ISSN 2542-6214

Volume 14 | Issue 3 | September-December 2021

INDIAN Journal of HEALTH SCIENCES AND BIOMEDICAL RESEARCH KLEU

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Quick Response Code:

Website: www.ijournalhs.org

DOI: 10.4103/kleuhsj.kleuhsj_390_20

Ofloxacin resistance in *Mycobacterium tuberculosis:* An increasing concern

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Abstract:

Multidrug resistance tuberculosis (MDR-TB) associated with the development of resistance to fluoroquinolones (FQs) especially ofloxacin is a matter of concern, as they had been earlier recommended drugs for usage in the MDR-TB treatment regimens, and moxifloxacin and other quinolones are still on the list. *Mycobacterium tuberculosis* acquires resistance to FQs mainly through mutations in the quinolone resistance determining regions (QRDRs) of the *gyrA* gene and less frequently in the *gyrB* gene. A literature search on the geographical distribution of ofloxacin resistance in TB shows that there is a mild surge in reporting of the resistance to ofloxacin in tuberculosis patients. Molecular tests demonstrating mutations in *gyrA* and *gyrB* genes is widely used to detect ofloxacin resistance and the broadly available commercial assay for the rapid detection of second-line-drug resistance, including FQ resistance, the GenoType MTBDRsl assay (Hain Life science, Nehren, Germany), detects the most common mutations found in the QRDR of *gyrA* while its new version 2.0 detects mutations in the *gyrB* as well. It has been shown that on reviewing the frequency and geographic distribution of *gyrA* and *gyr B* mutations associated with FQ resistance, there do exist geographic differences in the frequencies within and across countries. Cross-resistance to FQs is an area of concern, although some studies show that concordance in resistance among the FQ agents, lower level of cross-resistance has also been reported. The presence of ofloxacin resistance is an alarm signal while Moxifloxacin and other FQs are still the recommended drugs for the resistant TB cases. The WHO recommendation that ofloxacin be phased out from MDR-TB regimens is well justified. It is important that rationale usage of ofloxacin is needed for preventing ofloxacin resistance, to aid in the management of tuberculosis.

Keywords:

GyrA, gyrB, *Mycobacterium tuberculosis*, ofloxacin, resistance

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Received: 06 November 2020, **Revised:** 01 July 2021, **Accepted:** 11 August 2021, **Published:** 30 September 2021

Introduction

In recent years, the alarming increase in drug-resistant tuberculosis is a great concern in major parts of the world. Failure of adherence to treatment regimens and mismanagement of drugs[1] seems to be the major reasons for the increase in drug-resistant tuberculosis cases, especially in developing countries. Many concerns are in multidrug-resistant tuberculosis (MDR-TB) which is resistant to rifampicin and isoniazid, and extensively drug-resistant TB (XDR-TB), which is defined as MDR-TB plus resistant to at least one of the fluoroquinolones (FQs) and any one of the second-line injectable drugs (SLID). Globally, 3.5% of new TB cases and 18% of previously treated cases had MDR/rifampicin resistance (RR) and among the cases of MDR-TB in 2017, 8.5% (95% confidence interval, 6.2%–11%) were estimated to have XDR-TB.[2] Another form of drug-resistant TB, pre-XDR-TB, which is resistant to isoniazid, rifampicin and any one of the FQs or SLID, but not both FQ and SLID[3,4] is also a concern. Totally drug-resistant TB, resistant to all of the first-line and second-line drugs, and polypeptide, thioamide, cycloserine, and para-aminosalicylic acid,[3] have been reported in Italy, Iran, India, and South

How to cite this article: Kumar RS, Devi KR, Dusthackeer A, Nirmal CR. Ofloxacin resistance in *Mycobacterium tuberculosis*: An increasing concern. Indian J Health Sci Biomed Res 2021;14:302-9.

Africa.[5] The treatment for MDR/XDR-TB is long and challenged by the high frequency of adverse drug events, high costs, and low treatment success rates.[6] FQs and SLID play a crucial role in the successful treatment of MDR-TB. When resistance emerges to any one of these two groups of drugs among MDR-TB, the treatment becomes more complex. Both forms of pre-XDR-TB, ofloxacin-resistant and SLID-resistant pre-XDR-TB have been reported to be associated with poor survival[4] and so the XDR-TB.[7]

When patients are diagnosed initially with drug-resistant tuberculosis they are defined as primary drug resistance, and they are assumed to have transmitted with drug-resistant strains.[8] When wildtype pan-susceptible bacteria mutate into drug-resistant strains during the course of treatment, it is referred to as acquired resistance.[9] In the case of FQs, its usage in other respiratory infections is reported to be a cause for the emergence of FQ-resistant *Mycobacterium tuberculosis*.[10] Wang *et al*. reported that empirical use of FQs for presumed bacterial infection delays the treatment for TB and consequently results in poorer prognosis, and most likely associated with FQ resistance.[11] Similarly, Devasia *et al*. reported the high risk of FQ-resistant tuberculosis among individuals who received FQs for more than 10 days, particularly more than 60 days before tuberculosis diagnosis.[12] The proportion of MDR-TB cases with resistance to any of the FQs (ofloxacin, levofloxacin, and moxifloxacin) is reported to be 21%.[2] These findings strongly indicate the need for early and prompt diagnosis and appropriate usage of the antimicrobials to avoid the emergence of drug resistance.

The WHO treatment guidelines for drug-resistant tuberculosis, has recommended that ofloxacin be phased out from MDR-TB regimens.[13] A literature search on the ofloxacin resistance in TB across the world showed that there is an increase of ofloxacin resistance and we feel it was very appropriate that ofloxacin may be phased out from the MDR-TB regimens

Fluoroquinolones, Mechanism of Action, and Development of Resistance

FQs form an important class of antibiotics listed among the second-line drugs used for the management of MDR-TB. They belong to a family of broad-spectrum, systemic antibacterial agents and are commonly used as therapeutic agents for respiratory and urinary tract infections. They are active against a wide range of aerobic Gram-positive and Gram-negative organisms. The FQ are classified into four generations and OFX comes under second generation[14] while moxifloxacin and gatifloxacin come under third-generation FQs, the three FQs widely used for MDR-TB. The basic mechanism of action of all FQs is similar and they all inhibit DNA synthesis by targeting DNA gyrase.[15] In *M. tuberculosis* DNA gyrase is encoded by DNA gyrase subunits A (*gyrA*, Rv0006), and DNA gyrase subunits B (*gyrB*, Rv0005). Since the DNA gyrase is essential for DNA replication and transcription of the bacteria, its inhibition results in bactericidal activity. Therefore, mutations in the quinolone resistance determining region (QRDR) of *gyrA* predominantly and to a lesser extent in *gyrB*, cause resistance to FQ. Alternate mechanisms, namely, efflux pumps,[16,17] mutations in *parE* in *Streptococcus pneumonia*,[18] *parC* and *parE* genes in *Salmonella enterica*[19] have also been reported to cause FQ-resistance.

Ofloxacin in the Management of Tuberculosis

As per the WHO treatment guidelines for drug-resistant tuberculosis, 2016 update, it was recommended that ofloxacin be phased out from MDR-TB regimens and ciprofloxacin to be never used due to the limited evidence of their effectiveness and recommended the use of later-generation FQs (defined for these guidelines as high-dose levofloxacin, moxifloxacin, and gatifloxacin) as they significantly improve treatment outcomes in adults with RR-TB and MDR-TB. Regarding the usage of ofloxacin in drug-sensitive TB, in some clinical studies, ofloxacin[20] has been tried but had not made any big impact, and better and newer FQs containing regimens are being studied for TB. Also, WHO TB treatment guidelines[21] recommend not to use any 4-month FQ containing regimen for drug-sensitive TB. But it is to be reminded that WHO consistently recommends FQs for drug-sensitive TB in cases of intolerance of standard first-line drugs, particularly hepatotoxicity if there were no options, and there is a chance that ofloxacin may be used by the treating physician at that time due to its wider availability and that could be an occasion where ofloxacin is exposed to a patient with TB.

Geographical Distribution in OFX Resistance

In 2018, Zignol *et al.*,[22] reported in an analysis across Asian countries, and the overall pooled sensitivity values for genetic sequencing among all tuberculosis cases were 85% (77–91) for *gyrA* and *gyrB* combined (ofloxacin resistance), and 88% (81–92) for *gyrA* and *gyrB* combined (moxifloxacin resistance).[21] A literature search using PubMed, on the geographical distribution of ofloxacin resistance in TB shows that there is a mild surge in reporting of the resistance to ofloxacin in tuberculosis patients. The main findings of each of these studies are reported in Tables 1 and 2 show a summary of FQs resistance among various clinical isolates.

Table 1: Geographical distribution of ofloxacin resistance: summary from a literature review

Author (year)	Country	Study findings on OFX resistance	References
Willby *et al.,* (2015)	USA	MICs for MXF and LVX and susceptibility to the critical concentration of OFX were determined using the agar proportion method for 133 isolates of *M. tuberculosis*. Most isolates resistant to OFX had LVX MICs of >1 g/ml and MXF MICs of >0.5 g/ml	[23]
Selvakumar *et al.,* (2015)	India	Additional OFX resistance was reported among 18.2% of new and 28.7% of previously treated MDR-TB patients	[24]
Dalal *et al.,* (2015)	India	The proportion of patients with OFX resistance done at two points 2005 and 2015 reported to have significantly increased from 57.6% to 75.3% (P<0.05)	[25]
Verma *et al.,* (2011)	India	The results showed 85.1% of drug (first-line) sensitive *M. tuberculosis* isolates and 61.8% of MDR isolates were reported to be susceptible to OFX; MDR-TB patients were found to have 1.377 fold increased risk to become resistant to OFX than drug sensitive patients	[26]
Myneedu *et al.,* (2011)	India	Of the 223 patients of tuberculosis who were culture positive and *M. tuberculosis* was resistant to Rifampicin and Isoniazid during January 2007 to December 2009, 69% of the total (154 out of 253) were reported to have OFX resistance	[27]
Kamal *et al.,* (2015)	Bangladesh	Among the MDR-TB patients, 19.2% (exclusively previously treated) were reported to have resistance to OFX	[28]
Ghafoor *et al.,* (2015)	Pakistan	Out of 100 MDR-TB isolates, 97% were reported to be sensitive to amikacin, 53% to OFL, 87% to capreomycin, and 87% to ethionamide	[29]
Chan *et al.,* (2007)	Hongkong	71 out of 250 (28%) were reported to be OFX resistant, with or without resistance to other drugs	[30]
Zhang *et al.,* (2014)	China	Of the 138 *M. tuberculosis* isolates, the prevalence of resistance for FQ were: OFX: 3.76%; LVX: 3.18%; MXF: 3.12%; sparfloxacin: 1.91%; and gatifloxacin: 1.33%	[31]
Daniel *et al.,* (2011)	Nigeria	Among the 34 MDR-TB patients, OFX resistance was observed to be 11.8%	[32]
El Sahly *et al.,* (2011)	USA (2011)	Of the 557 *M. tuberculosis* isolates, 10 (1.8%) were reported to be resistant to OFX	[33]

M. tuberculosis: Mycobacterium tuberculosis, FQ: Fluoroquinolone, MDR-TB: Multidrug-resistant tuberculosis, MXF: Moxifloxacin, LVX: Levofloxacin, MIC: Minimum inhibitory concentration, OFX: Ofloxacin

Laboratory Methods for the Diagnosis of Ofloxacin Resistance

Phenotypic methods

There is uncertainty about how to interpret results from drug susceptibility testing (DST) against FQs. Three general methods used for determining drug susceptibility of *M. tuberculosis* are the proportion method, absolute concentration method (MIC method) and the resistance ratio method. Phenotypic DST for ofloxacin is generally carried out by the absolute concentration method or by proportion sensitivity method.[46] The WHO (2018) recommended critical concentrations for moxifloxacin was 0.25–1.0 μg/ml and for ofloxacin 1.0–2.0 μg/ml in various media.[47] MIC revaluation of the critical concentration for Ofloxacin should be attempted to validate the WHO recommended ones and this was suggested to be different in every geographical area and there is more literature evidence for this effect.[48]

Molecular Tests to Detect Resistance to OFX and *gyrA* and *gyrB* Mutations

Currently, the phenotype-based approach is the diagnostic gold standard in determining the FQ resistance. However, recent advances in molecular diagnostics technology enable us to assess the resistance rapidly. The *gyrA* and *gyrB* genes were directly amplified from the DNA of 41 Mtb clinical isolates and mutation were confirmed by sequencing.[49] Primers were designed for *M. tuberculosis* acquires resistance to the FQs mainly through mutations in the QRDRs of the *gyrA* gene and less frequently, in the *gyrB* gene. Agreement between genotypic and phenotypic susceptibility has been reported to be high (≥97%) by various studies.[49-51] The broadly available commercial assay for the rapid detection of second-line-drug resistance, including FQ resistance, the MTBDRsl assay (Hain Lifescience, Nehren, Germany), detects the most common mutations of the QRDR of *gyrA*.[52] The new version of GenoType MTBDR*sl* (v2.0) includes mutations in the *gyrB* at codons 536–541, in addition to *gyrA*.[53] The accuracy of the FQ resistance detection by MTBDR*sl* v2.0 was found to be 97% with sensitivity and specificity of 93.0% and 98.3%, respectively. For ofloxacin alone sensitivity and specificity were calculated to be 92.9% and 97.7%, respectively. The WHO expert group suggested GenoType MTBDRsl assay be used for "rule-in" test of FQ resistance though it cannot be used as a replacement test for conventional DST.[54]

Geographical Distribution of *gyrA* and *gyrB* Mutations

Globally, several studies have been undertaken to identify mutations in gyrA and *gyrB* to explain OFX-resistance from different parts of the world including from China[55,56] and India[57-61] and Russia[62-64] where more than half of the MDR-TB patients are reported.[65] In a study conducted in 41 clinical isolates, they have found

Table 2: Summary of fluoroquinolones resistance among various clinical isolates with 95% confidence intervals

Country	FQ tested	FQ resistant *M. tuberculosis*		FQ resistant MDR-TB		Susceptibility testing method	References
		n	Percentage of total MTB with (95% CI)	*n*	Percentage of total MDR-TB with (95% CI)		
India	OFX	107	8.8 (8.7-12.1)	41	5.7 (4.0-7.4)	Agar proportion method	[24]
Australia	OFX	2	0.6 (0.1-2.0)	1	11 (0.2-48)	Automated qualitative broth-based method (BACTEC MGIT 960)	[34]
Australia	MXF	2	0.6 (0.1-2.0)	1	11 (0.2-48)	Automated qualitative broth-based method (BACTEC MGIT 960)	[34]
Taiwan	OFX	36	1.3 (0.9-2)	28	32 (22-42)	Agar proportion method	[35]
Korea	OFX	94	3.4 (2.7-4.1)	83	30 (25-36)	Absolute concentration method	[36]
Ethiopia	FQ gene target (gyrA + gyrB	0	0.0 (0.0-1.4)	0	0 (0-25)	DNA hybridization technology (GenoType MTBDRsl)	[37]
Tanzania	Ciprofloxacin	2	0.7 (<0.01-2.5)	0	0 (0-71)	Automated qualitative broth-based method (BACTEC MGIT 960)	[38]
Tanzania	MXF	1	0.3 (<0.01-1.9)	0	0 (0-71)	Automated qualitative broth-based method (BACTEC MGIT 960)	[38]
Rwanda	OFX	4	0.6 (0.2-1.5)	3	9 (2-25)	Agar proportion method	[39]
Pakistan	Ciprofloxacin or OFX	12	5.9 (3.0-10)	5	11 (4-24)	Agar proportion method	[40]
Spain	OFX	13	6.0 (3.3-10)	7	47 (21-73)	Automated qualitative broth-based method (BACTEC 460TB)	[41]
USA	OFX	10	1.8 (0.9-3.3)	3	38 (9-76)	Agar proportion method	[33]
USA	MXF	10	1.8 (0.9-3.3)	3	38 (9-76)	Agar proportion method	[33]
USA and Canada	Ciprofloxacin	2	0.2 (<0.01-0.6)	N/A		Agar proportion method	[42]
USA	OFX	2	3.6 (0.4-13)	0	0 (0-98)	Automated qualitative broth-based method (BACTEC 460TB)	[43]
USA	MXF	1	1.8 (<0.01-10)	0	0 (0-98)	Automated qualitative broth-based method (BACTEC 460TB)	[42]
USA	OFX	16	2.5 (1.4-4.0)	0	0	Agar proportion method	[12]
India	OFX	181	35.2 (in the year 2004)	N/A	N/A	LJ medium proportion method	[28]
Phillipines	OFX and ciprofloxacin	54	35.3	18	51.4	Indirect proportion method utilizing the disk elution technique	[44]
Tunisian	Ciprofloxacin	4	0.8	N/A	N/A	Indirect proportion method with the agar dilution technique	[45]

LJ: Löwenstein-Jensen, *M. tuberculosis: Mycobacterium tuberculosis*, FQ: Fluoroquinolone, MDR-TB: Multidrug-resistant tuberculosis, MXF: Moxifloxacin, OFX: Ofloxacin, N/A: Not applicable, CI: Confidence interval, gyrA: Gyrase subunits A, gyrB: Gyrase subunits B, MGIT: Mycobacteria growth indicator tube

that mutation in gyrA, shows resistance to moxifloxacin and susceptible to ofloxacin.[49] In a meta-analysis which included 19 articles from 22 independent studies, the sensitivity and specificity of MTBDR*sl* were reported to be 86.9% and 97.3% respectively for FQs.[66] Similarly, Theron *et al*. reported pooled sensitivity and specificity of MTBDR*sl* based on (i) culture isolates confirmed as TB positive (indirectly) to 83.1% and 97.7% respectively and (ii) smear-positive sputum specimens (directly) to be 85.1% and 98.2%, respectively.[67] Furthermore, another systematic review revealed that only 80% OFX-resistant of *M. tuberculosis* strains to have mutations in *gyrA* that are also covered in GenoType MTBDR*sl*.[68]

The frequency and nature of drug-resistant mutations are known to be varying between countries or regions that add complexities in predicting the resistance precisely like that of phenotypic determination. Sandgren *et al*. established a comprehensive database of drug-resistant mutations for tuberculosis; the database also contains high-confidence drug-resistant mutations listed based on the relative frequency of the most common mutations associated with resistance to specific drugs. In this database, mutations from gyrA, at codons 74, 89-91, 94, and 102, and from *gyrB* at codon 510 are listed as high-confidence mutations conferring FQ-resistance.[69] The GenoType MTBDR*sl* is used widely now to detect FQ-resistant mutations rapidly based on the mutations at codons A90V, S91P, D94A, D94N/Y, D94G, and D94H.[70] The diversity and frequency of mutations by a systematic review conducted by Avalos *et al*.[68] included a total of 3846 unique clinical *M. tuberculosis* isolates with phenotypic resistance profile, from 18 different countries covered in 46 studies. Mutations A90V and D94G were reported as the most frequently occurring in 14 of the 18 countries included in this systematic review. However, the frequency of FQ-resistant mutations was reported to be different between India and China where drug-resistant TB is reported to be very high. In India, the most commonly reported mutations were D94A (20%)

followed by A90V (10%) and D94G (9%), which were all covered in MTBDR*sl*. On the other hand, in China, the frequency of D94A was only 8%, while D94G and A90V were 28% and 18%, respectively. Further, in the *gyrB*, mutation N538D (also reported as N510D), D500H, T539N, and A543V were reported to be rare (at frequencies of <1%) among ofloxacin-resistant isolates.[68]

The genotypes of *M. tuberculosis* have also been reported to influence the drug resistance, which also differs between various regions and countries. For example, CAS lineage predominantly present in North and North-West India (up to 62.3%) the EAI strains are dominant in South India (44% in Chennai) while the Beijing lineage are present in all over India (17.2%) through its presence is very high in North-Eastern parts of India (65.6% in Assam and 25.3% in Kolkata region).[71] Beijing genotype has been reported to be associated with multidrug resistance as well as extensively drug resistance TB.[72] Based on molecular surveillance of multidrug-resistant tuberculosis conducted during 2003-2007 in 24 European countries, it was found that majority of the MDR and XDR genotypes were Beijing.[73] Zhang *et al*. reported significantly higher proportions of ofloxacin-resistant and pre-XDR isolates in Beijing strains than non-Beijing strains.[74] A similar association of Beijing genotype of *M. tuberculosis* with FQ resistance was also reported from Vietnam.[75] These results indicate the need for exploring other plausible mechanisms, including the relevance of geography and genotype of the bacteria with drug resistance.

Other Methods of Diagnosing Ofloxacin Resistance

However, it must be reminded that as the conventional DST tests are slow and laborious, and molecular genetic tests are too expensive although fast, detection of ofloxacin resistance in *M. tuberculosis* by low-cost colorimetric methods, namely resazurin and nitrate reductase assays are good alternate options for future testing the susceptibility of ofloxacin, as study has shown that the results are concurrent with the standard and test results are available in an average of 10 days.[76]

Recent developments in whole-genome sequencing (WGS) and bioinformatics enable rapid determination of resistance for all of the drugs for which resistant mutations are known. For example, TB-Profiler, a new bioinformatics tool can predict resistant mutations for a total of 11 different TB drugs including OFX from WGS of *M. tuberculosis;* sensitivity and specificity of TB-Profiler for OFX are 85.5% and 94.9%, respectively.[77] The accuracy of prediction by TB-Profiler was reported to vary between countries[77] supporting the fact that there could be a difference in the frequency of geographical distribution of mutations causing drug resistance. Johana *et al.* determined the MIC of ciprofloxacin in 13 clinical isolates using new Raman spectroscopy.[78]

Cross-Resistance-a Matter of Concern

FQ DST is an important step in the design of effective treatment regimens for multidrug-resistant tuberculosis. Ofloxacin is also being used for other infectious diseases and they could pave a way for cross-resistance to other FQs. In most occasions, the DST pattern result obtained for anyone of the FQ is considered as the results applicable for any of the other FQs. While some studies support the occurrence of cross-resistance and suggest to avoid usage of any FQs if resistance is found in any one member, whereas others favor in the usage of other FQ even with resistance to anyone FQ. Studies have reported concordance in the DST pattern of FQs in *M. tb;* 100% concordance between resistance to moxifloxacin and ofloxacin[33] and high concordance among ofloxacin, moxifloxacin, and gatifloxacin resistance[49] have been reported. Similarly, Willby *et al*. observed a high degree of cross-resistance between OFX, MFX, and LVX from USA.[23] However, studies have demonstrated significantly better treatment outcomes among OFX-resistant MDR-TB when susceptible to moxifloxacin and treated with a regimen containing later generation of FQs (levofloxacin, gatifloxacin, and moxifloxacin, mainly the latter).[79] Sirgel *et al*. reported clinical relevance of using moxifloxacin in the treatment of OFX-resistant TB.[50] Furthermore, the WHO earlier recommended moxifloxacin for the therapy of ofloxacin-resistant TB.[80]

M. tuberculosis isolates with mutations at Ala90Val or Ser91Pro were reported to have higher MIC_{90} for OFX (4.0 μg/ml) and lower MIC_{90} (1.0 μg/ml) MXF thus, indicated for standard or increased MXF dosing for clinical MTB isolates shown to have these mutations.[81] Furthermore, a study from Peru demonstrated lower concordance in resistance among three FQ (CFX, OFX, and MFX), with one-third to half of the strains showing no agreement among the three agents.[82] The discrepancy observed, in particular, the high rates of CFX/OFX-resistant isolates that were intermediate or susceptible to MFX,[82] a finding which was also demonstrated from India,[83] suggest that DST should be performed for the specific FQ planned for clinical use. The WHO reports that while resistance-conferring mutations to FQs detected by the MTBDRsl assay are highly correlated with phenotypic resistance to ofloxacin and levofloxacin, the correlation with moxifloxacin (and gatifloxacin) is less clear and the inclusion of moxifloxacin in an MDR-TB regimen is best guided by phenotypic DST results. We feel that the surge in the ofloxacin resistance

is a matter of concern with the available literature on cross-resistance among the quinolones.

Public Health Relevance and Programmatic Implications of Ofloxacin Resistance

Public health and programmatic implications on this increase in ofloxacin resistance in TB are many. One must realize that this ofloxacin resistance TB stain is being transmitted from the infected person to the community and this increase in the prevalence of ofloxacin resistance is an area of concern, especially in high TB burden countries. At present, in most low- and middle-income countries, DST is done only in a fraction of all cases leading to a large number of undetected ofloxacin drug-resistant TB. And even if DST has done routinely, it is often only done in retreatment cases resulting in delayed diagnosis of ofloxacin resistance in *M. tuberculosis* DST methods for the second-line drugs, especially for the FQs are not wider application tests in the low and middle income, high TB burden countries, although it is being scaled up in the Indian TB control program. This leaves a need for improving the TB diagnostic laboratories to upgrade themselves in diagnosing the ofloxacin resistance. Also, ofloxacin being used to treat new TB patients at the time of severe adverse reaction to first-line drugs, given the scenario where ofloxacin resistance testing not being done routinely, could end up treating with inappropriate regimen as there could be a chance of them being affected by ofloxacin resistance stain, given the prevalence of increase in ofloxacin resistance as evidenced by this review. Most importantly with the available literature evidence of cross-resistance, this increase in ofloxacin resistance is a matter of concern.

To conclude, the surge of ofloxacin resistance is a concern and we feel that the WHO had rightly decided to phase out ofloxacin in the management of resistant TB. However, in high TB burden countries with limited resources, it is suggested that scaling up of DST facilities specific to all second-line TB drugs are need of the hour.

Acknowledgment

The authors would like to thank Dr.Soumya Swaminathan, former Director-General, Indian Council of Medical Research, for her support for developing this review. We would like to thank Dr.Jagdish Chandrabose Sundaramoorthi former Scientist C, ICMR-National Institute for Research in Tuberculosis, for contributing in the retrieval of relevant references.

Financial support and sponsorship

Institutional support.

Conflicts of interest

There are no conflicts of interest.

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