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4-month moxifloxacin containing regimens in the treatment of patients with sputum-positive pulmonary tuberculosis in South India – a randomised clinical trial

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Abstract

BACKGROUND Shortening tuberculosis (TB) treatment duration is a research priority. We tested the efficacy and safety of 3- and 4-month regimens containing moxifloxacin in a randomised clinical trial in pulmonary TB (PTB) patients in South India.

METHODS New, sputum-positive, adult, HIV-negative, non-diabetic PTB patients were randomised to 3- or 4-month moxifloxacin regimens [moxifloxacin (M), isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E)] or to a control regimen ($2H_3R_3Z_3E_3/4R_3H_3$) [C]. The 4 test regimens were $3R_7H_7Z_7E_7M_7$ [M3], $2R_7H_7Z_7E_7M_7/2R_7H_7M_7$ [M4], $2R_7H_7Z_7E_7M_7/2R_3H_3M_3$ [M4-I] or $2R_7H_7Z_7E_7M_7/2R_3H_3E_3M_3$ [M4-IE]. Treatment was directly observed. Clinical and bacteriological assessments were done monthly during treatment and for 24 months post-treatment. The primary end point was TB recurrence post-treatment.

RESULTS Of 1371 patients, randomised, modified intention-to-treat (ITT) analysis was done in 1329 and per-protocol (PP) analysis in 1223 patients. Regimen M3 was terminated due to high TB recurrence rates. 'Favourable' response at end of treatment was 96–100% in the moxifloxacin regimens and 93% in the control regimen. Among these, the TB recurrence occurred in 4.1% in the M4 regimen and in 4.5% in the control regimen and demonstrated equivalence within a 5% margin (95% CI −3.68, 4.55). Similar findings were observed in modified ITT analysis. The TB recurrence rates in the M4-I and M4-IE regimens did not show equivalence with the control regimen. Sixteen (1.4%) of 1087 patients in the moxifloxacin regimens required treatment modification.

CONCLUSION The 4-month daily moxifloxacin regimen [M4] was found to be equivalent and as safe as the 6-month thrice-weekly control regimen.

keywords tuberculosis, chemotherapy of tuberculosis, short course chemotherapy, moxifloxacin, fluoroquinolones

Sustainable Development Goals (SDGs): SDG 3 (good health and well-being), SDG 17 (partnerships for the goals)

Introduction

A shorter efficacious regimen for treating tuberculosis (TB) would be a boon for both patients and healthcare providers. The fluoroquinolones have shown promise towards achieving this goal [1]. Moxifloxacin (M) has bactericidal and sterilising activities in *in vitro* and murine studies [2,3]. Its addition to isoniazid (H) and rifampicin (R) enhanced their activity in exponential and stationery phase cultures of *Mycobacterium tuberculosis* [4]. In mice, a combination of R-M and pyrazinamide (Z) shortened the duration of therapy by 2 months [5,6]. We had earlier shown that 4- or 5-month regimens with ofloxacin (O), H, R and Z achieved high cure rates and low TB recurrence rates in pulmonary TB patients [7]. A subsequent study using 4-month thrice-weekly regimens with either M or gatifloxacin(G) had high TB recurrence rates, suggesting that the search for shorter TB regimens using fluoroquinolones should use daily treatment, at least initially [8]. We hypothesised that M added to R, H, E and Z, given for 3 or 4 months could achieve results comparable to those of the standard 6-month regimen. We assessed the efficacy and safety of 3- and 4-month regimens containing moxifloxacin in patients with newly diagnosed sputum-positive pulmonary TB in comparison with the 6-month thrice-weekly control regimen.

Methods

This randomised, open-label, parallel-arm, clinical trial was approved by the NIRT Scientific Advisory and Ethics Committees and monitored by a Data and Safety Monitoring Board (DSMB). It is registered in the Clinical Trials Registry of India (CTRI 2008/091/000024).

Study participants

Newly diagnosed, adult, sputum-positive pulmonary TB patients satisfying eligibility criteria were enrolled at Chennai, Madurai and Vellore in South India. Those with previous treatment for TB exceeding 30 days and or exceeding 1 week in the preceding 1 month of enrolment, weighing <30 kg, pregnant or lactating women and those with diabetes, epilepsy, extra-pulmonary TB, hepatic or renal disease or HIV infection were ineligible. Written informed consent was obtained before enrolment to the study.

Treatment regimens

Eligible patients were randomly allocated in a 1:1:1:1:1 ratio to one of four test regimens or a control regimen as follows:

- M3: Three-month moxifloxacin regimen (M + H + R + Z + E) daily
- M4: Four-month daily moxifloxacin regimen (M + H + R + Z + E daily for 2 months followed by M + H + R daily for 2 months)
- M4-I: Four-month moxifloxacin regimen with intermittent continuation phase (M + H + R + Z + E daily for 2 months followed by M + H + R thrice weekly for 2 months)
- M4-IE: Four-month moxifloxacin regimen with intermittent continuation phase along with ethambutol (M + H + R + Z + E daily for 2 months followed by M + H + R + E thrice weekly for 2 months)
- C: Six-month control regimen – H + R + Z + E thrice weekly for 2 months followed by H + R thrice weekly for 4 months.

Regimen allocation was stratified on sputum smear grading (0/1+ or 2+/3+), extent of chest X-ray involved (≤2 or >2 zones) and duration of previous anti-TB treatment (0–14 or 15–30 days). Restricted random allocation sequences were generated using random number tables for the six strata and sealed opaque envelopes were used to assign regimens.

Medication dosages (in mg) were as follows: R 450 or 600 based on body weight in kg (<60 or ≥60), H 300 (daily) or 600 (thrice-weekly), Z 1500; E 800 (daily) or 1200 (thrice-weekly) and M 400. Treatment was directly observed for 5 days of the week, and two doses were self-administered during weekends in the daily phase. The patient travelled to the clinic where the healthcare worker provided directly observed treatment (DOT). During the thrice-weekly phase, every dose was directly observed. Patients who missed treatment were visited and motivated to attend the clinic for treatment. Missed doses were compensated for up to 15 days.

Investigations

At enrolment, four sputum specimens (two spot and two overnight) were collected for microscopy and culture. Smears were prepared from raw sputum, stained with auramine–rhodamine, and read and graded using fluorescence microscopy [9]. Sputum was decontaminated and concentrated prior to culture by modified Petroff's method [10]. Positive cultures were identified as *Mycobacterium tuberculosis* by standard methods [11,12]. Cultures were graded based on quantum of growth in Lowenstein Jensen (LJ) medium [9]. Drug susceptibility tests (DST) were performed by MIC method for H, R, E and O based on WHO recommendations [11–13]. The definitions of drug resistance for H, R, E

and O were as used in previous studies; DST for M was not performed [14–16]. In addition, a posterior–anterior chest radiograph, electrocardiogram (ECG), urine examination for albumin, glucose, bile salts, acetyl INH, and R, total and differential leucocyte counts, haemoglobin estimation, erythrocyte count, platelet count, serum bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase, blood urea, serum creatinine, serum uric acid, random blood glucose and ELISA for HIV antibody were done.

Patients were evaluated initially and every month for symptom review, systemic examinations and adverse drug reactions. Sputum specimens were examined every month by microscopy and culture, three specimens (two overnight and one spot) during treatment phase and two specimens (one overnight and one spot) during the follow-up phase. Two additional specimens (one overnight and one spot) were collected at the 15th and 45th days of treatment. One positive sputum culture was tested each month for susceptibility to H, R, E and O. Bacteriological investigations were done by technicians who were blinded to the clinical status of the patient and the treatment regimen. Haemogram, random blood sugar, hepatic and renal function tests and ECG were done every month up to 4 months. Chest X-ray was done at the end of the intensive phase and end of treatment. Patients who had clinical deterioration or adverse reactions to anti-TB drugs were reviewed by a panel of physicians. Patients who had a 'Favourable' response at the end of treatment were followed up for 24 months after treatment completion. Patients with 'Unfavourable' response to treatment or those with TB recurrence were provided a full course of appropriate anti-TB treatment as per Revised National TB Control Programme (RNTCP) guidelines, and they were followed up until treatment completion.

Study outcomes

The primary outcome was recurrence of TB among those with 'Favourable' response at end of treatment. Secondary outcomes were (i) sputum culture conversion at 2 months, (ii) status at end of treatment ('Favourable' or 'Unfavourable') and (iii) adverse reactions to drugs.

Primary outcome

Recurrence of TB *(in those with 'Favourable' response at the end of treatment):*

1 *Bacteriological recurrence:* Defined as two positive sputum cultures in a 2-month period, one of which was at least 20 colonies, or positive sputum cultures during four consecutive monthly examinations, and none of which was 20 colonies or more
2 Clinical/Radiological recurrence: Defined as clinical or persistent radiological deterioration consistent with TB in the absence of bacteriological criteria defined above.

Secondary outcomes

1 *Sputum culture conversion at 2 months:* Proportion of patients in whom all sputum cultures were negative after 2 months of treatment.
2 *Status at the end of treatment:*

a 'Favourable' – Defined as all three sputum cultures being negative in the last month of treatment or if one culture was positive but subsequent monthly cultures were negative,
b 'Unfavourable' – Any of the following:

- Unfavourable bacteriological response – Defined as more than one sputum culture being positive in the last month of treatment, one of which was at least 20 colonies or more, or one culture being positive in the last month of treatment followed by positive cultures in subsequent months, or treatment was changed for persistent positive sputum cultures.
- Treatment changed for radiological or clinical deterioration
- Treatment changed for drug toxicity
- Patient died during treatment

3 *Adverse reactions to drugs:* Proportion of patients who developed adverse reactions attributable to drugs in the treatment regimens. They were classified as Mild (requiring only symptomatic treatment), Moderate (drug temporarily withheld and re-introduced) and Severe (treatment modified or patient required hospitalisation).

Sample size and statistical analysis

Using an equivalence design with margin of indifferency of 5%, we assumed the efficacy of the control regimen to be 95% TB recurrence-free survival over 24 months post-treatment. With a type I error of 0.05, and a type II error of 0.20, sample size was calculated to be 298 patients per regimen. Allowing for 10% attrition rate based on previous experience on randomised clinical trials conducted in NIRT with a minimum of 24 months of post-treatment follow-up, the final sample size was calculated to be 330 patients per regimen.

Data were analysed using IBM SPSS Statistics version 25.0. Armonk, NY: IBM Corp. Proportions of patients with TB recurrence, sputum culture conversion at 2 months, 'Favourable', 'Unfavourable' response at end of treatment and drug adverse events were compared between each test regimen and control regimen. The analysis of the outcomes of interest was both by intention-to-treat and per-protocol. Equivalence was defined as 95% confidence interval (CI) of the percentage points of difference in TB recurrence between the test and control regimen to be within 5% of the upper and lower boundary of two-sided 95% CI. Chi-square test was used to compare proportions. P value ≤ 0.05 was considered significant. A Kaplan–Meier survival curve was constructed for TB recurrence-free survival during 24 months of post-treatment follow-up.

Results

Patient population

Patients were recruited from May 2007 to October 2016. The DSMB recommended suspension of enrolment to the M3 regimen in October 2011 due to high TB recurrence rate. The allocation ratio was modified to 2:2:2:1 for the three 4-month test regimens and control regimen respectively in December 2012. Results up to 24 months post-treatment are presented.

A total of 1371 patients were enrolled out of 4382 screened. The population for modified intention-to-treat analysis was 1329 patients after exclusion of 42 patients post-randomisation. The reasons for exclusion include non-tuberculous *Mycobacteria* ($n = 14$), previous anti-TB treatment >1 month ($n = 4$), negative sputum culture ($n = 4$), multi-drug resistant TB ($n = 18$), associated spinal TB ($n = 1$) and history of psychiatric illness ($n = 1$). Per-protocol analysis included 1223 patients who had received ≥80% of the allocated treatment (Figure 1). The baseline demographic and clinical characteristics were similar among the treatment groups (Table 1).

Tuberculosis recurrence

In per-protocol analysis, of 292 patients treated with the M4 regimen who had a 'Favourable' response at the end of treatment, 12 (4.1%) had recurrence of TB during 24 months of follow-up, *vs.* 9 (4.5%) of 198 patients treated with the control regimen (Table 2). The difference in TB recurrence rate was 0.44% and 95% CI of the difference was −3.68, 4.55, thus demonstrating equivalence with the margin of indifferency being within 5%. Similar findings were observed in the modified intention-to-treat analysis favouring the M4 regimen (difference 0.42%; 95% CI −3.63, 4.48). The M3, M4-I and M4-IE regimens did not show equivalence with the control regimen with TB recurrence rates of 19.2%, 8.4% and 6.5%, respectively (Table 2). In 80 of 84 patients who had TB recurrence in per-protocol analysis, the recurrence was with pulmonary TB.

Tuberculosis recurrence rates in patients with drug susceptible TB were 20.7%, 3.1%, 7.8%, 6.1% and 4.5% in the M3, M4, M4-I, M4-IE and control regimens, respectively (Table 3); in those with drug-resistant TB, the corresponding figures were 8.3%, 13.3%, 11.9%, 9.1% and 5.3% in the M3, M4, M4-I, M4-IE and control regimens, respectively (Table 4).

Of the 82 patients with recurrence of pulmonary TB, in 76 (65 with initial drug susceptible and 11 with initial drug resistant cultures), the drug susceptibility profile of the sputum cultures at recurrence was similar to their initial cultures. One patient each with baseline susceptible organisms in M4 and M4-IE regimens had emergence of ofloxacin resistance whereas four patients with initial ofloxacin resistance and two patients with initial isoniazid resistance had susceptible organisms at TB recurrence. One patient with susceptible culture initially in the control regimen arm had emergence of resistance to H and R at the time of TB recurrence (Table 5).

Figure 2 describes a time-to-event analysis (Kaplan–Meier method) for post-treatment TB recurrence over 24 months. Sixty-two (72%) of the 86 TB recurrences occurred within 6 months of stopping treatment.

Sputum culture conversion upto 2 months

The proportion of patients with negative sputum cultures at day 15, 30, 45 and 60 for the moxifloxacin and control regimen arms is shown in Figure 3. Since all patients allocated to the moxifloxacin regimens received RHZEM for the first 2 months, results were amalgamated. Overall there were 1087 patients in the moxifloxacin regimens and 242 in the control regimen. Cultures were available for evaluation from 1044 patients in the moxifloxacin regimens and 225 patients in the control regimen. A total of 978 (94%) of 1044 patients treated with the moxifloxacin regimen and 173 (77%) of 225 patients treated with the control regimen had negative sputum cultures at 2 months ($P < 0.001$; Figure 3). The higher sputum culture conversion in the moxifloxacin regimens was statistically significant from the 15th day of treatment [17].

Status at the end of treatment

Of 1223 patients in per-protocol analysis, 96–100% treated with the moxifloxacin regimens had 'Favourable'

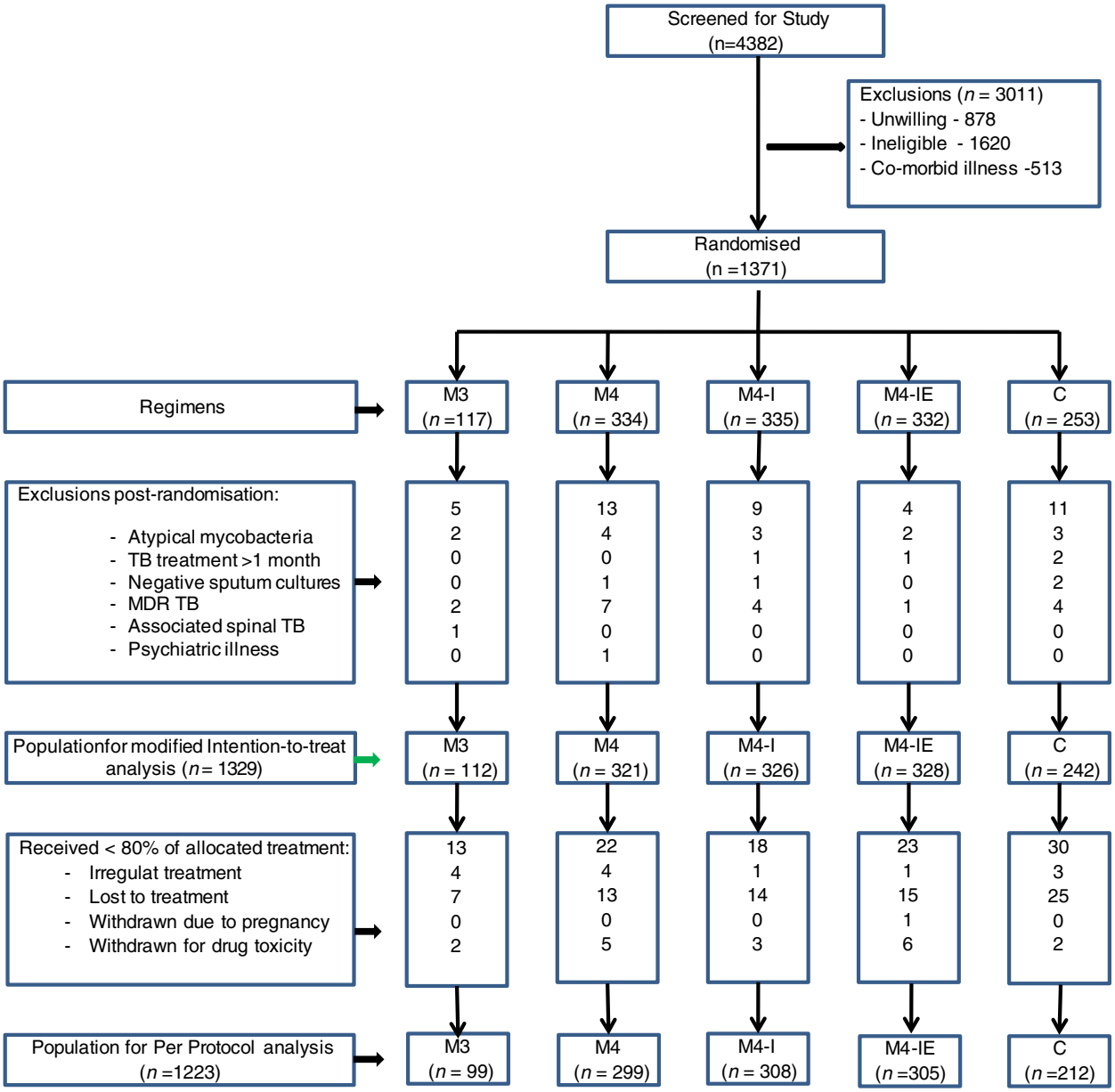


Figure 1 Flow diagram of patients from eligibility to analysis stage. [Colour figure can be viewed at wileyonlinelibrary.com]

response at the end of treatment compared to 93% in the control regimen (Table 2). Five (0.5%) of 1011 patients in the moxifloxacin regimens and 4 (1.8%) of 212 in the control regimen (P = 0.053) died.

Of 1075 drug susceptible patients, 97–100% in the moxifloxacin regimens and 96% in the control regimen had 'Favourable' response (Table 3). Resistance to H emerged in three patients with 'Unfavourable' response in the control regimen. In 148 patients with drug-resistant TB, 117 (95%) of 123 in the moxifloxacin regimens and 19 (76%) of 25 in the control regimen had 'Favourable' response (P = 0.005; Table 4). Two of six initially H resistant patients with 'Unfavourable' response in the control regimen developed additional resistance to R. One patient with initial R resistance in M4-IE regimen developed resistance to H and O.

Table 1 Baseline characteristics of 1329 pulmonary tuberculosis (TB) patients enrolled to study and found eligible (modified intention-to-treat group)

Patient characteristics	M3 *N* = 112 *n* (%)	M4 *N* = 321 *n* (%)	M4-I *N* = 326 *n* (%)	M4-IE *N* = 328 *n* (%)	Control *N* = 242 *n* (%)	Total *N* = 1329 *n* (%)
Gender						
Male	89 (79)	248 (77)	245 (75)	233 (71)	185 (76)	1000 (75)
Female	23 (21)	73 (23)	81 (25)	95 (29)	57 (24)	329 (25)
Age (years)						
<35	56 (50)	136 (42)	162 (50)	162 (49)	125 (52)	641 (48)
≥35	56 (50)	185 (58)	164 (50)	166 (51)	117 (48)	688 (52)
Body mass index* (kg/m^2)						
<16	42 (39)	121 (38)	130 (41)	141 (44)	98 (41)	532 (41)
16–18.49	51 (47)	120 (38)	131 (41)	126 (39)	100 (42)	528 (40)
18.5–22.9	14 (13)	69 (22)	48 (15)	48 (15)	38 (16)	217 (17)
>23	1 (1)	5 (2)	11 (3)	7 (2)	1 (<1)	25 (2)
Sputum culture† (maximum grade)						
≤1+	3 (3)	13 (4)	19 (6)	23 (7)	7 (3)	65 (5)
2+	11 (10)	48 (15)	56 (17)	56 (17)	54 (22)	225 (17)
3+	98 (87)	260 (81)	251 (77)	249 (76)	181 (75)	1039 (78)
Zones involved in chest radiograph						
≤2	25 (22)	64 (20)	65 (20)	69 (21)	51 (21)	274 (21)
>2	87 (78)	257 (80)	261 (80)	259 (79)	191 (79)	1055 (79)
Cavity in chest radiograph						
No	78 (70)	182 (57)	181 (56)	181 (55)	134 (55)	756 (57)
Yes	34 (30)	139 (43)	145 (44)	147 (45)	108 (45)	573 (43)
Drug susceptibility profile						
Susceptible to H, R, E, O	98 (88)	288 (90)	277 (85)	289 (88)	210 (87)	1162 (87)
Any resistance	14 (13)	33 (10)	49 (15)	39 (12)	32 (13)	167 (13)
Resistant to H	10 (9)	20 (6)	24 (7)	27 (8)	17 (7)	98 (7)
Resistant to O	3 (3)	11 (3)	22 (7)	11 (3)	14 (6)	61 (5)
Resistant to R	0 (0)	1 (0)	2 (1)	0 (0)	1 (0)	4 (0)
Resistant to HE	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)
Resistant to HO	0 (0)	0 (0)	1 (0)	1 (0)	0 (0)	2 (0)
Resistant to E	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	1 (0)
Duration of previous TB treatment						
Nil	107 (96)	303 (94)	309 (95)	302 (92)	230 (95)	1251 (94)
≤15 days	5 (4)	16 (5)	14 (4)	25 (8)	11 (5)	71 (5)
>15 to <30 days	0 (0)	2 (1)	3 (1)	1 (0)	1 (0)	7 (1)

H, Isoniazid, R, Rifampicin, O, Ofloxacin, E, Ethambutol.
*Body mass index: *N* = 1302; Missing values M3 = 4, M4 = 6, M4-I = 6, M4-IE = 6, C = 5.
†Sputum culture grading: Actual number up to 19 colonies, 20–100 colonies (1+), >100 colonies (2+) and confluent growth (3+).

In the intention-to-treat group, 'Favourable' bacteriological response ranged from 90% to 92% in the moxifloxacin regimens compared to 83% in the control regimen ($P < 0.05$; Table 2).

Adverse reactions to anti-TB drugs

Of 1087 patients treated with moxifloxacin regimens, 16 (1.4%) required treatment modification (Table 6). The commonest adverse events were arthralgia (21–32% in the moxifloxacin regimens compared to 5% in the control regimen, $P < 0.001$). Gastrointestinal symptoms occurred in 6–8% of patients in the moxifloxacin regimens and 4% in the control regimen.

Treatment was modified for five patients with cutaneous toxicity [three rifampicin induced (two in moxifloxacin and one in control regimen), one moxifloxacin induced and one unidentified]. Treatment was modified for hepatotoxicity in 11 patients in the moxifloxacin regimens.

Thirteen patients had seizures (10 in moxifloxacin regimens and three in control regimen). Treatment was withheld and re-introduced along with anti-convulsants in 10

Table 2 Status at the end of treatment and tuberculosis (TB) recurrence during follow-up in per-protocol and modified intention-to-treat populations

	Per-protocol analysis (N = 1223)					Modified intention-to-treat analysis (N = 1329)				
	M3	M4	M4-I	M4-IE	Control	M3	M4	M4-I	M4-IE	Control
Total patients	*N* = 99	*N* = 299	*N* = 308	*N* = 305	*N* = 212	*N* = 112	*N* = 321	*N* = 326	*N* = 328	*N* = 242
Favourable response to treatment, *n* (%)	99 (100)	292 (98)	297 (96)	294 (96)	198 (93)	103 (92)*	296 (92) *	298 (91) *	295 (90) *	201 (83) *
Unfavourable bacteriological response, *n* (%)	0	5 (2)	8 (3)	6 (2)	10 (5)	0	5 (2)	8 (2)	6 (2)	10 (4)
Treatment changed for clinical/radiological deterioration, *n* (%)	0	0	3 (1)	2 (1)	0	0	0	3 (1)	2 (<1)	0
Death due to TB, *n* (%)	0	1 (<1)	0	3 (1)	3 (1)	0	1 (<1)	0	3 (<1)	3 (1)
Death due to other cause, *n* (%)	0	1 (<1)	0	0	1 (<1)	0	1 (<1)	0	0	1 (<1)
Treatment changed for drug toxicity, *n* (%)	NA	NA	NA	NA	NA	2 (2)	5 (1)	3 (1)	6 (2)	2 (<1)
Withdrawn from treatment (missed >1 month of treatment continuously/pregnancy), *n* (%)	NA	NA	NA	NA	NA	7 (6)	13 (4)	14 (4)	16† (5)	25 (10)
TB recurrence in those with favourable response to treatment (*n*)	19‡	12	25δ	19‖	9¶	20‡	12	26δ	19‖	9¶
TB recurrence (%)	19.2	4.1	8.4	6.5	4.5	19.4	4.1	8.7	6.4	4.5
% Difference between the test and control regimen. (95% CI)	14.6 (5.61, 23.69)	0.4 (−3.68, 4.55)	3.9 (−0.84, 8.58)	1.9 (−2.54, 6.38)	Ref	14.9 (6.05, 23.83)	0.4 (−3.63, 4.48)	4.3 (−0.46, 8.96)	1.9 (−2.46, 6.38)	Ref

NA, Not applicable.
*Includes patients with inadequate treatment of <80% of prescribed dose: 4 in M3, 4 in M4, 1 each in M4-I and M4-IE and 3 in control regimen.
†Withdrawn from treatment due to pregnancy: *n* = 1.
Type of Extra-pulmonary TB recurrence: ‡Meningitis (*n* = 1), δTB lymphadenitis (*n* = 1), ‖Psoas abscess (*n* = 1),¶Pleural effusion (*n* = 1).

Table 3 Status at the end of treatment and tuberculosis (TB) recurrence during follow-up in per-protocol and modified intention-to-treat populations in patients susceptible to Isoniazid, Rifampicin, Ethambutol and ofloxacin

	Per-protocol analysis (*N* = 1075)					Modified intention-to-treat analysis (*N* = 1162)				
	M3	M4	M4-I	M4-IE	Control	M3	M4	M4-I	M4-IE	Control
Total patients	*N* = 87	*N* = 268	*N* = 263	*N* = 270	*N* = 187	*N* = 98	*N* = 288	*N* = 277	*N* = 289	*N* = 210
Favourable response to treatment, *n* (%)	87 (100)	262 (98)	255 (97)	261 (97)	179 (96)	91 (93)	265 (92)	256 (92)	262 (91)	181 (86)
Unfavourable bacteriological response, *n* (%)	0	4 (1)	6 (2)	4 (1)	4 (2)	0	4 (1)	6 (2)	4 (1)	4 (2)
Treatment changed for clinical/radiological deterioration, *n* (%)	0	0	2 (1)	2 (1)	0	0	0	2 (1)	2 (1)	0
Death due to TB, *n* (%)	0	1 (<1)	0	3 (1)	3 (2)	0	1 (<1)	0	3 (1)	3 (1)
Death due to other cause, *n* (%)	0	1 (<1)	0	0	1 (<1)	0	1 (<1)	0	0	1 (<1)
Treatment changed for drug toxicity, *n* (%)	NA	NA	NA	NA	NA	1 (1)	4 (1)	3 (1)	5 (2)	0
Withdrawn from treatment (Missed >1 month of treatment continuously/ Pregnancy), *n* (%)	NA	NA	NA	NA	NA	6 (6)	13 (5)	10 (4)	13* (4)	21 (10)
TB recurrence in those with favourable response to treatment (*n*)	18†	8	20‡	16δ	8‖	19†	8	21‡	16δ	8‖
TB recurrence %	20.7	3.1	7.8	6.1	4.5	20.9	3.0	8.2	6.1	4.4

NA, Not applicable.
*Withdrawn from treatment due to pregnancy: *n* = 1.
Type of Extra-pulmonary TB recurrence: †Meningitis (*n* = 1), ‡TB lymphadenitis (*n* = 1), δPsoas abscess (*n* = 1), ‖Pleural effusion (*n* = 1).

patients, and one was lost to treatment. Two of these patients in the moxifloxacin regimens were subsequently diagnosed with brain tuberculoma, and treatment was changed. In the moxifloxacin regimen, one patient had prior history of seizure. Two patients had a calcified lesion in the brain; one each in the moxifloxacin and control regimens. Nine patients (eight in moxifloxacin and one in control arm) had prolongation of QTc interval (>450 ms) in the ECG; moxifloxacin was temporarily withheld in eight and terminated in one patient (M4-IE regimen).

Discussion

This study shows that a daily 4-month moxifloxacin regimen (M4) is equivalent to a 6-month thrice-weekly regimen in new sputum-positive pulmonary TB patients with advanced disease (95% with sputum culture grading of ≥2+ and 79% with involvement of >2 zones in chest radiograph) without HIV infection, diabetes mellitus or other co-morbidities. The TB recurrence rate in patients who received this regimen and followed up for 24 months of post-treatment was 4.1% compared to 4.5% for the control regimen. The difference in the TB recurrence rate and its 95% CI satisfied our criteria of a 5% margin. The other two 4-month regimens (M4-I and M4-IE) did not show equivalence compared to the control regimen. The 3-month regimen had a high TB recurrence rate even though at the end of treatment the 'Favourable' response was 100%. Previous trials with 3-month regimens containing ofloxacin or streptomycin also documented high relapse rates [7,18].

Among patients with initial drug resistance, a greater proportion of patients treated with the moxifloxacin regimens had a 'Favourable' response at the end of treatment (95%) compared to those treated with the control regimen (76%) and this difference was statistically significant. This is in agreement with previous reports using ofloxacin or moxifloxacin containing regimens [7,8]. In this trial, drug resistance emerged in only one patient in the moxifloxacin regimens compared to five patients in the control regimen (3 to isoniazid and 2 to rifampicin).

Table 4 Status at the end of treatment and tuberculosis (TB) recurrence during follow-up in per-protocol and modified intention-to-treat populations in patients resistant to one or more drugs (Isoniazid, Rifampicin, Ethambutol and Ofloxacin) excluding resistance to Isoniazid and Rifampicin

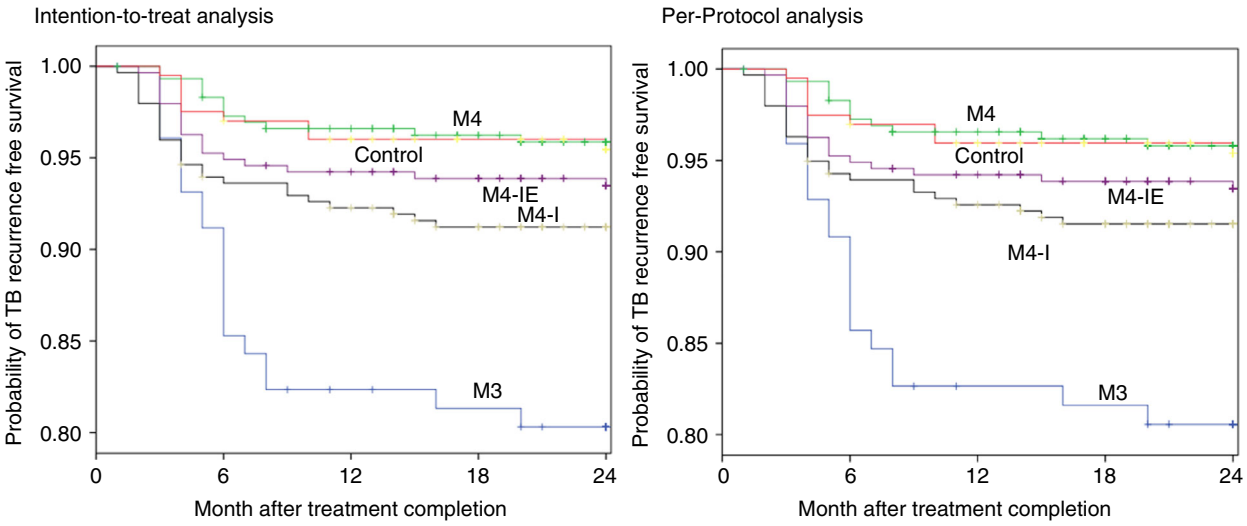
	Per – Protocol analysis (*N* = 148)					Modified intention-to-treat analysis (*N* = 167)				
	M3	M4	M4-I	M4-IE	Control	M3	M4	M4-I	M4-IE	Control
Total patients	*N* = 12	*N* = 31	*N* = 45	*N* = 35	*N* = 25	*N* = 14	*N* = 33	*N* = 49	*N* = 39	*N* = 32
Favourable response to treatment, *n* (%)	12 (100)	30 (97)	42 (93)	33 (94)	19 (76)	12 (86)	31 (94)	42 (86)	33 (85)	20 (63)
Unfavourable bacteriological response, *n* (%)	0	1 (3)	2 (4)	2 (6)	6 (24)	0	1 (3)	2 (4)	2 (5)	6 (18)
Treatment changed for clinical/ radiological deterioration, *n* (%)	0	0	1 (2)	0	0	0	0	1 (2)	0	0
Death due to TB, *n* (%)	0	0	0	0	0	0	0	0	0	0
Death due to other cause, *n* (%)	0	0	0	0	0	0	0	0	0	0
Treatment changed for drug toxicity, *n* (%)	NA	NA	NA	NA	NA	1 (7)	1 (3)	0	1 (2)	2 (6)
Withdrawn from treatment (missed >1 month of treatment continuously/pregnancy), *n* (%)	NA	NA	NA	NA	NA	1 (7)	0	4 (8)	3 (8)	4 (13)
TB recurrence in those with favourable response to treatment (*n*)	1	4	5	3	1	1	4	5	3	1
TB recurrence (%)	8.3	13.3	11.9	9.1	5.3	8.3	12.9	11.9	9.1	5.0

NA, Not applicable.

Table 5 Emergence of drug resistance or change in drug susceptibility pattern in patients during unfavourable bacteriological response at the end of treatment and at TB recurrence (per-protocol analysis)

Regimen	Baseline drug susceptibility	Number of patients	Change in drug susceptibility profile	
			Unfavourable bacteriological response	Recurrence
M3	Susceptible	87	Nil	Nil
	Resistant	12	Nil	Nil
M4	Susceptible	268	Nil	1 patient with initial susceptible culture developed resistance to O
	Resistant	31	Nil	2 patients with initial H resistance had drug susceptible cultures 2 patients with initial O resistance had drug susceptible cultures
M4-I	Susceptible	263	Nil	Nil
	Resistant	45	1 patient with initial R resistance developed H and O resistance	2 patients with initial O resistance had drug susceptible cultures
M4-IE	Susceptible	270	Nil	1 patient with initial drug susceptible culture developed resistance to O
	Resistant	35	Nil	Nil
Control	Susceptible	187	3 patients with initial drug susceptible cultures developed resistance to H	1 patient with initial drug susceptible culture developed resistance to H and R
	Resistant	25	2 patients with initial H resistance developed resistance to R	Nil

H, Isoniazid; R, Rifampicin; O, Ofloxacin.



Number at risk

Regimen	0	6	12	18	24
M3	103	93	82	80	77
M4	296	289	276	259	240
M4-I	298	278	272	257	240
M4-IE	295	281	272	262	239
Control	201	196	191	182	176

Number at risk

Regimen	0	6	12	18	24
M3	99	89	79	78	75
M4	292	285	272	255	237
M4-I	297	278	272	257	240
M4-IE	294	280	271	261	238
Control	198	193	188	179	173

Figure 2 Kaplan–Meier analysis of post-treatment tuberculosis (TB) recurrence-free survival over 24 months in the study regimens in patients with favourable response at the end of treatment. [Colour figure can be viewed at wileyonlinelibrary.com]

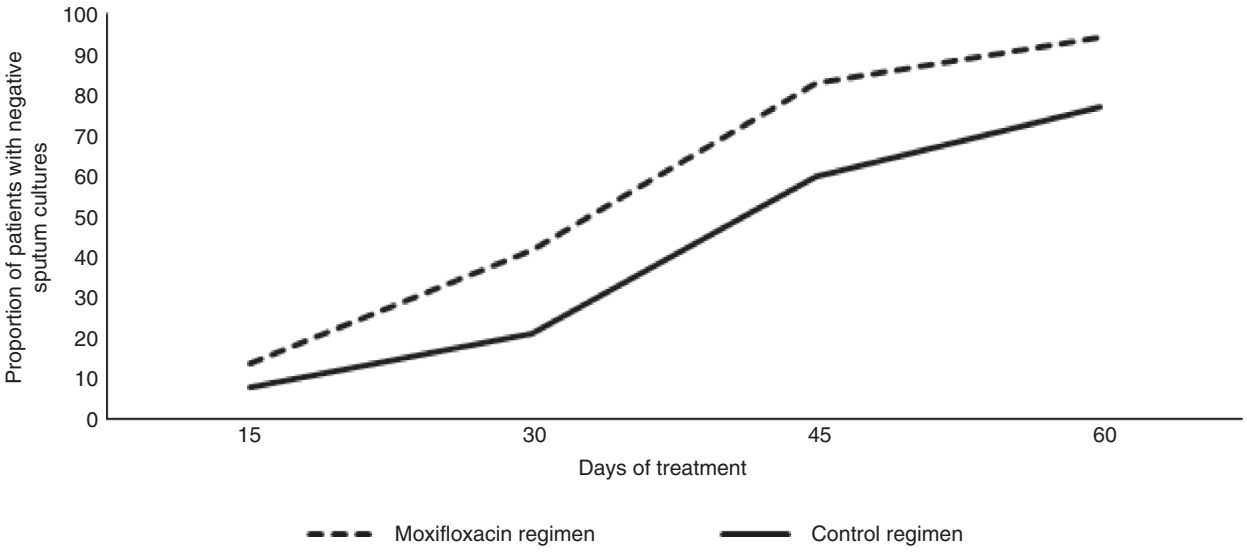


Figure 3 Sputum culture conversion during first 2 months of treatment – modified intention-to-treat analysis (Moxifloxacin regimen 1087, Control regimen 242).

Table 6 Adverse reactions attributable to anti-TB drugs in 1329 patients (modified intention-to-treat group)

Adverse drug reaction	Severity	M3 *N* = 112 *n* (%)	M4 *N* = 321 *n* (%)	M4-I *N* = 326 *n* (%)	M4-IE *N* = 328 *n* (%)	Control *N* = 242 *n* (%)
Arthralgia	Mild	36 (32)*	83 (26)*	70 (21)*	67 (20)*	12 (5)
	Moderate	0	0	2	1	0
	Severe	0	2	0	0	0
Gastro intestinal	Mild	9 (8)	19 (6)	16 (5)	26 (8)†	10 (4)
	Moderate	2	3	3	0	0
	Severe	0	0	1	4	0
Cutaneous	Mild	2 (2)	8 (2)	10 (3)	16 (5)	12 (5)
	Moderate	0	3	1	2	1
	Severe	0	1	2	2	1
Giddiness	Mild	3	3	7	3	1
	Moderate	0	0	1	1	0
	Severe	0	0	1	0	0
Hepatic	Moderate	1	3	5	4	3
	Severe	2	4	1	4	0
Cardiac	Moderate	1	4	1	1	1
	Severe	0	0	0	1	0
Seizures	Severe	0	0	8	2	3
Peripheral neuropathy	Mild	0	1	4	0	1
Renal	Moderate	0	0	0	0	1
	Severe	0	1	0	0	0
Thrombocytopenia	Severe	0	0	0	0	1

*$P < 0.001$ compared to control arm.
†$P = 0.03$ compared to control arm.

Three previous clinical trials that evaluated the efficacy of 4-month regimens with moxifloxacin (REMox TB and RIFAQUIN) or gatifloxacin (Oflotub study) in sputum-positive pulmonary TB patients did not establish non-inferiority to a 6-month regimen [19–21]. In contrast to those studies in which either moxifloxacin or gatifloxacin replaced one of the drugs in the 4-drug combination, in our study we used a 5-drug regimen, supplementing the standard 4-drug TB regimen (rifampicin, isoniazid, pyrazinamide and ethambutol) with moxifoxacin in the intensive phase [19–21]. This crucial difference plus the fact that we administered treatment under direct observation could be the reasons why we were able to show equivalence of the 4-month daily regimen with the control regimen. While making this comparison, we concede that the control regimen in our study was a 6-month thrice-weekly regimen, unlike the ReMox TB, RIFAQUIN and Oflotub trials where the control arm was a 6-month daily regimen. We used the thrice-weekly regimen as it was the standard of care in the RNTCP at the time the study was in operation. Nevertheless, we believe this is the first study that has demonstrated that a 4-month treatment regimen using moxifloxacin could be as effective as a 6-month regimen in patients with sputum-positive pulmonary TB.

Sputum culture conversion to negative at 2 months is an important parameter for assessing the efficacy of a TB drug regimen [22]. Though some earlier studies had shown no difference in 2nd month sputum culture conversion when moxifloxacin was substituted for either ethambutol [23] or isoniazid [24] as part of the standard 4-drug regimen for patients with sputum-positive pulmonary TB, our study showed a significantly higher sputum culture conversion for the moxifloxacin regimens compared to the control regimen at 2 months, and this is consistent with other reports [8,25]. We had earlier published this finding as a preliminary report [17]. Not only was the proportion of culture conversion higher in the moxifloxacin regimens but also the conversion occurred earlier. Rapid sputum culture conversion is important from the public health perspective in curtailing disease transmission in the community.

The moxifloxacin regimens were well tolerated with only 1.4% of the patients requiring modification of treatment for adverse drug reactions. Drug-related adverse events in the moxifloxacin regimens were primarily arthralgia and gastrointestinal symptoms. Most of these were mild and were managed with symptomatic measures. Arthralgia was reported by 32% of patients who

received pyrazinamide for 3 months (M3) as compared to 21–26% of those who received it for 2 months. Arthralgia attributable to pyrazinamide has been reported in 25–36% in the previous trial with ofloxacin-containing regimen [7]. Prolongation of QTc interval (>450 ms) was seen in 8 (<1%) of 1087 patients treated with moxifloxacin regimens, and only one required treatment modification. Although seizure was reported in 10 patients in moxifloxacin regimens, non-drug-related causes could be identified in four patients. Hepatotoxicity warranting treatment modification was observed in 1% of patients in moxifloxacin regimens.

A limitation of this study is that the control arm was a thrice-weekly regimen and we used this as it was the standard of care in the RNTCP till very recently, and has consistently given high cure rates in the RNTCP. The RNTCP now recommends a daily 6-month regimen with the addition of ethambutol in the continuation phase for patients with newly diagnosed tuberculosis. Admittedly this regimen is likely to be more effective than the 6-month thrice-weekly regimen that we used as the control arm. In addition, the programme now advocates drug sensitivity testing upfront so that any drug resistance is identified at initial diagnosis and appropriate treatment is provided. Notwithstanding these facts, we believe that our study has shown that the 4-month daily moxifloxacin regimen given under direct observation is effective and safe in patients with newly diagnosed advanced pulmonary TB without co-morbidities. Shortening treatment duration would have considerable benefits for both patients and healthcare providers in terms of saving of administrative costs and would ensure better treatment compliance. A treatment regimen that is safe, with a high cure rate and a recurrence rate of only 4.1% over 24 months post-treatment, and that shortens the currently recommended treatment duration by 33% would be a significant contribution to improving TB control.

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References

1. Gillespie SH, Kennedy N. Fluoroquinolones: a new treatment for tuberculosis? *Int J Tuberc Lung Dis* 1998: **2**: 265–271.
2. Ji B, Lounis N, Maslo C, Truffot-Pernot C, Bonnafous P, Grosset J. In vitro and in vivo activities of moxifloxacin and clinafloxacin against *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 1998: **42**: 2066–2069.
3. Hu Y, Coates AR, Mitchison DA. Sterilizing activities of fluoroquinolones against rifampin-tolerant populations of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2003: **47**: 653–657.
4. Sulochana S, Mitchison DA, Kubendiren G, Venkatesan P, Paramasivan CN. Bactericidal activity of moxifloxacin on exponential and stationary phase cultures of *Mycobacterium tuberculosis*. *J Chemother* 2009: **21**: 127–134.
5. Nuermberger EL, Yoshimatsu T, Tyagi S *et al*. Moxifloxacin-containing regimen greatly reduces time to culture conversion in murine tuberculosis. *Am J Respir Crit Care Med* 2004: **169**: 421–426.
6. Nuermberger EL, Yoshimatsu T, Tyagi S *et al*. Moxifloxacin-containing regimens of reduced duration produce a stable cure in murine tuberculosis. *Am J Respir Crit Care Med* 2004: **170**: 1131–1134.
7. Tuberculosis Research Centre. Shortening short course chemotherapy: a randomised clinical trial for treatment of smear-positive pulmonary tuberculosis with regimens using ofloxacin in the intensive phase. *Ind J Tuberc* 2002: **49**: 27–38.
8. Jawahar MS, Banurekha VV, Paramasivan CN *et al*. Randomized clinical trial of thrice-weekly 4-month moxifloxacin or gatifloxacin containing regimens in the treatment of new sputum positive pulmonary tuberculosis patients. *PLoS ONE* 2013: **8**: e67030.
9. ICMR. *Standard Operating Procedures for Mycobacteriology Laboratory*, Department of Bacteriology, Tuberculosis

Research Centre, ICMR, 2010. (Available from: http://nirt.res.in/pdf/bact/SOP.pdf) [28 Jun 2019].
10. Kent PT, Kubica GP. Isolation procedures. In: *Public Health Mycobacteriology. A Guide for Level 3 Laboratory*. US Department of Health and Human Services. Public Health CDC Service: Atlanta, GA; 1985; 42–43.
11. Allen B, Baker FJ. *Mycobacteria: Isolation, Identification and Sensitivity Testing*. Butterworths: London, 1968.
12. Kubica SP. Differential identification of mycobacteria. VII: Key features for identification of clinically significant mycobacteria. *Am Rev Respir Dis* 1973: **107**: 109.
13. World Health Organization. *Policy Guidance on Drug-Susceptibility Testing (DST) of Second-Line Antituberculosis Drugs*. WHO/HTM/TB: Geneva, Switzerland, 2008; 392.
14. Subbammal S, Nair NGK, Radhakrishna S, Tripathy SP. Comparison of various measures of sensitivity of *Mycobactenum tuberculosis* to Ethambutol. *Tubercle* 1978: **59**: 185–191.
15. Canetti G, Fox W, Khomenko A *et al.* Advances in techniques of testing mycobacterial drug sensitivity and the use of the sensitivity tests in tuberculosis control programs. *Bull World Health Organ* 1969: **41**: 21–43.
16. Sulochana S, Venkataraman P, Paramasivan CN. Evaluation of various methods of susceptibility to ofloxacin in strains of *Mycobacterium tuberculosis*. *Indian J Med Res* 1999: **110**: 186–189.
17. Velayutham BV, Allaudeen IS, Sivaramakrishnan GN *et al.* Sputum culture conversion with moxifloxacin-containing regimens in the treatment of patients with newly diagnosed sputum-positive pulmonary tuberculosis in South India. *Clin Infect Dis* 2014: **59**: e142–e149.
18. Tuberculosis Research Centre, Madras, and National Tuberculosis Institute, Bangalore. A controlled clinical trial of 3- and 5 month regimens in the treatment of sputum-positive pulmonary tuberculosis in south India. *Am Rev Respir Dis* 1986: **134**: 27.
19. Gillespie SH, Crook AM, McHugh TD *et al.* Four-month moxifloxacin-based regimens for drug sensitive tuberculosis. *N Engl J Med* 2014: **371**: 1577–1587.
20. Jindani A, Harrison TS, Nunn AJ *et al.* High-dose rifapentine with moxifloxacin for pulmonary tuberculosis. *N Engl J Med* 2014: **371**: 1599–1608.
21. Merle CS, Fielding K, Sow OB *et al.* A four-month gatifloxacin-containing regimen for treating tuberculosis. *N Engl J Med* 2014: **371**: 1588–1598.
22. Fox W, Ellard GA, Mitchison DA. Studies on the treatment of tuberculosis undertaken by the British Medical Research Council Tuberculosis Units, 1946–1986, with relevant subsequent publications. *Int J Tuberc Lung Dis* 1999: **3**: S231–S279.
23. Burman WJ, Goldberg S, Johnson JL *et al.* Moxifloxacin versus ethambutol in the first 2 months of treatment for pulmonary tuberculosis. *Am J Respir Crit Care Med* 2006: **174**: 331–338.
24. Dorman SE, Johnson JL, Goldberg S *et al.* Substitution of moxifloxacin for isoniazid during intensive phase treatment of pulmonary tuberculosis. *Am J Respir Crit Care Med* 2009: **180**: 273–280.
25. Conde MB, Efron A, Loredo C *et al.* Moxifloxacin versus ethambutol in the initial treatment of tuberculosis: a double-blind, randomised, controlled phase II trial. *Lancet* 2009: **373**: 1183–1189.

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