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Previous use of quinolones: a surrogate marker for first line anti-tuberculosis drugs resistance in HIV-infected patients?

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Objectives: Drug resistant *Mycobacterium tuberculosis* causes much higher rates of treatment toxicity, failure or relapse, and mortality. We determined the drug resistant profile of *Mycobacterium tuberculosis* strains isolated from a population of HIV-infected patients in southern Brazil and studied the potential factors associated with resistance.

Methods: We conducted a retrospective cohort study to determine the resistance profile of *Mycobacterium tuberculosis* isolated from HIV-infected patients and factors that could be associated with resistance from 2000 to 2005.

Results: 236 patients were included in the study. Resistance to at least one drug was observed in 32 (14.6%) isolates, and multi-drug resistance was observed in 4 (1.82%) isolates. On multivariate analysis, previous use of tuberculostatics and quinolones were related to any first-line drug resistance.

Conclusions: In our study, previous quinolone use was significantly associated to first-line anti-TB drugs resistance. Multi-drug-resistant tuberculosis (MDR-TB) is a major problem worldwide, and we believe quinolones should be used with caution in settings where TB is endemic.

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Introduction

Tuberculosis (TB) and human immunodeficiency virus (HIV) disease are the two leading causes of infectious disease-associated mortality worldwide.¹ According to the World Health Organization (WHO) report — published on World TB Day, March 24, 2009 — there were an estimated 9.27 million new cases of tuberculosis worldwide in 2007, and 1.37 million cases in HIV-infected persons.²

Resistance to antituberculosis drugs was first described soon after the introduction of streptomycin in 1944, and

is currently one of the most important threats to global tuberculosis control.³ Drug resistance causes much higher rates of treatment toxicity, costs, failure, and mortality.⁴ Co-infection with HIV has been associated with TB drug-resistance.⁵

Because of the poor data available and in order to detect changes in the patterns of resistance, this study was performed to determine the resistance profile of *Mycobacterium tuberculosis* isolated from a population of HIV-infected patients and factors associated with resistance in southern Brazil.

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Methods

A retrospective cohort study was performed at Hospital de Clínicas de Porto Alegre, a 735-bed tertiary care hospital in southern Brazil. From August 2000 to August 2005, all consecutive hospitalized HIV-infected patients with positive culture from any clinical specimens for *Mycobacterium tuberculosis* detected in the laboratory of microbiology were included.

Patient records were reviewed. Previous use of antituberculosis drugs was defined as any anti-TB treatment started before hospitalization. Previous use of quinolones was defined as the use of this medication for at least five days, in the six-month period before hospital arrival. Primary resistance was defined as resistance in isolates from patients who did not have history of TB treatment. The following variables were studied: gender, age, diagnostic infection site, length of time for *Mycobacterium tuberculosis* identification in specimens' culture, and TB-drugs susceptibility profile. We also reviewed CD4 lymphocyte count and HIV viral load tests performed within six months of hospitalization. Primary outcome was defined as hospital discharge or in-hospital death. Any drug resistance was defined as resistance to rifampin, isoniazid, pirazinamide, ethambutol, or streptomycin.

Mycobacterial identification and resistance profile were assessed according to hospital routine. Specimens from anatomic sites where normal bacterial flora were present were submitted to digestion with the mucolytic agent 5% N-acetyl-L-cysteine and to decontamination with 2% sodium hydroxide using the BBL MycoPrep kit (Becton Dickinson, Sparks, MD – USA) in 50 mL screw-cap polypropylene tubes. These samples were further concentrated by centrifugation (3,000 rpm for 15 min). Specimens from sterile sites were concentrated directly by centrifugation as above. A volume of 1 mL of the concentrated sample was used to inoculate liquid MiddleBrook medium (Bactec 12B with Bactec supplement, PANTA), which was processed by the radiometric method (Bactec 460TB System, Becton Dickinson) for culture of *Mycobacterium* spp. In parallel, a volume of 0.2 mL of the concentrated sample was also processed for conventional *Mycobacterium* spp. culture using Lowenstein-Jensen medium. The identification of TB complex was performed using the *p*-nitro-acetyl-amino-hydroxypropionophenone (NAP) test in the Bactec System (Becton Dickinson). Cultures positive for the TB complex were submitted to susceptibility tests for isoniazid (INH), rifampin (RIF), ethambutol (EMB) and streptomycin (SM), using the radiometric method (Bactec System). The susceptibility test for pyrazinamide (PZA) was carried out on a modified culture medium with pH set to 6.0 (Bactec, PZA). The TB strain H37Rv (susceptible to the five drugs tested) was used as a quality control to certify the activity of the first-line anti-TB agents. *Mycobacterium bovis* BCG (clinical strain identified at the Professor Hélio Fraga National Reference Center for Tuberculosis, Brazil) was used as a quality assurance of PZA resistance.

Pearson's chi-square and Fisher's exact test were used to evaluate the association between qualitative variables. Mann-Whitney's test was used to compare continuous data when normality of data could not be assured. Poisson regression multivariate analysis was performed including the following variables: previous use of RIF, INH, PZA, SM, EMB, previous use of tuberculostatics and previous use of quinolones. Two-tail level of significance was of 5%, and data analysis was performed on the Statistical Package for the Social Sciences (SPSS) version 16.0 software.

Results

The patients' characteristics are shown in Table 1. A total of 285 patients were included; 49 were initially excluded because of age under 18 or negative test for HIV infection or were not tested for HIV infection.

Data from resistance profile were available from 219 patient isolates (92.7%). Resistance to any drug was observed in 14.6% of patient isolates (n = 32). Four isolates (1.82%) were resistant to rifampin, 17 (7.76%) were resistant to isoniazid, seven (3.19%) were resistant to pyrazinamide, 16 (7.3%) were resistant to streptomycin and three (1.36%) were resistant to ethambutol. Multi-drug resistant (i.e. resistance to isoniazid and rifampin) TB was observed in four (1.82%) patient specimens. 28 patients with resistant isolates (87.5%) were considered as having primary resistance.

Table 1 - Demographic characteristics of patients

All patients	n = 236
Age – median, interquartile range (years)	33 (27-39)
Men [% (n)]	64.4 (152)
CD4 – median, interquartile range (cells/ μ L); n = 141	84 (38-172)
Viral load – median, interquartile range (log/mL); n = 50	4.88 (3.96-5.39)
Site of infection [% (n)]	
Pulmonary	52.5 (124)
Disseminated*	25.4 (60)
Lymph nodes	11.4 (27)
Pleural	4.7 (11)
CNS	3.4 (8)
Others**	2.6 (6)
Median time to TB therapy – interquartile range (days); n = 228***	1 (0-7)
Outcome [% (N)]	
In-hospital death	33.5 (79)
Discharge	66.5 (157)

*Defined as infection in two non-contiguous sites, or identification of *Mycobacterium tuberculosis* in blood, bone marrow or liver;
 urinary tract, pericardial, joints, mediastinal mass, abscesses, bone; *time in days counted from sample collection to therapy initiation.

In our analysis we included isolates from clinical specimens of different sites for the same patient with a positive culture showing resistance to any tuberculostatic drug. Resistance to any drug was more common in specimens from central nervous system TB (34.4%, n = 10), when compared to lymph nodes (17.6%, n = 18; p = 0.05), disseminated disease (16.8%, n = 16; p = 0.04), and respiratory tract (12.5%, n = 21, p < 0.01).

As shown in Table 2, 15 patients (6,84%) had previously used quinolones. On univariate analysis, prior quinolone treatment was associated with resistance to rifampin (OR 16.54; 95% CI 2.15-125.21; p < 0.01), to isoniazid (OR 3.94; 95% CI 1.02-15.79; p = 0.04), to streptomycin (OR 4.3; 95% CI 1.04-17.73; p = 0.02), to ethambutol (OR 33.27; 95% CI 2.79-395.89; p < 0.01) and to multidrug-resistance (OR 16.15; 95% CI 2.1-124.0; p < 0.01), but not with resistance to pyrazinamide (OR 2.66; 95% CI 0.29-23.57; p = 0.36). Only two patients had previous use of tuberculostatics and quinolones; one patient was previously treated with rifampin, isoniazid and pirazinamide and showed resistance to rifampin,

isoniazid, pirazinamide and ethambutol. The other patient had previous use of prophylactic isoniazid and showed resistance to isoniazid, streptomycin and ethambutol.

On multivariate analysis including previous use of tuberculostatics, previous rifampin, isoniazid, pirazinamide, streptomycin, ethambutol, or quinolone use, the following factors were independently associated with any drug resistance: previous use of rifampin (p < 0.01), isoniazid (p < 0.01), streptomycin (p < 0.01), and quinolone use (p < 0.01).

Discussion

In our setting, tuberculosis incidence and resistance profile to first line anti-TB drugs was low, according to the OMS classification.⁶ Besides, comparing to data published a few years before, it remained stable. A previous study (1997-2003) from our center showed that resistance to a single drug was observed in 12.6% of 398 patients, and MDR-TB was

Table 2 - Characteristics of patients with tuberculosis previously exposed with quinolones

	Age	Gender	Quinolone	Days of use	Days from therapy to culture ¹	Date of culture	Indication of use	Any drug resistance ²	Outcome
Patient 1	21	F	Levofloxacin	11	30	04-oct-00	Respiratory infection	No	Discharge
Patient 2	31	M	Ciprofloxacin	7	45	03-feb-01	Respiratory infection	Yes	Death
Patient 3	34	M	Levofloxacin	8	133	25-may-01	Respiratory infection	No	Discharge
Patient 4	19	M	Levofloxacin	5	9	03-aug-01	Respiratory infection	Yes	Discharge
Patient 5	60	M	Ciprofloxacin, levofloxacin	5	30	19-oct-01	Respiratory infection	No	Discharge
Patient 6	27	F	Ciprofloxacin	13	71	13-dec-01	Presumed NTM infection	No	Death
Patient 7	39	F	Levofloxacin	5	29	03-may-02	Respiratory infection	Yes	Death
Patient 8	31	F	Levofloxacin, ciprofloxacin	12	13	23-apr-02	Respiratory infection	No	Death
Patient 9	43	M	Gatifloxacin	> 5	< 6 months	04-sep-02	Respiratory infection	No	Discharge
Patient 10	40	F	Levofloxacin	10	10	31-oct-02	Respiratory infection	No	Discharge
Patient 11	27	F	Levofloxacin	10	17	26-dec-02	Respiratory infection	Yes	Discharge
Patient 12	34	M	Levofloxacin	5	29	06-mar-03	Respiratory infection	Yes	Death
Patient 13	33	M	Ciprofloxacin, levofloxacin	3	14	29-mar-04	Diarrhea Respiratory infection	No	Death
Patient 14	29	F	Levofloxacin	7	< 6 months	28-sep-04	Respiratory infection	No	Death
Patient 15	33	F	Levofloxacin	6	< 6 months	26-jul-05	Respiratory infection	No	Discharge

NTM, non-tuberculous mycobacteria; ¹days from therapy to culture, means the number of days from quinolone therapy initiation to date of culture collection; ²any drug resistance, means resistance to rifampin, isoniazid, pirazinamide, ethambutol or streptomycin.

found in 2% of patients.⁷ Two years later our local resistant profile did not change, and is lower than other parts of the country. The prevalence of TB resistance in HIV-infected patients in Rio de Janeiro was 16.6% (any first-line drugs),⁸ and more than 19% in São Paulo with 11.3% of multidrug resistance tuberculosis.⁹ Nonetheless, these data are from patients collected from a single tertiary care center and do not necessarily represent the resistance profile of the general population with TB.

In Estonia, a study from 2003 to 2005 found a strong association between previously treated tuberculosis and multidrug and extensive drug resistance.¹⁰ Also, a systematic review showed previous treatment as the strongest determinant of MDR-TB.⁵ In addition, age under 65 years, living outside Europe, male gender, and persons infected with HIV were associated with MDR-TB.¹¹

As shown in our data, other authors have demonstrated that previous use of quinolones was related to drug resistance. In the study by Park et al., previous ofloxacin use was not associated with ofloxacin resistance or with other antituberculosis drug resistance. One patient with previous use of levofloxacin developed ofloxacin, rifampin, isoniazid and pyrazinamid resistance.¹² In the study by Wang et al., the *Mycobacterium tuberculosis* isolates resistant to first-line antituberculosis drugs were more likely to have quinolone resistance, but they did not address a cause-effect relationship between previous quinolones use and first-line anti-TB drug resistance.¹³ Long et al. showed that multiple, but not single, quinolone prescriptions were associated with quinolone resistant TB. In this study, most patients who had received multiple quinolone prescriptions had TB with first-line drug resistance.¹⁴ So far, there is no evidence of cross-resistance between quinolones and anti-TB agents. The quinolones and first line TB drugs do not share similar mechanisms of resistance and there is a lack of data referring to this cause-effect relationship. However, we found a correlation between previous use of quinolones and first-line TB drugs resistance.

Our study has some obvious limitations that could threaten the validity of our results. This study analyzed inpatients' samples from a single reference center, and due to the retrospective design, confounding factors could have not been controlled. On the other hand, despite a lack of evidence regarding cross-resistance, previous quinolone use could be assumed as a surrogate marker of first-line TB drugs resistance.

Conclusions

In summary, previous use of quinolones was significantly related to first-line anti-TB drugs resistance. The identification of significant associations must be accompanied by biological plausibility. However, it is important to validate these results in prospective controlled studies. Nevertheless, MDR-TB is a major problem worldwide, and our study suggests that quinolones should not be used as first-line antibiotics to treat pneumonia in settings where TB is endemic.

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Conflict of interest

All authors declare to have no conflict of interest.

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