

In vitro effect of fluoroquinolones against *Mycobacterium tuberculosis* isolates from Agra & Kanpur region of north India

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Background & objectives: Fluoroquinolones (FQs) are important drugs used for treatment of drug resistant tuberculosis and are also now being considered as first line drugs to shorten the duration of treatment of tuberculosis (TB). In order to find out useful FQs for treatment of tuberculosis, the comparative efficacy of five FQs, namely, ofloxacin (OFL), ciprofloxacin (CIP), sparfloxacin (SPX), gatifloxacin (GAT) and levofloxacin (LEVX) was studied against *Mycobacterium tuberculosis* (MTB) isolates obtained from both treated and untreated patients from Agra and Kanpur regions of north India.

Methods: A total of 162 MTB isolates [including 110 MTB isolates obtained from untreated patients (Cat-I) and 52 isolates from treated patients (Cat-II)] were tested for their susceptibilities to FQs using standard minimum inhibitory concentration (MIC) method on Löwenstein-Jensen medium.

Results: Keeping in view the therapeutically achievable drug levels, it was found that in Cat-I 97.2 per cent (107/110) isolates were sensitive to GAT, 89 per cent (98/110) to LEVX at 1 µg/ml whereas 92.7 per cent (102/110) isolates were inhibited by OFL at 2 µg/ml and 73.6 per cent (81/110) to SPX at 0.5 µg/ml. Only 63.6 per cent (70/110) isolates were found to be sensitive to CIP at 2 µg/ml which increased to 89 per cent (98/110) at 4 µg/ml (higher than achievable peak serum level). On the other hand, among 52 isolates for Cat-II, 37 (71.2%) were found to be sensitive to GAT and 33 (63.5%) to LEVX at 1 µg/ml concentration, 28 (53.8%) to SPX at 0.5 µg/ml whereas 33 (63.5%) and 24 (46.2%) isolates were found to be sensitive to OFL and CIP at 2 µg/ml, respectively.

Interpretation & conclusions: It appears that GAT has higher activity against MTB isolates followed by OFL, LEVX and SPX whereas CIP showed the lowest activity. GAT was also found to be the most effective FQ against multi-drug resistant (MDR) isolates both from Cat-I and Cat-II patients. Thus, except CIP, other FQs showed potential to be included in the treatment regimens of tuberculosis including MDR-TB.

Key words Fluoroquinolones - *Mycobacterium tuberculosis* - resistance - sensitivity

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Tuberculosis (TB) is one of the serious public health problems in both developing and developed countries. Two billion people, 1/3rd of the world's population are estimated to be infected with *Mycobacterium tuberculosis*¹. As a consequence of the inappropriate use of essential anti-TB drugs, tubercle bacilli have increasingly become resistant to one or more of these drugs. Treatment of drug resistant, especially multi drug resistant tuberculosis (MDR-TB), is expensive and the mortality rate is usually high². The global project on anti-tuberculosis drug resistance surveillance revealed that the overall primary multi drug resistance (MDR-TB) was 1.1 per cent (range 0-14.2%) whereas acquired MDR-TB was estimated to be 7.0 per cent (range 0-58.3%)³. In India, an overall prevalence of about 3 per cent for MDR-TB has been estimated in untreated cases⁴. Higher rates of drug resistance necessitate the introduction of other drugs in TB treatment regimens.

Among the alternate drugs available for the treatment of drug resistant TB cases, fluoroquinolones (FQs) have prominent place and are often recommended^{5,6}. FQs have also been tried to shorten the duration of treatment⁷. However, there is a need for in depth studies to know the therapeutic potential of FQs in different geographical settings. Hence, the present study was planned to determine the minimum inhibitory concentrations (MICs) of five FQs *viz.*, ofloxacin (OFL), ciprofloxacin (CIP), sparfloxacin (SPX), gatifloxacin (GAT) and levofloxacin (LEVX) against *M. tuberculosis* isolates collected from patients from Agra and Kanpur region of North India.

Material & Methods

***M. tuberculosis* isolates:** A total of 162 isolates of *M. tuberculosis* from Mycobacterial Repository Centre of National JALMA Institute for Leprosy & other Mycobacterial Diseases, Agra, were included in this study. One hundred and ten isolates were obtained from untreated patients (Cat-I) and 52 from treated patients (Cat-II). Among the Cat-I isolates, 77 were collected during July, 2004 to February, 2005 from Kanpur district, India and 33 during February, 2005 to June, 2005 from Agra district. All 52 Cat-II isolates were obtained during February, 2005 to June, 2005 from Agra district, India. Identification of these isolates as *M. tuberculosis* was based on biochemical properties⁸.

Drug concentrations: Drug stocks of OFL (Sigma Chemical Co., USA) and GAT (Mankind Pharmaceuticals, India) were prepared in distilled water acidified with minimal amount of 1N HCl to facilitate the

dissolution. SPX (Fluka, Canada) was initially dissolved in 0.1N NaOH and subsequently diluted with distilled water⁹. CIP (Hi-Media, India), LEVX (Fluka, Canada) and isoniazid (INH) (Sigma Chemical Co., USA) drug stocks were prepared in distilled water. Rifampicin (RIF) (Sigma Chemical Co., USA) was dissolved in dimethyl sulphoxide to prepare the stock solution. These drugs were added aseptically to the Löwenstein-Jensen (LJ) medium to give the desired final preinoculation concentrations of 2, 4 and 6 µg/ml for OFL and CIP¹⁰, 0.5, 1 and 2 µg/ml for SPX^{11,12} and LEVX^{10,13,14}, 0.25, 0.5 and 1 µg/ml for GAT¹⁴, 1 µg/ml for INH¹⁵ and 64 µg/ml for RIF^{16,17}.

Drug susceptibility testing: *M. tuberculosis* isolates were tested for their susceptibilities to FQs, INH and RIF using standard minimum inhibitory concentration (MIC) method¹⁸. The standard bacterial suspension of 4 mg/ml was prepared¹⁹ and used as inoculum with the help of a loop (3 mm internal diameter) to inoculate on to LJ slants. The culture bottles were incubated at 37°C and readings were taken after 4 wk of incubation. The MIC was determined by counting the colony forming units (cfu) and comparing with control cultures. The standard strain of *M. tuberculosis* H37Rv was also tested which were found to be sensitive at <1 µg/ml levels for OFL and CIP, <0.25 µg/ml GAT, LEVX and SPX. An isolate was considered resistant if it yielded a growth of 20 colonies or more at a particular concentration of drug. The term MDR refers to those bacilli that are resistant to RIF and INH. To assess the quality of inoculum, culture control was read as growth of ++ (more than 100 colonies, usually 150-200 colonies) and considered essential for reading the results, otherwise the procedure is repeated. MIC was defined as the minimum drug concentration inhibiting the growth of *M. tuberculosis* isolate^{17,18}. The corresponding cut-off concentrations were 64 µg/ml for RIF, 1 µg/ml for INH, 2 µg/ml for OFL and CIP, 1 µg/ml for LEVX, GAT and 0.5 µg/ml for SPX.

Statistical analysis: Data of susceptibility tests were analyzed with Stata-7 statistical software (Stata Corporation, TX, USA). Chi square (χ^2) test and Fisher's exact test were used at 5 per cent level of significance to test the significance difference in activity of different FQs (GAT, OFL, SPX, LEVX and CIP) at their critical concentration.

Results & Discussion

Drug susceptibility results of the tested FQs are summarized in Table I. Of the 162 isolates tested, 33 (20.3%) were found to be MDR (*i.e.*, simultaneously resistant to RIF and INH). Of these 33 MDR isolates,

8/110 (7.2%) were found to be Cat-I cases while 25/52 (48%) were among Cat-II cases (Table I).

In developing countries like India, LJ medium is being used as standard medium for susceptibility testing of mycobacteria^{10,20,21}. For the drugs which have low MIC/MBC (minimum inhibitory concentration/minimum bactericidal concentration) ratio, for example, FQs, the MICs have been reported to be in the same range when determined in egg medium²², agar medium²³ or in broth²⁴. Moreover, due to high cost, tedious procedure and difficulties in interpretation of results, the MIC determination in broth has not been used widely²⁵. MIC determinations for CIP, OFL, GAT and SPX have also yielded similar MIC ranges in both 7H11 and LJ media²¹.

In our study susceptibility testing for quinolones was performed by the absolute concentration method as according to the WHO it is also one of the standard methods for performing drug sensitivity testing (DST)^{18,26}. The proportion method is much more definitive but

MIC method is more convenient and fairly reliable. Moreover, proportional susceptibility testing (PST) is more time consuming than a minimum inhibitory concentration determination^{18,27}. MIC method has earlier been standardized by others for quinolones and used satisfactorily^{10,21}. We have also tested the same in our laboratory and observed good concordance with PST.

Table II depicts the inhibition abilities of FQs; of the 110 isolates from Cat-I patients, 107 (97.2%) were found to be sensitive to GAT and 98 (89%) to LEVX at 1 µg/ml, 81 (73.6%) to SPX at 0.5 µg/ml concentration whereas 102 (92.7%) and 70 (63.6%) isolates were respectively found to be sensitive to OFL and CIP drugs at 2 µg/ml respectively. On statistical analysis, these five drugs showed a significant difference in their inhibition abilities. A decreasing order of *M. tuberculosis* isolates inhibited at critical concentration of various FQs was GAT>OFL>LEVX>SPX>CIP. While in the case of 8 MDR isolates of Cat-I cases, 7 (87.5%) were sensitive to GAT and 4 (50%) to LEVX at 1 µg/ml, 3 (37.5%)

Table I. Determination of fluoroquinolone MICs against MTB isolates

Fluoroquinolone	No. (%) of isolates with MIC (µg/ml)					
	0.25	0.5	1	2	4	6
<i>Cat I patients</i> (n=110)						
GAT	52 (47.27)	92 (83.63)	107 (97.2)	ND	ND	ND
SPX	ND	81 (73.6)	105 (95.4)	108 (98.1)	ND	ND
LEVX	ND	65 (59)	98 (89.09)	106 (96.3)	ND	ND
OFL	ND	ND	ND	102 (92.7)	107 (97.2)	107 (97.2)
CIP	ND	ND	ND	70 (63.6)	98 (89.09)	107 (97.2)
<i>Cat II patients</i> (n=52)						
GAT	21 (40.4)	32 (61.5)	37 (71.2)	ND	ND	ND
SPX	ND	28 (53.8)	36 (69.2)	41 (78.8)	ND	ND
LEVX	ND	22 (42.3)	33 (63.5)	36 (69.2)	ND	ND
OFL	ND	ND	ND	33 (63.5)	35 (67.3)	37 (71.2)
CIP	ND	ND	ND	24 (46.2)	30 (57.6)	35 (67.3)

ND, not determined. GAT, gatifloxacin; SPX, sparfloxacin; LEVX, levofloxacin; OFL, ofloxacin; CIP, ciprofloxacin

Table II. Inhibition of Cat-I isolates at critical concentrations of FQs

Isolates	No. (%) of isolates with MIC (µg/ml)					P values
	OFL 2	CIP 2	SPX 0.5	GAT 1	LEVX 1	
Non MDR (n=102)	96 (94.12)	67 (65.39)	78 (76.4)	100 (98.04)	94 (92.16)	P<0.0001
MDR (n=8)	6 (75)	3 (37.5)	3 (37.5)	7 (87.5)	4 (50)	P*=0.267
Total (n=110)	102 (92.73)	70 (63.6)	81 (73.6)	107 (97.27)	98 (89.09)	P<0.0001

p (χ^2 with 4 degree of freedom); P* (Fisher's exact test)

MDR, multidrug resistant; remaining abbreviations as in Table I

to SPX at 0.5 µg/ml concentration, 6 (75%) isolates were found to be sensitive to OFL and 3 (37.5%) to CIP at 2 µg/ml. There was no statistically significant difference between inhibition abilities of these drugs in case of MDR isolates from Cat I cases ($P=0.267$; Table II). In pair-wise comparison of drug activity of GAT and OFL, OFL and LEVX, LEVX and GAT and CIP and SPX, no significant difference was found. GAT, OFL and LEVX showed significantly better activity in comparison to CIP and SPX ($P<0.05$).

Of the 52 isolates from Cat-II patients, 37 (71.2%) were found to be sensitive to GAT and 33 (63.5%) to LEVX at 1µg/ml, 28 (53.8%) to SPX at 0.5 µg/ml concentration whereas 33 (63.5%) and 24 (46.2%) isolates were respectively found to be sensitive to OFL and CIP at 2 µg/ml (Table III). Among these 52 isolates from Cat-II patients, 25 were MDR. Of these, 14 (56%) were sensitive to GAT and 10 (40%) to LEVX at 1 µg/ml concentration, 10 (40%) to SPX at 0.5 µg/ml whereas 9 (36%) isolates were found to be sensitive to both OFL and CIP at 2 µg/ml (Table III). In the remaining 27 non MDR isolates, 24 (88.8%) were inhibited by OFL and 15 (55.5%) by CIP at 2 µg/ml whereas 18 (66.6%) were inhibited by SPX at 0.5 µg/ml, 23 (85.2%) each by GAT and LVX at 1 µg/ml concentration, respectively. There was significant difference ($P<0.002$) among FQs in non MDR isolates in this category. Almost similar findings in Cat-II isolates have been reported by other investigators^{21,28,29}. The Philippines study²⁹ showed that 64.7 per cent isolates were sensitive to OFL, 73.2 per cent to CIP and 48.6 per cent MDR isolates were sensitive to CIP and OFL. Similarly, one study from India reported that 40 per cent isolates were sensitive to OFL and CIP (at 8 µg/ml conc.), 41.81 per cent to SPX (at 2 µg/ml), while GAT (at 2 µg/ml) sensitivity was found in 60 per cent isolates²¹.

Of the 33 MDR isolates from both Cat-I and Cat-II, 45.5 per cent were susceptible to OFL, 36.6 per cent to CIP at 2 µg/ml, 39.4 per cent to SPX at 0.5 µg/ml, while 42.4 per cent to LVX, and 63.6 per cent were sensitive to GAT at 1µg/ml. Among MDR group GAT (63.6%) showed higher activity than other FQs but there was no significant difference.

Our results showed that FQs were more effective in Cat-I cases whereas these drugs might be comparatively less useful in Cat-II cases. The reason of this lower efficacy may be prior use of these drugs alone in the failing regimens of Cat-II cases resulting higher resistance rates. This also suggests the need to include FQs in MDR cases only after proper DST.

It is evident from our findings that among FQs, GAT had higher activity at low concentrations (leading to inhibition of 97.2% isolates at 1 µg/ml) followed by OFL (93% inhibition at 2 µg/ml), LEVX (89% inhibition at 1 µg/ml) and SPX (73.6% inhibition at 0.5 µg/ml), while CIP showed the least inhibition ability (inhibiting 63.3% isolates at 2 µg/ml concentration). Even though number of isolates being inhibited at 4 and 6 µg/ml concentration of CIP was much higher (89 and 97.2% respectively), these concentrations are higher than the peak serum level (C_{max}) of the drug in human *i.e.*, 3.5 µg/ml³⁰.

In a Spanish study, 93 per cent isolates were reported to be inhibited by OFL at 1µg/ml concentration³¹ and 98.2 per cent at 4 µg/ml³². Jain *et al*¹⁰ have reported the concentration of 2.04 µg/ml OFL as the MIC of *M. tuberculosis* isolates. In our study it was found that in Cat-I cases, 92.7 per cent isolates were inhibited at 2 µg/ml concentration of OFL whereas 97 per cent isolates were inhibited at 4 µg/ml. These concentrations of OFL are below the peak serum level achievable in humans *i.e.*, 10 µg/ml¹¹.

Table III. Inhibition of Cat-II isolates at critical concentrations of FQs

Isolates	No. (%) of isolates with MIC (µg/ml)					P values
	OFL 2	CIP 2	SPX 0.5	GAT 1	LEVX 1	
Non MDR (n= 27)	24 (88.8)	15 (55.5)	18 (66.6)	23 (85.18)	23 (85.18)	$P<0.002$
MDR (n=25)	9 (36)	9 (36)	10 (40)	14 (56)	10 (40)	$P=0.586$
Total (n=52)	33 (63.5)	24 (46.2)	28 (53.8)	37 (71.2)	33 (63.5)	$P*=0.09$
P^*	$P<0.001$	$P=0.177$	$P=0.09$	$P<0.021$	$P<0.001$	

p (χ^2 with 4 degree of freedom); P^* (Fishers exact test); Abbreviations as in Table II

The activity of GAT and LEVX at their MIC level (*i.e.*, 1 µg/ml) was 97.2 per cent and 89 per cent respectively. This concentration was lower than the peak serum level *i.e.*, 3.8-4.2 µg/ml for GAT and 6.2 µg/ml for LEVX³³. In the present study, the MIC level of SPX was 0.5 µg/ml (inhibiting 73.6% isolates) which was lower than the mean peak serum level *i.e.*, 1.4 µg/ml after 200 mg daily doses¹¹. Though at 1 µg/ml concentration of SPX, 95.4 per cent isolates were inhibited, but this concentration was near to the mean serum peak level. Since peak serum level of GAT is 4.2 µg/ml after 400 mg daily doses, the 'Cmax/MIC' value for GAT is also more than that for SPX. It has been reported that SPX was more active than LEVX, OFL and CIP^{11,12,34-37}. Lubasch *et al*³⁵ reported that SPX was an effective and safe alternative agent in the treatment of complicated tuberculosis and the suitable alternative drug for some cases of MDR tuberculosis. According to Alvirez-Freites *et al*¹⁴, GAT appeared to have sufficient activity alone and in combination with ethanamide with or without pyrazinamide for treatment of tuberculosis. One Indian study has also reported low MIC for GAT²¹. Hence based on the findings of present study and the published literature^{14,21}, GAT appears to have higher activity against *M. tuberculosis* isolates.

Among the 8 MDR isolates (from Cat-I) tested, GAT showed highest activity inhibiting 7 (87.5%) at 1 µg/ml concentration. While comparing the inhibition ability of LEVX at higher concentration (2 µg/ml), which is still much below the peak serum value (*i.e.*, 6.2 µg/ml), 87.5 per cent (7/8) isolates were found to be inhibited. Similarly, 75 per cent (6/8) isolates were sensitive to CIP at 4 and 87.5 per cent (7/8) and SPX 2 µg/ml concentrations, respectively. However, these concentrations were slightly higher than the peak serum levels *i.e.*, 3.5 µg/ml for CIP and 1.4 µg/ml for SPX. In studies based on isolates from treated patients, GAT along with moxifloxacin was found to be most effective followed by SPX, OFL, CIP and lomefloxacin^{11,12,21,34-37}.

In conclusion, fluoroquinolones used in our study showed promising activity (up to 97.2% inhibition for *M. tuberculosis* isolates from untreated patients) at concentrations lower than the peak serum levels. The present study provides the base line data about *in vitro* activity of various quinolones against *M. tuberculosis* from north India. Similar findings have been reported by several investigators from different parts of the world^{12,13,33,38,39}. Also FQs showed better activity in Cat-I cases in comparison to Cat-II. These

drugs may be useful in the treatment of tuberculosis including MDR tuberculosis. Other quinolones, like moxifloxacin which have shown promising results in *in vitro* and animal studies^{32,40}, need further testing.

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References

1. IUATLD. Tuberculosis. In: Activity Report of the International Union Against Tuberculosis and Lung Disease; 2004. p. 6-7.
2. Espinal MA, Laszlo S, Simonsen L, Boulahbat F, Kim SJ, Reniero A, *et al*. Global trends in resistance to antituberculosis drugs. *N Engl J Med* 2001; 344 : 1294-303.
3. World Health Organization. The WHO/IUATLD. *Global Project on Antituberculosis Drug Resistance Surveillance: Antituberculosis drug resistance in the world*. Report No.3. Geneva: Switzerland; 2004. WHO/CDS/TB/2004.
4. Paramasivan CN, Venkataraman P. Drug resistance in tuberculosis in India. *Indian J Med Res* 2004; 120 : 377-86.
5. Tomioka H, Sato K, Kajitani H, Akaki T, Shishido S. Comparative antimicrobial activities of the newly synthesized quinolone WQ-3034, levofloxacin, sparfloxacin and ciprofloxacin against *Mycobacterium tuberculosis* and *Mycobacterium avium* complex. *Antimicrob Agents Chemother* 2000; 44 : 283-6.
6. Aubry A, Pan X-S, Fisher LM, Jarlier V, Cambau E. *Mycobacterium tuberculosis* DNA gyrase: Interaction with quinolones and correlation with antimycobacterial drug activity. *Antimicrob Agents Chemother* 2004; 48 : 1281-8.
7. Jawahar MS, Rahman F. Shortening short course chemotherapy: A randomized clinical trial for treatment of smear positive pulmonary tuberculosis with regimens using ofloxacin in the intensive phase. *Indian J Tuberc* 2002; 49 : 27-38.
8. Vestal AL. In: *Procedure for the isolation and identification of mycobacteria*. US Department of Health, Education and Welfare Pub no. (CDC) 77 - 8230. Atlanta, Georgia: Centers for Disease Control and Prevention; 1977. p. 15-90.
9. Guillemin I, Jarlier V, Cambau E. Correlation between quinolone susceptibility patterns and sequences in the A and B subunits of DNA gyrase in mycobacteria. *Antimicrob Agents Chemother* 1998; 42 : 2084-8.
10. Jain NK, Surpal BB, Khanna SP, Fatima T. Drug activity of ofloxacin against clinical isolates of *Mycobacterium tuberculosis*. *Indian J Tuberc* 1996; 3 : 183-6.
11. Rastogi N, Goh KS. *In vitro* activity of the new difluorinated quinolone sparfloxacin (AT-4140) against *Mycobacterium tuberculosis* compared with activities of ofloxacin and ciprofloxacin. *Antimicrob Agents Chemother* 1991; 35 : 1933-6.

12. Ruiz-Serrano MJ, Alcalá L, Martínez L, Díaz M, Marin M, Gonzalez-Abad MJ, *et al.* *In vitro* activities of six fluoroquinolone against 250 clinical isolates of *Mycobacterium tuberculosis* susceptible or resistant to first line antituberculosis drugs. *Antimicrob Agents Chemother* 2000; 44 : 2567-8.
13. Tomioka H, Sato K, Akaki T, Kajitani H, Kawahara S, Sakatani M. Comparative *in vitro* antimicrobial activities of the newly synthesized quinolone HSR-903, sitafloxacin (DU-6859a), gatifloxacin (AM-1155) and levofloxacin against *Mycobacterium tuberculosis* and *Mycobacterium avium* complex. *Antimicrob Agents Chemother* 1999; 43 : 3001-4.
14. Alvarez-Freites EJ, Carter JL, Cynamon MH. *In vitro* and *in vivo* activities of gatifloxacin against *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2002; 46 : 1022-5.
15. Srivastava K, Das R, Sharma VD, Singh D, Singh HB, Katoch VM. Relevance of degree of rifampicin resistance in *Mycobacterium tuberculosis*. *Indian J Med Microbiol* 2001; 19 : 36-9.
16. Paramasivan CN, Chandrasekaran V, Santha T, Sudarsanam NM, Prabhakar R. Bacteriological investigations for short course chemotherapy under the tuberculosis programme in two districts of India. *Tuber Lung Dis* 1993; 74 : 23-7.
17. Srivastava K, Das R, Jakhmola P, Gupta P, Chauhan DS, Sharma VD, *et al.* Correlation of mutations detected by INNO-LiPA with levels of rifampicin resistance in *Mycobacterium tuberculosis*. *Indian J Med Microbiol* 2004; 120 : 100-5.
18. Canetti G, Fox W, Khomenko A, Mahler HT, Menon NK, Mitchison DA, *et al.* Advances in techniques of testing mycobacterial drug sensitivity and the use of sensitivity tests in tuberculosis control programmes. *Bull World Health Organ* 1969; 41 : 21-43.
19. Gupta P, Jadaun GPS, Das R, Gupta UD, Srivastava K, Chauhan A, *et al.* Simultaneous ethambutol & isoniazid resistance in clinical isolates of *Mycobacterium tuberculosis*. *Indian J Med Res* 2006; 123 : 125-30.
20. Venkataraman P, Paramasivan CN, Prabhakar R. *In vitro* activity of ciprofloxacin and ofloxacin against south Indian isolates of *Mycobacterium tuberculosis*. *Indian J Tuberc* 1994; 41 : 87-90.
21. Sulochana S, Rahman F, Paramasivan CN. *In vitro* activity of fluoroquinolones against *Mycobacterium tuberculosis*. *J Chemother* 2005; 17 : 169-73.
22. Collins CH, Uttley AH. *In vitro* susceptibility of mycobacteria to ciprofloxacin. *J Antimicrob Chemother* 1985; 16 : 575-80.
23. Gay JD, DeYoung DR, Roberts GD. *In vitro* activities of norfloxacin and ciprofloxacin against *Mycobacterium tuberculosis*, *M. avium* complex, *M. chelonae*, *M. fortuitum* and *M. kansasii*. *Antimicrob Agents Chemother* 1984; 26 : 94-6.
24. Heifets LB, Lindholm-Levy PJ. Bacteriostatic and bactericidal activities of ciprofloxacin and ofloxacin against *Mycobacterium tuberculosis* and *Mycobacterium avium* complex. *Tubercle* 1987; 68 : 267-76.
25. Heifets LB. Qualitative and quantitative drug susceptibility tests in mycobacteriology. *Am Rev Respir Dis* 1988; 137 : 1217-22.
26. Canetti G, Froman S, Grosset J, Handusoy P, Langerova M, Mahler HT, *et al.* Mycobacteria laboratory methods for testing drug sensitivity and resistance. *Bull World Health Organ* 1963; 29 : 565-78.
27. Mitchison DA. Drug resistance in tuberculosis. *Eur Respir J* 2005; 25 : 376-9.
28. Kant L. Using fluoroquinolones for shortening SCC. *Indian J Tuberc* 2002; 49 : 123-4.
29. Grimaldo ER, Tupasi TE, Rivera AB, Quelapio Ma ID, Cardano RC, Derilo JO, *et al.* Increased resistance to ciprofloxacin and ofloxacin in multi drug resistant *Mycobacterium tuberculosis* isolates from patients seen at a tertiary hospital in the Philippines. *Int J Tuberc Lung Dis* 2001; 5 : 546-50.
30. Andersson MI, Mac Gowan AP. Development of quinolones. *J Antimicrob Chemother* 2003; 51 : 1-11.
31. Casal M, Ruiz P, Herreras A, Spanish study group of *Mycobacterium tuberculosis* resistance. Study of the *in vitro* susceptibility of *Mycobacterium tuberculosis* to ofloxacin in Spain. *Int J Tuberc Lung Dis* 2000; 4 : 588-91.
32. Rodriguez JC, Ruiz M, Climent A, Royo G. *In vitro* activity of four fluoroquinolones against *Mycobacterium tuberculosis*. *Int J Antimicrob Agents* 2001; 17 : 229-31.
33. Wright DH, Brown GH, Peterson ML, Rotschafer JC. Application of fluoroquinolone pharmacodynamics. *J Antimicrob Chemother* 2000; 46 : 669-83.
34. Lalande V, Truffot-Pernot C, Paccaly-Moulin A, Grosset J, Ji B. Powerful bactericidal activity of sparfloxacin (AT-4140) against *Mycobacterium tuberculosis* in mice. *Antimicrob Agents Chemother* 1993; 37 : 407-13.
35. Lubasch A, Erbes R, Mauch H, Lode H. Sparfloxacin in the treatment of drug resistant tuberculosis or intolerance of first line therapy. *Eur Respir J* 2001; 17 : 641-6.
36. Hu Y, Coates ARM, Mitchison DA. Sterilizing activities of fluoroquinolones against rifampin-tolerant populations of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2003; 47 : 653-7.
37. Hoffner SE, Gezelius L, Olsson-Liljequist B. *In vitro* activity of fluorinated quinolones and macrolides against drug resistant *Mycobacterium tuberculosis*. *J Antimicrob Chemother* 1997; 40 : 885-8.
38. Dam T, Isa M, Bose M. Drug-sensitivity profile of clinical *Mycobacterium tuberculosis* isolates - a retrospective study from a chest - disease institute in India. *J Med Microbiol* 2005; 54 : 269-71.
39. Ji B, Lounis N, Truffot-Pernot C, Grosset J. *In vitro* and *in vivo* activities of levofloxacin against *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 1995; 39 : 1341-4.
40. Yoshimatsu T, Nuermberger E, Tyagi S, Chaisson R, Bishai W, Grosset J. Bactericidal activity of increasing daily and weekly doses of moxifloxacin in murine tuberculosis. *Antimicrob Agents Chemother* 2002; 46 : 1875-9.

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