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Synergistic activities of clofazimine with moxifloxacin or capreomycin against *Mycobacterium tuberculosis* in China

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ABSTRACT

Clofazimine (CFZ) is a promising candidate drug for use in the management of multidrug-resistant tuberculosis (MDR-TB) patients. In this study, the minimum inhibitory concentration (MIC) method and checkerboard method were used to investigate potential synergies between CFZ and moxifloxacin (MOX) or capreomycin (CAP). Thirty *Mycobacterium tuberculosis* strains were collected, including 13 MDR strains, 2 extensively drug-resistant (XDR) strains, 3 pan-sensitive strains and 12 strains resistant to other drugs. When the minimum fractional inhibitory concentration indexes (FICIs) were calculated, synergy was found in 21 (70.00%) *M. tuberculosis* strains against the CFZ/CAP combination and 29 (96.67%) against the CFZ/MOX combination. When the maximum FICIs were calculated, 10 of 15 MDR/XDR strains and 2 of 15 other drug-resistant or pan-sensitive strains showed antagonism against the CFZ/CAP combination, whilst 8 of 15 MDR/XDR strains and 1 of 15 other drug-resistant or pan-sensitive strains showed antagonism against the CFZ/MOX combination, respectively. In conclusion, these findings demonstrate that the combination of CFZ and MOX shows better synergism than the combination of CFZ and CAP. The MDR/XDR isolates are more likely to show antagonism than the other drug-resistant or pan-sensitive strains in both the CFZ/MOX and CFZ/CAP combinations. CFZ in combination with MOX may be a promising drug regimen for the treatment of MDR-TB, particularly for susceptible *M. tuberculosis* infections.

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1. Introduction

The emergence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) poses a serious problem for tuberculosis control [1,2]. In 2016, 4.1% of new and 19% of previously treated tuberculosis cases were estimated to have had MDR-TB/RR-TB (rifampin resistance-TB cases including MDR-TB cases), and an estimated 6.2% of patients with MDR-TB had XDR-TB [3]. Several new treatment strategies are being evaluated and have shown excellent results [3,4]. However, more effort is needed to provide information about resistant tuberculosis control.

Clofazimine (CFZ), a riminophenazine antimicrobial agent, was initially used to treat multibacillary leprosy as a component of the World Health Organization (WHO)-recommended triple-drug regimen [5]. CFZ was also listed as one of the group five drugs in the treatment of MDR-TB [6]. Several studies have shown that CFZ has excellent activity against *Mycobacterium tuberculosis* [7,8]. Further studies of synergistic activities of CFZ with other antibiotics are needed to establish a reasonable drug combination against *M. tuberculosis*.

Capreomycin (CAP) is one of the main second-line antituberculous drugs and has been used for almost 50 years. It is an injectable, primarily bacteriostatic drug and is thought to exert antimicrobial effects by inhibiting protein synthesis. The resistance mechanism of CAP has not been clarified; mutations in *rrs, tlyn* and *eis* promoter have been reported to be associated with CAP resistance. The fluoroquinolone, moxifloxacin (MOX) has been reported to show excellent activity against *M. tuberculosis*. MOX was listed by the WHO as a component of several new regimens being tested in Phase II or Phase III trials [3].

The purpose of this work was to assess the activity of CFZ in combination with CAP or MOX against clinical strains of

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Table 1
Drug susceptibility patterns of 29 clinical *M. tuberculosis* isolates

susceptibility or resistance	number of strains
fully susceptible	2
R-monoresistant	6
H-monoresistant	2
Over poly-resistant	4
HS	1
RO	1
OE	1
HSCKO	1
Overall MDR	13
HR	3
HRS	4
HREO	1
HRSO	1
HRSEO	4
Overall XDR	2
HRSKO	1
HRSOCKA	1

Note, H, isoniazid; R, rifampicin; S, streptomycin; E, ethambutol; K, kanamycin; O, ofloxacin; C, capreomycin; A, amikacin.

M. tuberculosis. Synergistic activity has been reported with 8 and 5 of 24 MDR strains against CFZ/MOX combination and CFZ/CAP combination, respectively; however, maximum fractional inhibitory concentration index (FICI) was not reported [8]. The data reported from the current study will increase our knowledge of CFZ.

2. Methods

2.1. Bacteria

The laboratory strain, *M. tuberculosis* H37Rv and 29 clinical strains of *M. tuberculosis* were included in this study. The isolate profiles of drug susceptibility were evaluated by the proportional method using Lowenstein-Jensen slants with the following: isoniazid, 0.2 µg/mL; rifampicin, 40 µg/mL; streptomycin, 4 µg/mL; ethambutol, 2 µg/mL; kanamycin, 30 µg/mL; ofloxacin, 2 µg/mL; CAP, 40 µg/mL [9]; and amikacin, 30 µg/mL [10], comprising 2 fully susceptible, 6 rifampicin-monoresistant, 2 isoniazid-monoresistant, 4 poly-resistant and 15 MDR strains. A full susceptibility profile for all strains is shown in Table 1.

2.2. Antimicrobial agents

CFZ, CAP and MOX were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Initially, CAP and MOX were dissolved in distilled water to create stock solutions (1.28 and 0.32 mg/mL, respectively), and CFZ was dissolved in dimethyl sulphoxide (DMSO) at a concentration of 4 mg/mL. The stock solutions were stored at -80°C until they were used. Working solutions were prepared from the stock solutions by dilution with 7H9 broth medium.

2.3. Minimum inhibitory concentration (MIC) determination

The MICs of CFZ, CAP and MOX for 30 strains were determined by Microplate alamar blue assays using Middlebrook 7H9 broth medium [11]. Briefly, 0.1 mL serial two-fold dilutions of each drug solution (ranging from 0.016 to 32 µg/mL) were prepared with 7H9 broth medium. These were dispensed into the wells of a 96-well microplate, followed by inoculation of 1.5×10^7 CFU/well of each *M. tuberculosis* strain suspended in 7H9 broth medium. Thus, the final drug concentrations ranged from 0.008 to 16 µg/mL. The MIC was defined as the lowest concentration of drug that completely inhibited bacterial growth. Each drug susceptibility test was performed in triplicate.

2.4. Combination testing

A checkerboard method [12] was performed to determine potential synergistic or additive effects of combinations of CFZ with CAP or MOX. These drugs were tested in combination at the MIC of 4-fold MIC to 1/32-fold MIC. The fractional inhibitory concentrations (FIC) were calculated as follows:

FIC_a= MIC of A in combination/MIC of A alone
FIC_b= MIC of B in combination/MIC of B alone
A means CAP or MOX, B means CFZ.

The FICI of the two compounds in the combination was calculated as follows: FICI= FIC_a+FIC_b.

FICIs were interpreted as follows: <0.5 synergy; 0.5~4 indifference; and >4 antagonism [13-15].

2.5. Data analysis

Statistical analysis was performed using SPSS v.16.0 (SPSS Inc.,Chicago, IL). Differences were considered to be statistically significant at $P < 0.05$.

3. Results

3.1. Minimum inhibitory concentrations

The MIC distribution of 30 *M. tuberculosis* strains against CFZ ranged from 0.016 µg/mL to 2 µg/mL. Using the resistance breakpoint of ≥2 µg/mL for CFZ, all 30 strains were classified as susceptible.

The MIC distributions of 30 *M. tuberculosis* strains against CAP ranged from 0.25 µg/mL to 4 µg/mL. Using the resistance breakpoint of ≥2.5 µg/mL for CAP, 27 strains were classified as susceptible, and 3 strains were classified as resistant. The MIC distribution of 30 *M. tuberculosis* strains against MOX ranged from 0.016 µg/mL to 1 µg/mL. Using the resistance breakpoint of ≥2 µg/mL for MOX, all 30 strains were classified as susceptible.

3.2. Synergistic effect of CFZ combined with CAP

A total of 30 *M. tuberculosis* strains were tested for the synergistic effects of CFZ and CAP. As showed in Table 2, for the minimum FICIs, synergy was found in 21 isolates (70.00%), indifference in 9 isolates (30.00%), and antagonism in 0 isolates; for the maximum FICIs, synergy was found in 1 isolate (3.33%), indifference in 17 isolates (56.67%), and antagonism in 12 isolates (40.00%). According to the minimum FICIs, synergy most often occurred when the concentration of CFZ in combination was 1/32 MIC of that tested alone (found in 19/21 stains) and the concentration of CAP in combination was 1/4 MIC (found in 11/21strains) of that tested alone. When the concentration of CAP was fixed, increasing the CFZ concentration from 1/32- to 4-fold MIC of that tested alone led to an antagonistic effect in CFZ/CAP combination in 9 *M. tuberculosis* strains.

According to the minimum FICIs, the prevalence of a synergistic effect between CFZ and CAP in the MDR/XDR group was 67.7% (10/15), which was similar to that of the other drug-resistant and pan-sensitive group (11/15; 73.3%) (P=1.000), see Table 3.

According to the maximum FICIs, the prevalence of an antagonistic effect in the MDR/XDR group was 67.7% (10/15), which was significantly higher than that of the other drug-resistant and pan-sensitive group (2/15; 13.3%) (P=0.003), see Table 4.

3.3. Synergistic effect of CFZ combined with MOX

A total of 30 *M. tuberculosis* strains were tested for the synergistic effects of CFZ and MOX. As showed in Table 5, for the minimum

Table 2
Minimum inhibitory concentrations (MICs) and fractional inhibitory concentration indexes (FICIs) of clofazimine (CFZ) and capreomycin (CAP) against 30 *M. tuberculosis* isolates

Strain no.	Drug resistance*	MIC (μg/mL)		minimum FICI				maximum FICI			
		CFZ	CAP	FICI	relationship	MIC fold in combination		FICI	relationship	MIC fold in combination	
						CFZ	CAP			CFZ	CAP
FJ09127	RO	256	1	0.156	SYN	1/32	1/8	1.25	ID	1	1/4
H37Rv	pan-sensitive	0.031	0.5	0.156	SYN	1/32	1/8	0.25	SYN	1/8	1/8
FJ14031	HRSEO	0.5	1	0.281	SYN	1/32	1/4	1.25	ID	1	1/4
FJ07003	HRSEO	0.5	1	0.281	SYN	1/32	1/4	2.063	ID	2	1/16
HN11213	HRSO	0.125	1	0.281	SYN	1/32	1/4	4.25	AN	4↑	1/4
XZ06212	HR	0.125	1	0.281	SYN	1/32	1/4	4.25	AN	4↑	1/4
XZ06209	HRS	0.125	1	0.281	SYN	1/32	1/4	4.125	AN	4	1/8
HN04054	R	0.063	1	0.281	SYN	1/32	1/4	0.75	ID	1/2	1/4
FJ05415	H	0.063	1	0.281	SYN	1/32	1/4	0.75	ID	1/2	1/4
HeN05030	R	0.25	1	0.281	SYN	1/32	1/4	1.25	ID	1	1/4
SC06157	R	0.5	1	0.281	SYN	1/32	1/4	2.125	ID	2	1/8
XZ06115	R	0.5	1	0.281	SYN	1/32	1/4	2.25	ID	2	1/4
XZ09033	pan-sensitive	0.125	1	0.281	SYN	1/32	1/4	2.25	ID	2	1/4
XZ06107	HRS	0.125	1	0.531	SYN	1/32	1/2	4.5	AN	4↑	1/2
XZ06118	HR	0.25	1	0.531	SYN	1/32	1/2	4.5	AN	4↑	1/2
XZ10223	HR	0.5	1	0.531	SYN	1/32	1/2	4.25	AN	4	1/4
XZ06125	R	0.5	1	0.531	SYN	1/32	1/2	1	ID	1	1/2
XZ06196	R	0.5	1	0.531	SYN	1/32	1/2	2.016	ID	2	1/64
XZ09109	pan-sensitive	0.063	2	0.531	SYN	1/32	1/2	1.5	ID	1	1/2
HN11240	HREO	0.5	0.5	1.031	ID	1/32	1	2	ID	1	1
FJ09125	HRSEO	0.5	0.5	1.031	ID	1/32	1	5	AN	4↑	1
XZ06147	H	0.25	0.5	1.031	ID	1/32	1	2	ID	1	1
FJ05161	HRSKO	1	0.5	1.031	ID	1/32	1	5	AN	4↑	1
SC06120	HRS	0.5	4	1.5	ID	1	1/2	4.5	AN	4↑	1/2
SC06038	HS	0.5	0.5	2.031	ID	1/32	2	6	AN	4↑	2
FJ14005	HRSOCKA	0.5	4	2.031	ID	1/32	2	6	AN	4↑	2
HN11198	HRS	0.031	0.25	<0.502	SYN	1/2	1/512	2.25	ID	1/4	2
AH11291	HRSEO	256	1	<0.127	SYN	1/8	1/512	1.063	ID	1/16	1
FJ09133	OE	256	0.5	<2.002	ID	2	1/512	3	ID	1	2
ZJ06019	HSCKO	0.25	4	<2.002	ID	2	1/512	4.063	AN	1/16	4

Note, *H, isoniazid; R, rifampicin; S, streptomycin; E, ethambutol; K, kanamycin; O, ofloxacin; C, capreomycin; A, amikacin. SYN, synergy; ID, indifference; AN, antagonism. ↑ when the concentration of CAP was fixed, higher CFZ concentrations led to an antagonistic effect in the CAP/CFZ combination.

Table 3
Synergy analysis (according to the minimum FICIs) of CFZ/CAP combination between MDR/XDR and other *M. tuberculosis* strains

Group	No. of strains showing synergy	No. of strains showing indifference	χ^2	P
MDR/XDR strains(15)	10	5	0.000	1.000
other strains(15)	11	4		

Note, CAP, capreomycin; CFZ, clofazimine; FICIs - fractional inhibitory concentration indexes; MDR – multidrug-resistant; XDR, extensively drug-resistant

Table 4
Antagonism analysis (according to the maximum FICIs) of CFZ/CAP combination between MDR/XDR and other *M. tuberculosis* strains

Group	No. of strains showing antagonism	No. of strains showing no antagonism	χ^2	P
MDR and XDR strains(15)	10	5	8.89	0.003
other strains(15)	2	13		

Note, CAP, capreomycin; CFZ, clofazimine; FICIs - fractional inhibitory concentration indexes; MDR – multidrug-resistant; XDR, extensively drug-resistant

FICI of each isolate, synergy was found in 29 isolates (96.67%), indifference in 1 isolate (3.33%), and antagonism in 0 isolates. For the maximum FICI of each isolate, synergy was found in 4 isolates (13.33%), indifference in 17 isolates (56.67%), and antagonism in 9 isolates (30.00%). According to the minimum FICIs, synergy most often occurred when the concentration of CFZ in combination was 1/32 MIC of that tested alone (found in 29/29 stains) and the concentration of MOX in combination was 1/32 MIC (found in 11/29 strains) or 1/64 MIC (found in 10/29 strains) of that tested alone. According to the maximum FICIs, there were 9 *M. tuberculosis* strains that showed antagonism, while the concentration of CFZ of 9 strains was 4-fold MIC of that tested alone, combined with the concentrations of MOX of 8 strains and 1 strain were from 1/8 to 1/64 MIC and 2-fold MIC of that tested alone, respectively. When the concentration of MOX was fixed, increasing the CFZ concentration from 1/32- to 4-fold MIC of that tested alone led to an antagonistic effect in CFZ/MOX combination in 4 *M. tuberculosis* strains.

According to the minimum FICIs, the prevalence of synergy between CFZ and MOX in the MDR/XDR group was 93.33% (14/15), which was similar to that of the other drug-resistant or pan-sensitive group (15/15; 100.00%) ($P = 1.000$), see Table 6.

According to the maximum FICIs, the prevalence of an antagonism effect in the MDR/XDR group was 53.33% (8/15), which was significantly higher than that of the other drug-resistant or pan-sensitive group (1/15; 6.67%) ($P = 0.005$), see Table 7.

3.4. Comparing results of the CFZ/CAP and CFZ/MOX combinations

According to the minimum FICIs, the CFZ/MOX combination was more likely to show synergy against *M. tuberculosis* than the CFZ/CAP combination, $P = 0.006$, see Table 8.

According to the maximum FICIs, there was no statistically significant difference in the antagonistic effect between CFZ/CAP and CFZ/MOX combination against *M. tuberculosis,* $P = 0.417$, see Table 9.

Table 5
Minimum inhibitory concentrations (MICs) and fractional inhibitory concentration indexes (FICIs) of clofazimine (CFZ) and moxifloxacin (MOX) against 30 *M. tuberculosis* isolates

Strain no.	Drug resistance*	MIC (μg/mL)		Minimum FICI				Maximum FICI			
		CFZ	MOX	FICI	relationship	MIC fold in combination		FICI	relationship	MIC fold in combination	
						CFZ	MOX			CFZ	MOX
FJ14031	HRSEO	0.5	1	0.047	SYN	1/32	1/64	1.063	ID	1	1/16
FJ14005	HRSOCKA	0.5	0.0625	0.063	SYN	1/32	1/32	4.031	AN	4↑	1/32
AH11291	HRSEO	256	0.0625	0.094	SYN	1/32	1/16	0.188	SYN	1/8	1/16
FJ05161	HRSKO	1	0.25	0.063	SYN	1/32	1/32	4.063	AN	4	1/16
FJ05415	H	0.063	0.0625	0.047	SYN	1/32	1/64	1.031	ID	1	1/16
FJ09103	HR	0.5	0.031	0.281	SYN	1/32	1/4	1.250	ID	1	1/4
FJ09125	HRSEO	0.5	0.25	0.094	SYN	1/32	1/16	4.125	AN	4	1/8
FJ09127	RO	256	0.0625	0.047	SYN	1/32	1/64	1.016	ID	1	1/64
FJ09133	OE	256	0.0625	0.063	SYN	1/32	1/32	1.031	ID	1	1/32
H37Rv	pan-sensitive	0.031	0.125	0.063	SYN	1/32	1/32	0.266	SYN	1/4	1/64
HeN05030	R	0.25	0.031	0.063	SYN	1/32	1/32	1.031	ID	1	1/32
HN04054	R	0.063	0.031	0.063	SYN	1/32	1/32	1.063	ID	1	1/16
HN11198	HRS	0.031	0.016	0.063	SYN	1/32	1/32	0.531	SYN	1/2	1/32
HN11213	HRSO	0.125	1	0.047	SYN	1/32	1/64	2.031	ID	2	1/32
HN11240	HREO	0.5	1	0.047	SYN	1/32	1/64	1.031	ID	1	1/32
SC06038	HS	0.5	0.016	0.094	SYN	1/32	1/16	2.031	ID	2	1/32
SC06120	HRS	0.5	0.5	0.063	SYN	1/32	1/32	1.063	ID	1	1/16
SC06157	R	0.5	0.0625	0.047	SYN	1/32	1/64	4.016	AN	4↑	1/64
XZ06107	HRS	0.125	0.125	0.047	SYN	1/32	1/64	4.016	AN	4↑	1/64
XZ06115	R	0.5	0.25	0.047	SYN	1/32	1/64	2.031	ID	2	1/32
XZ06118	HR	0.25	0.016	0.094	SYN	1/32	1/16	4.063	AN	4↑	1/16
XZ06125	R	0.5	0.0625	0.063	SYN	1/32	1/32	1.031	ID	1	1/32
XZ06147	H	0.25	0.016	0.047	SYN	1/32	1/64	0.531	SYN	1/2	1/32
XZ06196	R	0.5	0.031	0.094	SYN	1/32	1/16	2.125	ID	2	1/8
XZ06209	HRS	0.125	0.031	0.047	SYN	1/32	1/64	4.031	AN	4	1/32
XZ09033	pan-sensitive	0.125	0.031	0.063	SYN	1/32	1/32	2.063	ID	2	1/16
XZ09109	pan-sensitive	0.063	0.0625	0.094	SYN	1/32	1/16	1.063	ID	1	1/16
XZ10223	HR	0.5	0.016	0.094	SYN	1/32	1/16	4.125	AN	4	1/8
ZJ06019	HSCKO	0.25	0.25	0.063	SYN	1/32	1/32	2.016	ID	2	1/64
XZ06212	HR	0.125	0.031	1.031	ID	1/32	1	6.000	AN	4	2

Note, *H, isoniazid; R, rifampicin; S, streptomycin; E, ethambutol; K, kanamycin; O, ofloxacin; C, capreomycin; A, amikacin. SYN, synergy; ID, indifference; AN, antagonism. ↑when the concentration of MOX was fixed, higher CFZ concentrations led to antagonistic effects in the MOX/CFZ combination.

Table 6
Synergy analysis (according to the minimum FICIs) of CFZ/MOX combination between MDR/XDR and other *M. tuberculosis* strains

Group	No. of strains showed synergy	No. of strains showed antagonism or indifference	χ^2	*P*
MDR/XDR strains(15)	14	1	0.000	1.000
other strains(15)	15	0		

Note, CFZ, clofazimine; FICIs - fractional inhibitory concentration indexes; MDR – multidrug-resistant; MOX, moxifloxacin; XDR, extensively drug-resistant

Table 7
Antagonism analysis (according to maximum FICIs) of CFZ/MOX combination between MDR/XDR and other *M. tuberculosis* strains

Group	No. of strains showed antagonism	No. of strains showed synergy or indifference	χ^2	*P*
MDR/XDR strains(15)	8	7	7.78	0.005
other strains(15)	1	14		

Table 8
Comparison results of the minimum FICIs between CFZ/CAP and CFZ/MOX combinations against *M. tuberculosis*

Group	No. of strains showed synergy	No. of strains showed indifference	χ^2	*p*
CFZ/CAP combination	21	9	7.68	0.006
CFZ/MOX combination	29	1		

Table 9
Comparison results of the maximum FICIs between CFZ/CAP and CFZ/MOX combinations against *M. tuberculosis*

Group	*No.* of strains showed antagonism	*No.* of strains showed synergy or indifference	χ^2	*P*
CFZ/CAP combination	12	18	0.659	0.417
CFZ/MOX combination	9	21		

4. Discussion

CFZ, an antimicrobial agent against *Mycobacterium leprae* and non-tuberculous mycobacteria, was introduced to cure MDR-TB because of the impressive treatment outcome for MDR-TB patients [7]. Zhang et al. reported that >70% of MDR-TB isolates collected from the national drug resistance survey conducted in China were susceptible to CFZ [8]. In the current study, 29 *M. tuberculosis* clinical isolates, including 15 MDR isolates, were susceptible to CFZ, which indicates CFZ may be a potential candidate for treating MDR-TB in China.

In addition to its primary antimicrobial activity, CFZ has anti-inflammatory/immunosuppressive properties and is used in chronic inflammatory disorders that are predominantly cutaneous in origin [7,16], and in non-cutaneous autoimmune disorders, such as multiple sclerosis and type I diabetes mellitus [17]. The secondary immunosuppressive properties of CFZ may be detrimental or potentially beneficial in tuberculosis treatment [7]. Chaisson et al. reported that the addition of CFZ to a regimen of clarithromycin and ethambutol for *Mycobacterium avium* complex

(MAC) bacteremia in acquired immunodeficiency syndrome (AIDS) patients did not contribute to the clinical response and was associated with higher mortality [18]. Therefore, it is important to consider the excellent antimicrobial activity and immunosuppressive properties of CFZ in tuberculosis treatment, particularly in patients with severe disease associated with advanced immunosuppression.

Multidrug combination chemotherapy containing fluoroquinolones and second-line injectable drugs is often used for MDR-TB treatment [19]. Thus, we determined the in vitro activities of MOX and CAP in combination with CFZ against MDR/XDR-TB isolates and other strains with different susceptibility patterns to explore the clinical utility of different combination regimens against MDR-TB. Synergies between MOX and other antituberculous drugs have been reported [8]. In this study, according to the minimum FICIs, there was synergy in 29 strains (96.67%) against CFZ/MOX combination and 21 strains (70.00%) against CFZ/CAP combination; therefore, both combinations showed excellent synergy. In a previous study, synergy was found in only 33.3% (8/24) and 20.8% (5/24) of MDR strains against CFZ/MOX and CFZ/CAP combination, respectively [8]. The reasons for the difference may be that in this study we used the checkerboard method, the concentration range was from 4-fold to 1/32-fold of separate CFZ MIC *value* and from 4-fold to 1/512-fold of separate CAP or MOX MIC value, which was larger than the range reported in the study by Zhang et al. in which the drugs were tested in combination at the MIC of each and at four 2-fold dilutions lower [8].

In the current study, according to the minimum FICIs, the percentage of synergy of the CFZ/CAP combination was lower than that of the CFZ/MOX combination, which indicated that MOX has better synergistic activity against *M. tuberculosis* than CAP when combined with CFZ. Although the exact mechanism(s) of CFZ-mediated antimicrobial activity remains to be established, as reviewed by Cholo et al. [7], the outer membrane appears to be the primary site of action of this agent. We speculated that CFZ increases the permeability of the bacterial envelope by disrupting membrane structure and function [16], thereby enhancing the penetration of other drugs.

However, according to the maximum FICIs, there was no statistically significant difference in the percentage of antagonism between CFZ/MOX and CFZ/CAP combinations. The concentrations of each drug for calculating the maximum FICIs were much higher than for the minimum FICIs for most of the strains, and the number of strains showing indifference or antagonism of both combinations were increased. We speculated that the doses of drugs in the regimen were important and that higher concentrations may lead to antagonism between drugs in the chemotherapy. Also, according to the maximum FICIs, the prevalence of antagonistic effect in MDR/XDR strains was higher than that in other drug-resistant strains or pan-sensitive strains, whether in CFZ/CAP or CFZ/MOX combination. Velayati et al. demonstrated that the cell wall of MDR-TB isolates was significantly thicker compared with that of susceptible strains [20]. The intrinsic feature of the cell envelope in MDR-TB isolates may influence the synergistic effect between CFZ and CAP or MOX.

In conclusion, CFZ/MOX and CFZ/CAP combinations both showed excellent synergism against *M. tuberculosis,* with MOX showing better synergism than CAP combined with CFZ. In addition, the MDR/XDR isolates were more likely to show antagonism than the other drug-resistant or pan-sensitive strains in both the CFZ/MOX and CFZ/CAP combinations. CFZ in combination with MOX may be a promising drug regimen for the treatment of MDR-TB, particularly for non-MDR-TB. The synergistic effect between CFZ and CAP or MOX in this study requires clinical validation; more laboratory and clinical studies are needed to quantify the doses of drugs in the regimen against *M. tuberculosis*.

Declarations

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Declaration of Competing Interests

None declared.

Ethical Approval

Not required.

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