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Limited fluoroquinolone resistance among *Mycobacterium tuberculosis* isolates from Rwanda: results of a national survey

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***Objectives*: There is an increasing interest in the possible role of fluoroquinolone antibiotics for the treatment of tuberculosis (TB), but widespread use of these antibiotics for the treatment of other bacterial infections may select for fluoroquinolone-resistant *Mycobacterium tuberculosis* strains.**

***Methods*: We evaluated fluoroquinolone susceptibility using the proportion method (ofloxacin, critical concentration 2.0 mg/L) in isolates from patients enrolled in a national drug resistance survey in Rwanda from November 2004 to February 2005.**

***Results*: Of the 701 *M. tuberculosis* isolates studied, 617 (88%) were susceptible to all first-line drugs, 32 (4.6%) were multidrug-resistant (MDR) and 52 (7.4%) were resistant to one or more first-line drugs but not MDR. Ofloxacin resistance was found in four (0.6%) of the isolates; three of them being MDR and one susceptible to all first-line drugs. Mutations in the *gyrA* gene were found in all ofloxacin-resistant strains at codons 80 and 94.**

***Conclusions*: Our finding is not alarming for Rwanda, but highlights the general risk of producing resistance to fluoroquinolones, jeopardizing the potential for these drugs to be used as second-line anti-TB agents in the programmatic management of drug-resistant TB and creating incurable TB strains.**

Keywords: susceptibility tests, ofloxacin, tuberculosis

Introduction

Fluoroquinolones have been demonstrated to be active *in vitro* against mycobacterial species and effective in the treatment of infections caused by *Mycobacterium tuberculosis*, *Mycobacterium leprae* and atypical mycobacteria such as *Mycobacterium fortuitum*.[1] However, mycobacteria were shown to be intrinsically less susceptible to fluoroquinolones than other bacteria like *Escherichia coli*,[2] and, curiously, the level of susceptibility to these drugs differs markedly according to the mycobacterial species.

Fluoroquinolones are widely used for treating bacterial infections other than mycobacterial infections, but the extent to which this use can have an impact on the selection of fluoroquinolone-resistant mutants of *M. tuberculosis* is not known.[3]

Resistance to fluoroquinolones in tuberculosis (TB) is not routinely assessed, particularly not in isolates that are susceptible to first-line drugs. We therefore assessed the rate of ofloxacin resistance in *M. tuberculosis* isolates from Rwanda to investigate the effects of common fluoroquinolone use on TB resistance and to assess the appropriateness of standardized treatment regimens before implementation of a programmatic management of drug-resistant TB (commonly called DOTS-plus programme; where DOTS stands for directly observed therapy short course) by the National Tuberculosis Programme (NTP).

Materials and methods

Study area and population

Rwanda is a landlocked country in East Africa, with an estimated population of 8.6 million. The NTP has applied the DOTS strategy since its inception in 1990. In Rwanda, TB is one of the leading causes of mortality and morbidity,[4] with an annual incidence of

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161 new sputum-smear-positive cases per 100 000 population and a prevalence (all types) of 664/100 000 in 2003.[4] The incidence has doubled over the last decade mainly due to the HIV/AIDS epidemic.[4]

This prospective study was undertaken as part of a nationwide survey using the WHO/International Union Against TB and Lung Disease (IUATLD) guidelines for surveillance of TB drug resistance.[4] The required sample size was determined to be 633 patients based on the number of new sputum-smear-positive TB cases notified in 2002 through the NTP ($n = 3956$), a 95% confidence interval and 1% precision and on an expected multidrug resistance (MDR) prevalence of 2%. An additional 20% was included to overcome loss of cases due to negative or contaminated cultures, yielding a final sample size of 760 new smear-positive cases.[4]

Laboratory methods

All sputum samples were mixed with 1% cetylpyridinium chloride and stored at ambient temperature. The samples were collected on a weekly basis from health districts and transported to the National Reference Laboratory in Kigali for analysis. Every sputum sample was accompanied by a shipment form that contained information on the date of sputum collection, sample identification number, type of case (new or retreatment) and quantified result of microscopy examinations. Each sample was cultured on the Löwenstein–Jensen (LJ) medium after decontamination using the Petroff method.[5]

Primary cultures of *M. tuberculosis* were sent to the Mycobacteriology Unit of the Institute of Tropical Medicine, Antwerp, Belgium (ITM) for species identification and drug susceptibility testing (DST).

All isolates were identified by conventional biochemical tests.[5] DST was performed on all *M. tuberculosis* isolates for isoniazid (0.2 mg/L), rifampicin (40 mg/L), streptomycin (4 mg/L) and ethambutol (2 mg/L) using the proportion method on the LJ medium.[6]

Ofloxacin susceptibility testing was performed by the proportion method on 7H11 (2 mg/L; Sigma-Aldrich, Bornem, Belgium).[7] Results were read after 28 days of incubation and resistance was reported when the colonies in the drug-containing tubes were $\geq 1\%$ compared with the drug-free control. Internal quality control of media was performed using a reference susceptible (Mt14323) and an MDR *M. tuberculosis* strain from the ITM collection. For ofloxacin-resistant strains, detection of mutations responsible for resistance was performed by PCR amplification and sequencing of genes encoding gyrase A and B using the method described previously.[8]

Data analysis

Data were double-entered on a weekly basis during the inclusion period using SPSS (version 11.5) by two different people to ensure accuracy. These data were later linked with DST results from the ITM. Both data sets were compared using Epi info (version 6.04d) and cleaned by verifying the paper-based questionnaires, sample transportation forms and DST results. Data analysis was done according to WHO/IUATLD recommendations.[9]

Results

The results of first-line drug resistance have been published by Umubyeyi *et al.*[4] and will therefore not be discussed here. In summary, DST results were available for 701 *M. tuberculosis* isolates from the same number of patients, of whom 616 (87.9%) were new cases of TB and 85 (12.1%) were retreatment cases.

Of the 616 new cases, 65 (10.6%) were resistant to at least one of the four drugs tested (Table 1). Mono-resistance was observed in 34 cases (5.5%), non-MDR poly-resistance in 7 cases (1.1%) and MDR in 24 (3.9%) cases. Among the 85 previously treated cases 19 (22.4%) showed resistance; with 10 (11.8%) mono-resistant cases, 1 case of non-MDR poly-resistance and 8 (9.4%) MDR cases.[4]

Ofloxacin resistance was found in four (0.6%) of the isolates, three of them being MDR and one susceptible to all first-line drugs, resulting in a 9.4% ofloxacin resistance rate among MDR-TB and 0.2% among non-MDR-TB. Two of these patients were infected with HIV but did not have advanced AIDS (CD4 cells counts 242 and 447 cells/mm^3, respectively).

The three MDR patients received a fluoroquinolone (ciprofloxacin) during TB treatment with a median duration of fluoroquinolone intake of 1 month (range of 1–8 months), whereas the patient whose isolate was only resistant to ofloxacin received <14 days of fluoroquinolone therapy for respiratory symptoms before TB treatment.

Sequencing of the *gyrA* gene showed that three had mutations encoding a change from Thr-80→Ala and one from Asp-94→Ala (Table 2).

Discussion

This prospective nationwide study on fluoroquinolone resistance among smear-positive pulmonary TB cases is the first of its kind in Rwanda. Fluoroquinolone resistance was found to be uncommon. This finding is similar to previous results from sample isolates from the general population of Kazakhstan, Azerbaijan and Kinshasa (Democratic Republic of Congo), where ofloxacin resistance was not detected.[10] This is in contrast to reports

Table 1. Patterns of drug resistance among *M. tuberculosis* isolates from the national survey, November 2004 to February 2005

Pattern of resistance	Total number of isolates (%)	Number of new cases (%)	Number of previously treated cases (%)	Number of ofloxacin-resistant isolates
Susceptible to all first-line drugs[a]	617 (88)	551 (89.4)	66 (77.7)	1
Resistant to ≥ 1 first-line drugs but not to H and R	52 (7.4)	41 (6.7)	11 (12.8)	0
Resistant to H and R	32 (4.6)	24 (3.9)	8 (9.4)	3
Total	701 (100)	616 (100)	85 (100)	4

[a]Isoniazid (H), rifampicin (R), ethambutol (E) or streptomycin (S).

Table 2. Results of DNA sequencing of the *gyrA* gene in ofloxacin-resistant isolates of *M. tuberculosis*

Codon	Wild-type amino acid	Amino acid encoded in mutant isolate	Number of isolates ($n = 4$) (%)
94	Asp	Ala	1 (25)
80	Thr	Ala	3 (75)

from Thailand, Spain and India, where high rates of fluoroquinolone-resistant TB were observed.[10]

In general, fluoroquinolone resistance occurs primarily among MDR-TB patients, as was the case in our study. Strains showing *in vitro* resistance to fluoroquinolones and an injectable aminoglycoside (e.g. kanamycin) are defined as extensively drug-resistant (XDR-TB).[11] Recent reports of the CDC and the WHO documented an increasing number of XDR-TB cases around the world. In South Africa, a recent outbreak of XDR-TB among HIV-infected patients highlighted the risk of acquisition and transmission of drug-resistant TB, including resistance to fluoroquinolones.[11]

Our data have shown that the situation in Rwanda is not so dramatic, but the non-MDR-TB case that probably developed fluoroquinolone resistance in <2 weeks prior to the onset of TB treatment emphasizes the possible danger of development of fluoroquinolone resistance if a patient co-infected with *M. tuberculosis* is treated with a fluoroquinolone for another infection. Vigilance is required. It has been documented before that fluoroquinolone resistance in *M. tuberculosis* seems to develop very rapidly.[11,12]

Mutations in the *gyrA* gene were the most common mechanism of fluoroquinolone resistance in *M. tuberculosis* in this and previous studies. In our study, all fluoroquinolone-resistant isolates yielded a single mutation, which generally confers a 2–8-fold increase in the MIC.[7] In addition, we observed a Ser-95→Thr substitution in the *gyrA* gene in almost all isolates tested, regardless of the phenotypic result for ofloxacin. This is consistent with the previous finding that Ser-95→Thr is a marker for evolutionary genetics and does not correlate with fluoroquinolone resistance.[12]

However, our study does have some limitations. First, we only evaluated the susceptibility of isolates to ofloxacin, a fluoroquinolone that is less potent against *M. tuberculosis* than newer members of this class (such as levofloxacin, moxifloxacin and gatifloxacin). Second, we largely lack data on details of prior TB treatment and fluoroquinolone usage. Finally, HIV testing was not done for all patients because of operational complications, and it is therefore not possible to describe exactly how HIV seropositivity was linked to the resistance patterns observed.

Conclusions

Fluoroquinolone resistance is not common among *M. tuberculosis* isolates from Rwanda and occurs primarily among MDR isolates. These results support the implementation of programmatic management of MDR-TB in Rwanda, through the Global Fund to Fight AIDS, TB and Malaria and the Green Light Committee for access to second-line drugs.

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Transparency declarations

None to declare.

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