Late diagnosis of histoplasmosis in a Brazilian patient with acquired immunodeficiency syndrome

Bactericidal activity of moxifloxacin against pneumococci

Moxifloxacin is a new fluoroquinolone with a good activity against Gram-negative and Gram-positive bacteria. Against *Streptococcus pneumoniae*, moxifloxacin remains active on penicillin-resistant isolates [1,2].

In this work, we first tested the susceptibility of 150 isolates of pneumococci against moxifloxacin sampled from patients with otitis or sinusitis: 50 penicillin-susceptible isolates (minimal inhibitory concentrations [MIC] < 0.125 mg/L), 50 penicillin-intermediate isolates (MIC 0.125–1 mg/L) and 50 penicillin-resistant isolates (MIC > 1 mg/L). MICs were determined in Mueller-Hinton agar supplemented with 5% horse blood, according to CASFM guidelines [3]. Suspensions with a turbidity equal to a 0.5 MacFarland standard were prepared in Suspension Medium (Bio Mérieux, Mary-L'Etoile, France) and spotted in microplates. Suspensions were inoculated with a Steers replicator and incubated at 35 °C under CO_2 during 18 h. The lowest concentration of antibiotic inhibiting growth was considered as the MIC value. We then studied the bactericidal kinetics for five penicillin-susceptible, five penicillin-intermediate and five penicillin-resistant isolates, respectively. The bactericidal kinetics of the 15 isolates was studied by using concentrations based upon the MIC of moxifloxacin in the different isolates: 0.125 mg/L for 11 isolates, 0.25 mg/L for two, 0.06 mg/L and 2 mg/L for one. Isolates were grown in Mueller-Hinton broth and diluted in the same medium to obtain a final concentration of 10^6–10^7 of µ/mL. The concentrations used for moxifloxacin were adjusted at 1 and 4 times the MIC. A control without antibiotic was analyzed in the same conditions. A test for carry-over was performed in these conditions. Cultures were shaken, incubated at 35 °C under CO_2 and 100 µL were removed at 0 h, 1 h, 4 h, 7 h, 9 h, 24 h, plated on Columbia agar with 5% horse blood (Bio Mérieux) after dilution in NaCl 0.15 M and incubated at 35 °C under CO_2. Colony-forming units (CFU) were counted after serial dilutions. Bactericidal activity was defined as a reduction of 3 $\log_{10}$ of CFU.

The MIC values are shown in Table 1. Each isolate of *Strep. pneumoniae* was susceptible to moxifloxacin, except one with a MIC at 2 mg/L. This isolate was defined as resistant to moxifloxacin according to a recent multicenter study which reported the MIC breakpoint for moxifloxacin of 1 mg/L [4]. The susceptibility of penicillin G did not influence the result of MIC 50 and 90. Moxifloxacin displayed a better activity than penicillin against penicillin-resistant isolates. In the majority of cases, moxifloxacin had lower MIC against penicillin-intermediate isolates.

We found that the killing rate of moxifloxacin was faster at 4 times the MIC than at the MIC (Table 2). Although there were variations between isolates, penicillin-intermediate isolates were less susceptible to the bactericidal effect of the quinolone. Indeed, the decrease of CFU/mL was higher in penicillin-resistant isolates as compared to penicillin-susceptible and penicillin-intermediate isolates of *Str. pneumoniae*. At the MIC, there was occasionally no bactericidal activity after 24 h, whereas at 4 times the MIC, only one isolate was not killed after 9 h and none at 24 h. The time of 3 $\log_{10}$ decrease of the moxifloxacin-resistant isolate was similar to the others. The bactericidal effect was very different between these isolates. Nevertheless, we can say that each isolate, except one, was killed at 4 times MIC after 9 h of incubation. As previously demonstrated in other studies, moxifloxacin shows a concentration-dependent killing rate against *Strep. pneumoniae* but also against other organisms that commonly cause respiratory, urinary, skin and soft-tissue infections [2,5–7]. Reductions in viable counts of 3 $\log_{10}$ occurred in most cases, except three, within 24 h. These results indicate that moxifloxacin is active

Table 1 MICs of moxifloxacin (mg/L) against *Streptococcus pneumoniae*

	Range MIC	MIC 50	MIC 90
Strep. pneumoniae Penicillin-susceptible MIC < 0.125 mg/L (50 isolates)	0.125–2	0.125	0.25
Strep. pneumoniae Penicillin-intermediate MIC 0.125–1 mg/L (50 isolates)	0.06–0.5	0.125	0.25
Strep. pneumoniae Penicillin-resistant MIC > 1 mg/L (50 isolates)	0.06–0.25	0.125	0.25

Table 2 Median bactericidal activity of moxifloxacin at 1 and 4 times MIC after 1, 4, 7, 9 h and 24 h of incubation. Results are expressed in decrease $\log_{10}$ CFU/mL

	Moxifloxacin 1 x MIC	Moxifloxacin 4 x MIC
Strep. pneumoniae		
Penicillin-susceptible		
1 h	0.25 (0.02–0.67)	0.51 (– 0.08–1.70)
4 h	1.37 (0.62–2.92)	2.39 (1.82–3.21)
7 h	2.11 (0.92–3.11)	4.10 (3.19–5.11)
9 h	2.87 (1.07–5.11)	4.66 (3.92–5.41)
24 h	4.11 (2.39–5.42)	5 (4.4–5.5)
Strep. pneumoniae		
Penicillin-intermediate		
1 h	0.13 (– 0.19–0.92)	0.04 (– 0.11–0.22)
4 h	1 (0.84–1.37)	1.98 (1.35–2.52)
7 h	1.79 (1.18–2.31)	3.03 (2.08–4.09)
9 h	2.09 (1.28–2.52)	3.68 (2.65–4.86)
24 h	3.38 (2.56–4.93)	5.10 (4.93–5.15)
Strep. pneumoniae		
Penicillin-resistant		
1 h	0.05 (– 0.05–0.16)	0.15 (– 0.03–0.65)
4 h	2 (0.61–2.60)	2.42 (1.86–3.21)
7 h	3.40 (1.83–4.84)	4.28 (3.13–5.14)
9 h	3.95 (2.43–5.14)	4.9 (3.65–5.41)
24 h	4.87 (3.43–5.5)	5.23 (4.73–5.5)

on wild-type isolates of penicillin-resistant isolates of *Strep. pneumoniae*.

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Late diagnosis of histoplasmosis in a Brazilian patient with acquired immunodeficiency syndrome

Histoplasmosis is frequent in areas where *Histoplasma capsulatum* var. *capsulatum* is present in soil containing starling and bat droppings (America, South–Central Africa, Southeastern Asia) [1]. The increased number of immunocompromised subjects, such as patients with human immunodeficiency virus (HIV) infection, and visits to or emigration from endemic areas have led to more frequent reports of histoplasmosis even in Europe [2]; some cases [3, 4] have been reported in Italian subjects who have never been abroad.

This report concerns a late-diagnosed case in a 24-year-old Brazilian female who had been in Italy for 18 months; she presented with fever and chills, tachycardia, sore throat, and oral candidiasis. Laboratory abnormalities such as leukopenia, lymphopenia, anemia, thrombocytopenia, hypoalbuminemia, hypergammaglobulinemia, increased erythrocyte sedimentation rate and increased lactate dehydrogenase level were found; an HIV test was positive, with a $CD4^+$ count of 2 cells/μL.

Therapy with co-trimoxazole was initiated, but after 2 days of negative clinical response, ceftazidime plus amikacin, ethambutol plus clarithromycin as anti-*Mycobacterium avium* complex (MAC) treatment, nystatin for oral candidiasis and methylprednisolone were started, with subsequent reduction of fever.

Chest X-ray and CT scan, brain CT scan and abdominal ultrasonography were negative, whereas heart ultrasonography showed mild hypokinesia of the left ventricle and moderate reduction of ejection fraction. Cultures from blood, urine and bone marrow (BM) were negative, as were tests for *Leishmania* (IgG antibody, culture from BM), mycobacteria (microscopy and culture from sputum, culture from blood and BM), and *Cryptococcus* (serum antigen). Sputum culture was positive for *Candida albicans*, and serum anticytomegalovirus IgG antibodies were found. Microscopy of the BM biopsy revealed interstitial plasmocytosis and increase of megakaryocytes.

Following positive results of early antigen test and blood culture for cytomegalovirus, ganciclovir was initiated. The patient remained afebrile for 2 weeks, but subsequently multiple daily fever peaks of 39–40 °C and oral candidiasis were observed; anti-MAC therapy was withdrawn, and imipenem and fluconazole (400 mg/day) were started, followed by reduction but not disappearance of fever and by recovery from oral candidiasis. The patient was discharged at her request, in order to go back to Brazil, but 3 days after arriving she died from heart and respiratory failure.

After the patient's death, four blood cultures processed by the BACTEC radiometric system for mycobacteria grew a fungus. Subcultures on Sabouraud agar and blood agar were prepared. After incubation at 28 °C for 20 days, felt-like colonies approximately 5 mm in size, whitish on top and ochre-brown on the back, developed. Hyphae with tuberculate macroconidia could be seen by microscopic examination. Subcultures on blood agar plates incubated at 37 °C for 20 days grew whitish colonies of approximately 1–3 mm, which microscopic examination revealed as consisting of yeasts. The organism was identified as *H. capsulatum*. Identification was confirmed by transformation at the Pasteur Institute, Paris, France.

The case we described highlights the difficulty in diagnosing histoplasmosis in non-endemic areas such as Italy. In the absence of specific clinical suspicion, cultures for fungi are eliminated after an incubation period too short for growing *H. capsulatum*. We luckily isolated the fungus from blood cultures incubated for a long period in order to grow mycobacteria. Even in the presence of clinical suspicion of histoplasmosis, the diagnostic process is long and hard. The availability of antigen or antibody tests is limited in non-endemic countries, and interpretation of results by inexperienced personnel is difficult. Histopathologic techniques must be subsequently confirmed by culture, and only in endemic areas are blood cultures important diagnostic tools, with the use of lysis centrifugation techniques [5]. Moreover, cross-reactions are possible between *H. capsulatum*, *Paracoccidioides brasiliensis*, *Blastomyces dermatitidis*, and *Coccidioides immitis*. Therefore, diagnosis should be based on a combination of case history, clinical picture recording, and laboratory tests.

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