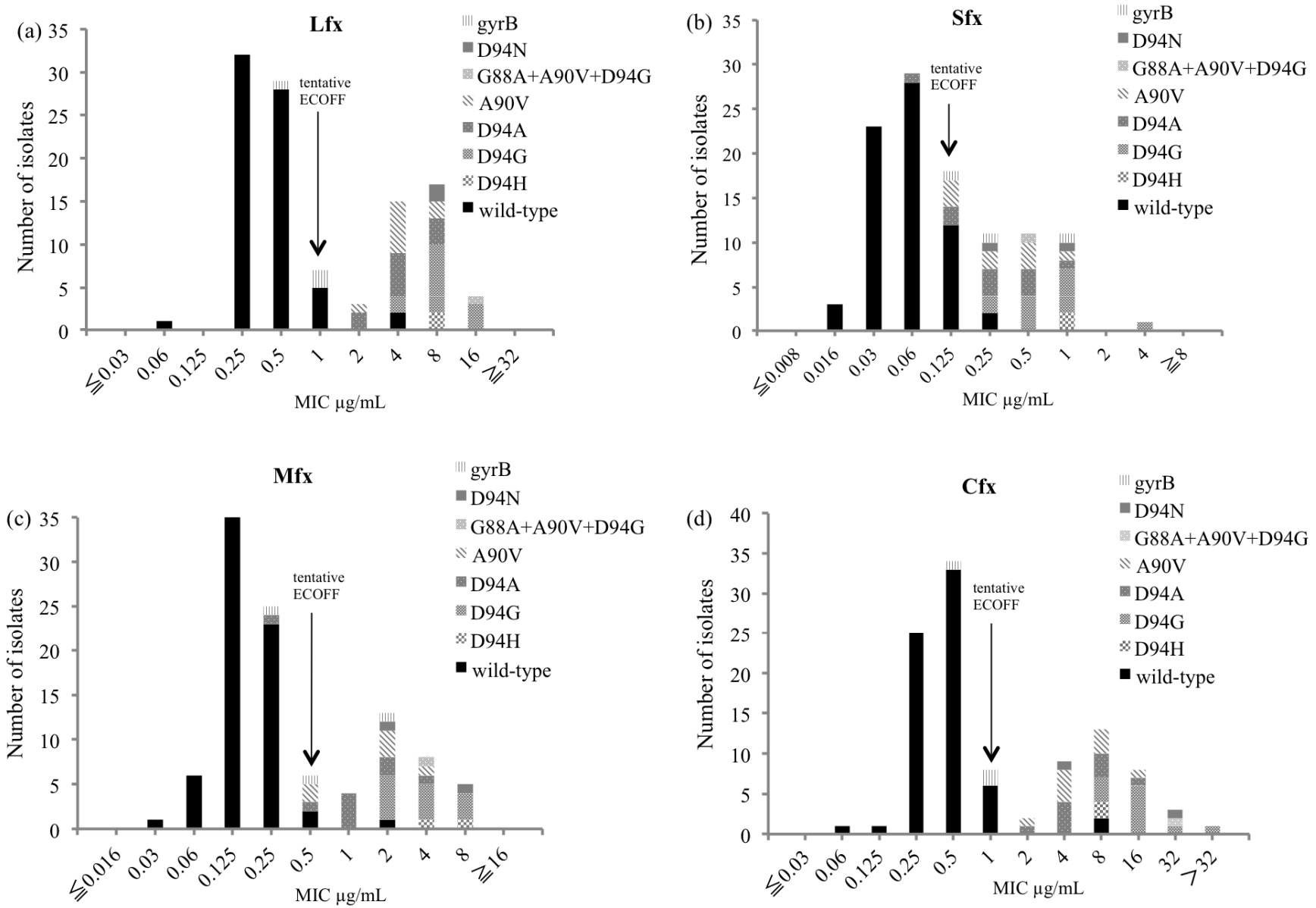
(a)
Lfx
Number of isolates
MIC µg/mL
tentative ECOFF
gyrB
D94N
G88A+A90V+D94G
A90V
D94A
D94G
D94H
wild-type
≦0.03
0.06
0.125
0.25
0.5
1
2
4
8
16
≧32
(b)
Sfx
≦0.008
0.016
0.03
0.06
0.125
0.25
0.5
1
2
4
≧8
(c)
Mfx
≦0.016
0.03
0.06
0.125
0.25
0.5
1
2
4
8
≧16
(d)
Cfx
≦0.03
0.06
0.125
0.25
0.5
1
2
4
8
16
32
>32

Table 1. The minimum inhibitory concentrations (MICs) of fluoroquinolones against *gyrA*-mutant isolates.

Mutation patterns	Isolates, n (%)[#]	MIC of Lfx (range), μg/mL	MIC of Mfx (range), μg/mL	MIC of Sfx (range), μg/mL	MIC of Cfx (range), μg/mL	Number of MDR &(non-MDR) isolates, n
A90V (GCG→ GTG)	7 (18.9)	(2–4)	(0.5–4)	(0.125–0.5)	(2–16)	3 (4)
D94G (GAC→GGC)	13 (35.1)	(4–16)	(2–8)	(0.25–4)	(8–32)	12 (1)
D94H (GAC→CAC)	2 (5.4)	8	(4–8)	1	8	1 (1)
D94N (GAC→GGC)	2 (5.4)	8	(2–8)	(0.25–1)	(4–32)	1 (1)
D94A (GAC→GCC)	8 (21.6)	(2–8)	(0.25–2)	(0.06–1)	(2–16)	8 (0)
A90V(GCG→GTG) &D500A(GAC→GCC)	1 (2.7)	8	1	1	8	1 (0)
A90V(GCG→GTG) &A543V(GCG→GTG)	1 (2.7)	8	2	0.125	8	1 (0)
D94A(GAC→GCC) &A543V(GCG→GTG)	2 (5.4)	8	(2–4)	0.25	8	2 (0)
G88A (GGC→GCC) &A90V (GCG→GTG) &D94G(GAC→GGC)	1 (2.7)	16	4	0.5	32	1 (0)

[#]percentage of total *gyrA* mutants.

Lfx, levofloxacin; Mfx, moxifloxacin; Sfx, sitafloxacin; Cfx, ciprofloxacin; MDR, multidrug-resistant.

Table 2. Minimum inhibitory concentrations (MICs) of fluoroquinolones against *gyrB*-mutant isolates without *gyrA* mutations.

Mutation patterns	Isolates, n (%)[#]	MIC of Lfx, μg/mL	MIC of Mfx μg/mL	MIC of Sfx, μg/mL	MIC of Cfx, μg/mL	Number of MDR isolates, n
R485C (CGT→TGT)	1 (12.5)	1	0.5	0.125	1	1
A543T (GCG→ACG)	1 (12.5)	0.5	0.25	0.06	0.5	1
E540D (GAA→GAC) &A543V (GCG→GTG)	1 (12.5)	1	0.5	0.125	1	1
I552S (ATC→AGC) &D577A (GAT→GCT)	1 (12.5)	0.5	0.25	0.125	0.5	1

[#]percentage of total eight *gyrB* mutants.

Lfx, levofloxacin; Mfx, moxifloxacin; Sfx, sitafloxacin; Cfx, ciprofloxacin; MDR, multidrug-resistant.

No.	Lfx(μg/mL)	Mfx(μg/mL)	Sfx(μg/mL)	Cfx(μg/mL)	INH(0.2μg/mL	RFP(40μg/mL)	Lfx(1.0μg/mL)	MDR isolate or non-MDR isolate	mutation in gyrA	mutation in gyrB
1	8	1	1	8	R	R	R	MDR	A90V	D500A
2	0.25	0.06	0.03	0.25	R	R	S	MDR		
3	0.5	0.125	0.03	0.5	R	R	S	MDR		
4	0.5	0.06	0.016	0.5	R	R	S	MDR		
5	0.5	0.25	0.06	0.5	R	R	S	MDR		A543T
6	1	0.25	0.06	1	R	R	S	MDR		
7	8	8	1	8	R	R	R	MDR	D94H	
8	4	4	0.5	16	R	R	R	MDR	A90V	
9	0.25	0.25	0.125	0.5	R	R	S	MDR		
10	8	8	1	16	R	R	R	MDR	D94G	
11	1	0.25	0.03	0.5	R	R	S	MDR		
12	4	2	0.25	8	R	R	R	MDR	D94G	
13	0.5	0.25	0.06	0.5	R	R	S	MDR		
14	0.25	0.25	0.06	0.5	R	R	S	MDR		
15	2	1	0.5	2	R	R	R	MDR	D94A	
16	0.25	0.125	0.03	0.25	R	R	S	MDR		
17	0.5	0.125	0.06	0.5	R	R	S	MDR		
18	0.25	0.06	0.06	0.25	R	R	S	MDR		
19	16	8	1	32	R	R	R	MDR	D94G	
20	0.25	0.06	0.016	0.25	R	R	S	MDR		
21	0.25	0.06	0.03	0.125	R	R	S	MDR		
22	4	2	1	8	R	R	R	MDR	D94A	
23	8	8	1	32	R	R	R	MDR	D94N	
24	0.25	0.125	0.06	0.25	R	R	S	MDR		
25	0.25	0.125	0.03	0.25	R	R	S	MDR		
26	4	2	0.25	8	R	R	R	MDR	D94A	
27	0.25	0.125	0.03	0.25	R	R	S	MDR		
28	0.25	0.125	0.03	0.5	R	R	S	MDR		
29	0.5	0.25	0.03	0.5	S	S	R	non-MDR		
30	4	2	0.25	8	R	R	R	MDR	D94G	
31	0.25	0.125	0.03	0.25	R	R	S	MDR		
32	1	0.5	0.125	1	S	S	R	non-MDR		
33	0.5	0.25	0.06	0.5	R	R	S	MDR		
34	0.5	0.125	0.06	0.5	S	S	R	non-MDR		
35	0.5	0.25	0.125	0.5	R	R	S	MDR		
36	0.5	0.125	0.03	0.5	R	R	S	MDR		
37	16	8	4	>32	R	R	R	MDR	D94G	
38	0.5	0.25	0.06	0.5	S	S	R	non-MDR		
39	2	0.5	0.125	2	R	R	R	MDR	A90V	
40	0.25	0.125	0.06	0.25	R	R	S	MDR		
41	4	2	0.25	4	S	S	R	non-MDR	A90V	
42	4	1	0.25	4	S	S	R	non-MDR	A90V	
43	0.25	0.125	0.03	0.25	R	R	S	MDR		
44	8	2	0.25	8	S	S	R	non-MDR	D94G	
45	8	4	1	8	R	S	R	non-MDR	D94H	
46	0.25	0.125	0.06	0.25	R	R	S	MDR		
47	0.25	0.125	0.03	0.25	S	S	R	non-MDR		
48	8	2	0.25	4	S	S	R	non-MDR	D94N	
49	0.25	0.25	0.03	0.25	R	R	S	MDR		
50	4	2	0.5	8	S	S	R	non-MDR	A90V	
51	4	1	0.5	4	S	S	R	non-MDR	A90V	
52	0.25	0.125	0.03	0.25	S	S	S	non-MDR		
53	0.25	0.125	0.06	0.25	S	S	S	non-MDR		
54	0.25	0.125	0.06	0.25	S	S	S	non-MDR		
55	0.25	0.125	0.06	0.25	S	S	S	non-MDR		
56	0.5	0.25	0.125	1	S	S	S	non-MDR		
57	0.25	0.125	0.06	0.5	S	S	S	non-MDR		
58	0.5	0.25	0.06	0.5	S	S	S	non-MDR		
59	0.5	0.125	0.03	0.5	R	S	S	non-MDR		
60	0.25	0.125	0.06	0.5	S	S	S	non-MDR		
61	0.25	0.125	0.06	0.5	S	S	S	non-MDR		
62	0.5	0.25	0.125	0.5	S	S	S	non-MDR		
63	0.5	0.25	0.125	1	S	S	S	non-MDR		
64	0.5	0.25	0.06	0.5	S	S	S	non-MDR		
65	0.5	0.25	0.125	0.5	S	S	S	non-MDR		
66	0.5	0.25	0.06	0.5	S	S	S	non-MDR		
67	0.5	0.25	0.06	0.5	S	S	S	non-MDR		
68	0.5	0.25	0.06	0.5	S	S	S	non-MDR		
69	0.5	0.25	0.125	0.5	S	S	S	non-MDR		
70	0.5	0.25	0.125	0.5	S	S	S	non-MDR		
71	0.25	0.125	0.03	0.25	S	S	S	non-MDR		
72	1	0.5	0.06	1	S	S	S	non-MDR		
73	0.25	0.125	0.03	0.25	S	S	S	non-MDR		
74	0.5	0.25	0.125	0.5	S	S	S	non-MDR		
75	0.25	0.125	0.06	0.25	S	S	S	non-MDR		
76	16	8	1	32	R	R	R	MDR	D94G	
77	8	2	1	16	R	R	R	MDR	D94G	
78	16	4	0.5	32	R	R	R	MDR	G88A A90V D94G	
79	1	0.5	0.125	1	R	R	R	MDR		E540D A543V
80	8	4	0.25	8	R	R	R	MDR	D94A	A543V
81	4	1	0.125	4	R	R	R	MDR	A90V	
82	8	4	0.5	16	R	R	R	MDR	D94G	
83	4	2	0.25	8	R	R	R	MDR		
84	4	1	0.5	4	R	R	R	MDR	D94A	
85	8	2	0.125	8	R	R	R	MDR	A90V	A543V
86	8	4	1	16	R	R	R	MDR	D94G	
87	4	1	0.5	4	R	R	R	MDR	D94A	
88	4	0.5	0.125	4	R	R	R	MDR	A90V	
89	8	4	0.5	8	R	R	R	MDR	D94G	
90	2	0.5	0.06	4	R	R	R	MDR	D94A	

91	8	4	0.5	16	R	R	R	MDR	D94G	
92	8	2	0.25	8	R	R	R	MDR	D94A	A543V
93	4	0.125	0.25	8	R	R	R	MDR		
94	0.5	0.125	0.03	0.5	R	R	S	MDR		
95	0.06	0.03	0.016	0.06	R	R	S	MDR		
96	0.5	0.25	0.125	0.5	R	R	R	MDR		I552S D577A
97	0.5	0.125	0.125	0.5	R	R	S	MDR		
98	0.5	0.125	0.06	0.5	R	R	S	MDR		
99	1	0.25	0.125	1	R	R	S	MDR		
100	1	0.5	0.125	1	R	R	R	MDR		R485C
101	0.25	0.06	0.03	0.25	R	R	S	MDR		
102	0.25	0.125	0.06	0.25	R	R	S	MDR		
103	0.5	0.125	0.03	0.5	R	R	S	MDR		
104	0.5	0.125	0.03	0.5	R	R	S	MDR		
105	0.25	0.125	0.06	0.25	R	R	S	MDR		
106	0.25	0.125	0.03	0.25	R	R	S	MDR		
107	8	0.25	0.125	16	R	R	R	MDR	D94A	
108	0.25	0.125	0.06	0.25	R	R	S	MDR		
109	8	2	0.5	16	R	R	R	MDR	D94G	
Rv	0.5	0.5	0.03	0.125	S	S	S	MDR		