Ofloxacin resistance among *Mycobacterium Tuberculosis* isolates in two states of south-west Nigeria

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Abstract

The fluoroquinolones are an important class of antibiotics used in the management of bacterial diseases and drug-resistance tuberculosis (TB). Although resistance to fluoroquinolones has been described among common bacterial pathogens in Nigeria, little is known about resistance among TB patients. The study was embarked upon to determine the occurrence of fluoroquinolone resistance among TB patients in south-west Nigeria.

This is a retrospective study of all patients from Oyo and Osun States who had their Drug Susceptibility Test (DST) results from the Institute of Tropical Medicine, Antwerp, Belgium between 2007 and 2009. The treatment cards of the patients were reviewed to extract the personal data of the patients and the DST results.

The prevalence of ofloxacin resistance among 67 *Mycobacterium tuberculosis* isolates of patients from Oyo and Osun states was 7.5%, which represented 4.3% among new patients and 9.1% among previously treated TB patients. Among the 34 multi-drug-resistant-TB patients, ofloxacin prevalence was 11.8%. There was no significant difference in resistance to ofloxacin between new and previously treated TB patients.

The study described the occurrence of fluoroquinolone resistance in both new and previously treated TB patients. There is the need for healthcare workers to be aware of this and take precaution in adding a fluoroquinolone to patients who have been previously treated with first-line anti TB medications.

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Introduction

Nigeria currently ranks fourth among the high-burden countries with tuberculosis (TB), which account for 80% of the TB burden worldwide.[1] With the global emergence of multi-drug-resistant TB (MDR-TB), the fluoroquinolones have become increasingly used in combination with other drugs for the clinical management of MDR-TB patients.[2] Fluoroquinolone resistance has however been described in *Mycobacterium tuberculosis* isolates. This has been linked majorly to mutations in the gyrA and gyrB genes of the A and B sub units in the quinolones-resistance determining regions (QRDRs) of gyrase enzyme.[3,4] Under clinical and programmatic conditions, studies have shown that the risk that a TB patient will develop fluoroquinolone resistance is associated with the patient's previous exposure to fluoroquinolones and resistance to any first-line anti-TB drugs.[5,6]

There are currently no data available in Nigeria on fluoroquinolone resistance in TB patients. Studies from some countries have shown ofloxacin resistance to vary from 0.6 to 1.8% among new TB patients and 4.1 to 9.3% among previously treated TB patients.[6–8] The study was embarked upon to assess the frequency of the resistance to the common fluoroquinolone (ofloxacin) in *M tuberculosis* isolates from two states in south-west Nigeria in readiness for the programmatic and clinical management of MDR-TB patients.

Subjects and methods

The study is a retrospective study of all patients from Oyo and Osun States who had Drug Susceptibility Test (DST) results from the Institute Of Tropical Medicine in Antwerp, Belgium between 2007 and 2009. The study was carried out in the two states of Oyo and Osun with a population of about 8 million people. The TB programme in the two states is supported by the Damien Foundation Belgium.

Sample collection and procedure

Three sputum samples were obtained from each patient for routine diagnostic purposes in the field, but for the study we obtained another two sputum specimens from the positive patients. The two sputum specimens obtained

from each patient with a positive acid-fast bacillus (AFB) result were collected and kept in a 50 ml screw cap centrifuge tube (Falcon). Then 5–7 ml of sputum with an equal volume of 1% cetylpyridinium chloride (CPC) and sodium chloride (NaCl) was added to the sputum to inhibit the growth of fungi and other bacteria present. The containers were securely capped and shaken by hand until specimens were liquefied. The caps were properly sealed and specimens were sent to Antwerp. The average period from collection to delivery to the Supranational Lab (SNL) in Belgium was 5–7 days by express courier.

At the SNL, liquefaction and decontamination were carried out using the modified Petroff method. The specimens were inoculated onto three Lowenstein–Jensen (LJ) slopes containing 0.5% pyruvate, glycerol, and *p*-nitro benzoic acid (PNB), 500 mg/l), and were incubated at 37°C for up to 8 weeks. *M tuberculosis* was confirmed by colony morphology, absence of pigment, and failure to grow in PNB. The simplified proportional method on LJ was used for the identification of the first-line drugs. The concentrations used for the first-line drugs were: isoniazid (INH) (0.2 µg/ml–1.0 µg/ml), rifampicin (RMP) (40 µg/ml), ethambutol (EMB) (2 µg/ml), and streptomycin (SM) (4 µg/ml). The reading time was 28 and 42 days after inoculation for the final results. The concentration for ofloxacin: 2 µg/ml, DST was received on average 3–4 months after the sample was sent.

Data collection and analysis

Details about socio-demographic characteristics and previous treatment history of the patients were obtained from their treatment cards and TB registers at their respective treatment centres. Data were initially entered into an Excel spreadsheet and analysis was done using EPI Info 2002.

Results

DST results for first- and second-line anti-TB drugs for 67 *M tuberculosis* patients were available. There were 37 (55.2%) males and 30 (44.8%) females. The overall mean age was 34.2±9.22 years. The mean age for males was 33.1±8.96 years while the mean age for females was 30.1±0.65; p=0.9). Twenty-three (34.2%) patients were new cases of TB and 44 (65.8%) were retreatment cases. The age and sex distribution of the patients are shown in Table 1.

Of the 23 new TB patients, two (8.7%) were resistant to at least one of the four first-line anti-TB drugs tested while 21 were susceptible to all the first-line anti-TB drugs. Mono-resistance was observed in two patients (5.5%), while poly-resistance and MDR-TB were not observed among the new TB patients. Among the 44 previously treated TB patients, 38 (86.4%) showed resistance to at least one anti-TB drug while 6 were susceptible to all the first-line anti-TB drugs. There were four (10.5%) patients with poly-resistant TB and 34/38 (89.5%) MDR-TB patients

Ofloxacin resistance was found in five (7.5%) of the isolates. Among the 23 new TB patients, only 1 (4.3%) had isolates resistant to ofloxacin while among the 44 previously treated TB patients 4 (9.1%) had isolates resistant to ofloxacin. Of the 34 patients with MDR-TB, four (11.8%) had isolates resistant to ofloxacin as shown in Table 2. There was no significant difference in resistance to ofloxacin between new and previously treated TB patients (p = 0.43, Fisher exact test).

Age (years)	Female (n= 29)	Male (n=34)	Total (N=63)
10–20	2	1	3
21–30	10	10	20
31–40	9	16	25
41–50	7	5	12
51–60	1	2	3
Total	**29**	**34**	**63**

NB The ages for three males and 1 female were missing in the data set

Table 1 Age and sex distribution of study participants

Discussion

Fluoroquinolone resistance was found in this study to be 7.5% in general and 11.8% among MDR-TB isolates. This is higher than that reported in Rwanda (0.6% vs 9.3%)[7] and in the USA and Canada (1.8% vs 4.1%),[8] among general and MDR-TB isolates, respectively. The high frequency may be due to the fact that a larger proportion of the study sample isolates are from MDR-TB patients among previously treated patient groups. This is consistent with previous studies where fluoroquinolone resistance is primarily seen in patients with MDR-TB.[9,10]

Several studies have shown an association between the development of fluoroquinolone-resistant *M tuberculosis* and prior use of fluoroquinolone.[8,9] The high resistance observed in this study may be a result of the indiscriminate use of some commonly available quinolones, e.g ofloxacin and sparfloxacin, in the open market, especially among TB patients who have failed category II regimens on the DOTS (Directly Observed Treatment Short course) programme, thereby leaving the fluoroquinolone as the only active drug in the regimen.

There is a need to control the use of fluoroquinolones, especially the newer class, in order to sustain its continued use in the management of MDR-TB patients. All MDR-TB suspects should routinely have DST done to first- and second-line anti-TB drugs, including fluoroquinolones, so that effective individualised treatment can be instituted based on the pattern of resistance.

The study, however, has some limitations. Firstly a lack of data on the history of fluoroquinolone intake by the patients and secondly the results may not be generalised to the whole country, because of the small sample size. Hence there is a need to carry out a nationwide survey to estimate the actual fluoroquinolone resistance pattern among new and previously treated TB patients.

Pattern of resistance	New patients		Previously treated patients		Total number of patients	Total no. of isolates resistant to ofloxacin
	No. of patients	No. of isolate(s) resistant to ofloxacin	No of patients	No of isolate(s) resistant to ofloxacin		
Susceptible to all first-line drugs	21	1 (4.8%)	6	0 (0%)	27	1 (3.7%)
Resistant to ≥1 first-line (mono-resistant TB)	2	0 (0%)	4	0 (0%)	6	0 (0%)
Resistant to isoniazid and rifampicin (MDR-TB)	0	0 (0%)	34	4 (11.8%)	34	4 (11.8%)
Total	**23**	**1 (4.3%)**	**44**	**4 (9.1%)**	**67**	**5 (7.5%)**

Table 2 Prevalence of ofloxacin resistance among new and previously treated TB patients in south-west, Nigeria, 2007–2009.

Acknowledgement

The authors wish to acknowledge the Local Government TB supervisors and the State TB and Leprosy Control Officers in the two states of Oyo and Osun in south-west Nigeria. Also, the laboratory team at University College Hospital are acknowledged for their support during the collection and processing of sputum samples from the patients. Their efforts towards the success of this study are highly appreciated.

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