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Evaluation of the antibacterial effect *in vitro* of moxifloxacin and linezolid on nontuberculous *mycobacterium.*

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Abstract

Objective: To evaluate the antibacterial effect *in vitro* of moxifloxacin and linezolid on nontuberculous *mycobacterium* (NTM), and provide scientific evidence for treatment of NTM disease.
Materials and methods: From January 2012 to June 2014, a total of 98 NTM strains were collected from suspected tuberculosis patients (n=98) in our hospital. The minimal inhibitory concentration (MIC) of the moxifloxacin and linezolid against NTM isolates were detected by microdilution broth method.
Results: The sensitivity of moxifloxacin to *Mycobacterium avium* complex was 84.5% (49/58), which was significantly higher than that of *Mycobacterium abscess* (11.5%, 3/26) ($\chi 2$=40.505, P=0.000). The sensitivity of linezolid to *Mycobacterium avium* complex was 65.5% (38/58), which was lower than that 88.5% (23/26) of *Mycobacterium abscess.* The difference was statistically significant (χ^2=4.753, P=0.029). Among *Mycobacterium avium* complex, the sensitivity of Moxifloxacin to *Mycobacterium avium* (96.2%, 25/26) was higher than that of *Mycobacterium* intracellulare (75%, 24/32), the difference was statistically significant (Fisher test, P=0.033); The sensitivity of linezolid to *Mycobacterium avium* and *Mycobacterium* intracellulare were 69.2% (18/26) and 62.5% (20/32) respectively. There was no significant difference between the two groups (χ^2=0.288, P=0.592).
Conclusion: Different species of NTM differed in the drug susceptibility profiles. It is necessary to identify and perform drug sensitivity test in the diagnosis and treatment of NTM diseases.

Keywords: Nontuberculous *mycobacterium*, Moxifloxacin, Linezolid, Antibiotics.

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Introduction

In recent years, the incidence of nontuberculous *mycobacterium* (NTM) has increased in many countries [1-3]. The result of China's epidemiological survey of tuberculosis showed that, the percentage of NTM isolates among *mycobacterium* isolates increased from 4.3% in 1979 to 11.1% in 2000, while in 2010, the ratio increased to 22.9% [4,5]. For many NTMs, especially the rapid growth of nontuberculous *mycobacterium* (RGM), they have not only high drug resistance rate but also extensive drug resistance, leading to limited alternative drugs and difficulties in clinical treatment. Therefore, searching for new drugs or exploring conventional drugs in new use becomes the urgent task for current treatment of NTM disease.

Linezolid is an oxazolidinone antibiotic used for the treatment of gram-positive cocci infection, which was found in recent research that it has satisfactory antibacterial activity against multidrug-resistant tuberculosis and *Mycobacterium avium* complex (MAC) [6,7]. Moxifloxacin is a new class of quinolones, which has certain curative effect on MAC disease [8]. However, there are few reports on research about the drug sensitivity of different NTMs to linezolid and moxifloxacin *in vitro* in China. To explore the possibilities of using linezolid and moxifloxacin drugs for the treatment of NTM disease, we have collected 98 NTM clinical isolates to detect and compare the minimal inhibitory concentration (MIC) of the two anti-infective drugs.

Materials and Methods

Materials

Strain source: 98 NTM strains were collected from clinical tuberculosis patients in Microbiology Laboratory, College of Medicine, the First Affiliated Hospital of Zhejiang University (China) from January 2012 to June 2014.

Strain identification: Jingxin Mycobacteria Identification Diagnostic Kit (Capital Bo Corporation, China) was used for Mycobacteria strains identification. According to the identification results, there were 32 strains of *Mycobacterium intracellulare*, 26 strains of *Mycobacterium avium*, 26 strains of *Mycobacterium abscessus*, 6 strains of *Mycobacterium Gordonae*, 2 strains of *Mycobacterium* chelonae, 2 strains of *Mycobacterium kansasii*, 2 strains of *Mycobacterium fortuilum*, 1 strain of *Mycobacterium* toad and 1 strain of *Mycobacterium smegmatis*.

Antimicrobial agents: linezolid (Sigma Company, the U.S.) and moxifloxacin (Dalian Merro Pharm, China).

Quality-control strains: *Mycobacterium* intracellulare ATCC 700898 and *Staphylococcus aureus* ATCC 29213.

Methods

Preparation of inoculated strain: Inoculate the retained strains in 7H9 liquid medium (containing 10% OADC addictive), culture them at 37 for about 7 days (for slow growing *Mycobacterium*) or 3 days (for rapid growing *Mycobacterium*). Until the strains grow to a certain degree of turbidity, and during their logarithmic growth phase, perform drug sensitive test.

Drug preparation: Linezolid and moxifloxacin were dissolved with sterile deionized water, with the parent solution concentration of 1280 mg/L. Diluted them by 7H9 broth under doubling dilution after 10 fold dilution , with the concentration ranging from 0.5~128 μg/mL; added 100 μL to each well.

Inoculation, incubation and result: Adjust the strains to be tested to the turbidity of 0.5 McFarland with 0.85% normal saline, then dilute the bacterial suspension for 100 times by 7H9 medium, and add 10 μL to each well. Place the reaction plate in a sealed bag containing wetted cotton to prevent evaporation of the culture medium from the 96-well culture plate. Place the sealed bag in a 37 incubator and culture it for 7 days or 3 days, and observe the growth of bacteria in the 96-well culture plate. Record the minimal inhibitory concentration (MIC) of drugs against bacteria when the bacteria in the positive control well grow well and on significant bacterial growth was observed in the negative control well.

Interpretation of drug sensitivity breakpoint: Refer to the interpretation standard for NTM drug sensitivity test of the United States Clinical and Laboratory Standards Institute (CLSI M24-A2 2011) (Table 1) [9].

Table 1. *The MIC Drug Sensitivity Interpretation Breakpoint of Mycobacterium.*

***Mycobacterium* Strains**	**Drugs**	**MIC (μg/mL) Interpretation Point**		
		S	**I**	**R**
Mycobacterium avium complex	moxifloxacin	≤ 1	2	≥ 4
	linezolid	≤ 8	16	≥ 32
Mycobacterium kansasii	moxifloxacin			>2
	linezolid			>16
Rapid Growing *Mycobacterium*	moxifloxacin	≤ 1	2	≥ 4
	linezolid	≤ 8	16	≥ 32

Statistical analysis

Statistical analysis was performed using SPSS17.0 software, and chi-square test or Fisher test was used to compare the rates. $P < 0.05$ was considered statistically significant.

Result

Demographic and clinical characteristics

The demographic and clinical features of 98 NTM cases were showed in Table 2. Among the 98 cases, pulmonary NTM (PNTM) infections accounted for 87.8% 86/98, and 37.2% (32/86) of PNTM patients had chronic lung comorbidities. Chronic lung disease and diabetes mellitus were the two major comorbidities, and a total of 18 patients had more than two comorbidities.

Table 2. Demographic and clinical features of 98 NTM cases.

Variable	Patients (n=98), n (%)
Age, y, median (range)	59 (26-90)
Sex, male: female	70:28
Fever	82 (83.7)
Comobidity diseases	88 (89.8)
Chronic lung disease	32 (32.7)
Diabetes mellitus	17 (17.3)
Chronic liver disease	15 (15.3)

Hypertension	12 (12.2)
Malignancy	12 (12.2)
Transplantation	5 (5.1)
HIV-infected	5 (5.1)
Chronic kidney disease	4 (4.1)
Syhilis	4 (4.1)
Limb paralysis	1 (1.0)
Infection site	
Lung	86 (87.8)
Cervical lymph node	4 (4.1)
Intracalvarium	2 (2.0)
Urinary system	2 (2.0)
Antrum auris	1 (1.0)
Lumbar vertebrae	1 (1.0)
Wound	2 (2.0)
Positive acid-fast bacillus (n=68)	68 (69.4)

Antibacterial effect in vitro of moxifloxacin and linezolid

The MIC of moxifloxacin and linezolid to 98 NTM isolates were showed in Table 3. The MIC90 of moxifloxacin to the clinical common strains *Mycobacterium* intracellulare, *Mycobacterium avium* and *Mycobacterium abscesses* were 4 µg/mL, 1 µg/mL and 8 µg/mL respectively; while the MIC90 of linezolid to the above three kinds of strains were 64 µg/mL, 32 µg/mL and 16 µg/mL. The sensitivity of moxifloxacin to *Mycobacterium avium* complex was 84.5%, which was significantly higher than that of *Mycobacterium abscesses* (11.5%) (χ^2=40.505, P=0.000). Among *Mycobacterium avium* complex, the sensitivity of Moxifloxacin to *Mycobacterium avium* (96.2%) was higher than that of *Mycobacterium* intracellulare (75%), the difference was statistically significant (Fisher test, P=0.033). The sensitivity of linezolid to *Mycobacterium avium* complex was 65.5%, which was lower than that of *Mycobacterium abscess* (88.5%), and the difference was statistically significant (χ^2=4.753, P=0.029). Among *Mycobacterium avium* complex, the sensitivity of linezolid to *Mycobacterium avium* and *Mycobacterium intracellulare* were 69.2% and 62.5% respectively, and the difference was not statistically significant (χ^2=0.288P=0.592).

The MIC90 of moxifloxacin to *Mycobacterium avium* was lower that its position on the concentration-accumulative inhibition curve was inclined to the left, while MIC 90 of moxifloxacin to *Mycobacterium abscesses* was higher that its position on the concentration-accumulative inhibition curve was inclined to the right (Figure 1). The MIC90 of linezolid to *Mycobacterium abscesses* was lower that its position on the concentration-accumulative inhibition curve is inclined to the left, while the MIC90 of linezolid to *Mycobacterium intracellulare* is higher that its position on concentration-accumulative inhibition curve is inclined to the right (Figure 2).

Table 3. *MIC Strain Distribution of Antibacterial Drugs to NTMs.*

Mycobacterium Strains	Quantity	The Quantity of inhibited strains by different concentration of antibacterial drugs (µg/mL)									MIC50	MIC90	S%	I%	R%
		≤ 0.5	1	2	4	8	16	32	64	≥ 128					
Mycobacterium intracellulare															
Moxifloxacin	32	21	3	4	4	0	0	0	0	0	0.5	4	75	12.5	12.5
Linezolid	32	1	2	5	6	6	2	4	4	2	8	64	62.5	6.3	31.2
Mycobacterium avium															
moxifloxacin	26	18	7	0	1	0	0	0	0	0	0.5	1	96.2	0	3.8
linezolid	26	6	2	2	2	6	4	2	2	0	8	32	69.2	15.4	15.4
Mycobacterium abscessus															
Moxifloxacin	26	0	3	9	11	1	1	1	0	0	4	8	11.5	34.6	53.9
Linezolid	26	0	1	4	13	5	3	0	0	0	4	16	88.5	11.5	0
Mycobacterium Gordonae															
Moxifloxacin	6	4	0	1	1	0	0	0	0	0	0.5	4	-	-	-
Linezolid	6	2	1	0	1	0	1	0	1	0	1	64	-	-	-
Mycobacterium															

chelonae															
Moxifloxacin	2	1	0	0	0	1	0	0	0	0	-	-	-	-	-
Linezolid	2	1	0	0	1	0	0	0	0	0	-	-	-	-	-
Mycobacterium kansasii															
moxifloxacin	2	2	0	0	0	0	0	0	0	0	-	-	-	-	-
linezolid	2	0	1	0	0	0	0	0	0	1	-	-	-	-	-
Mycobacterium fortuilum															
moxifloxacin	2	1	1	0	0	0	0	0	0	0	-	-	-	-	-
linezolid	2	0	1	0	0	0	0	1	0	0	-	-	-	-	-
Mycobacterium toad															
moxifloxacin	1	1	0	0	0	0	0	0	0	0	-	-	-	-	-
linezolid	1	1	0	0	0	0	0	0	0	0	-	-	-	-	-
Mycobacterium megmatis															
moxifloxacin	1	0	0	1	0	0	0	0	0	0	-	-	-	-	-
linezolid	1	0	0	1	0	0	0	0	0	0	-	-	-	-	-
Slow Growing *Mycobacterium*															
moxifloxacin	67	46	10	5	6	0	0	0	0	0	0.5	2	-	-	-
linezolid	67	10	6	7	9	12	7	6	7	3	8	64	-	-	-
Rapid Growing *Mycobacterium*															
moxifloxacin	31	2	4	10	11	2	1	1	0	0	2	8	19.3	32.3	48.4
linezolid	31	1	2	5	14	5	3	1	0	0	4	16	87.1	9.7	3.2

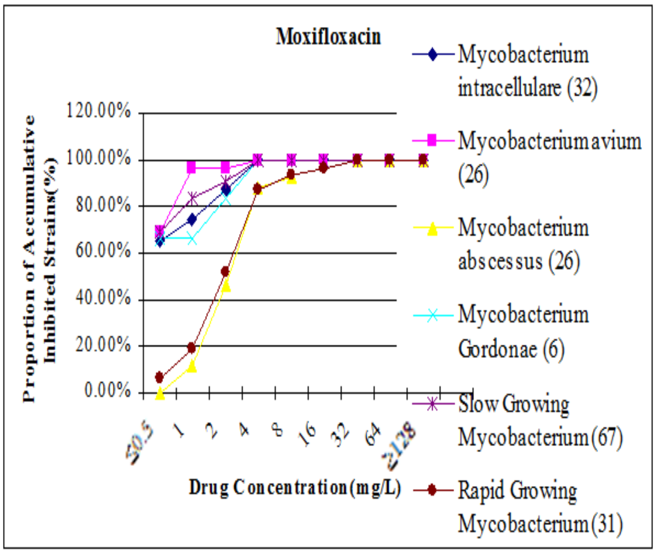

Figure 1. Concentration-accumulative inhibition curve of moxifloxacin to different species of NTM isolates.

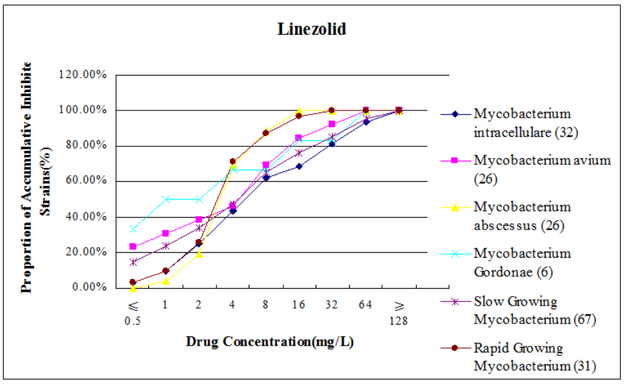

Figure 2. Concentration-accumulative inhibition curve of linezolid to different species of NTM isolates.

Discussion

Currently, there are more than 150 species of NTM strains have been found, some of which have the capability to cause human opportunistic infection. The result showed that prevalent NTM strains differed in different regions, and different species of NTM also differed in their pathogenic types, which were closely related to the local climate factors, geographical location and the patients' personal working and living conditions [10]. Each NTM isolate has its unique drug susceptibility profile, which requires different antimicrobial agents for treatment, and thus it is necessary to identify the strain types and perform NTM drug sensitivity test in vitro before the treatment.

In this study, the MIC value of moxifloxacin against NTM was detected by micro-broth dilution method, and the result showed that the MIC50 and MIC90 of moxifloxacin against *Mycobacterium intracellulare* were 0.5 μg/mL and 4 μg/mL respectively, and the drug resistance rate was 12.5%; the MIC50 and MIC90 of moxifloxacin against *Mycobacterium avium* was 0.5 μg/mL and 1 μg/mL, and the drug resistance rate was 3.8%. Duan et al. have detected the isolated strains from Japanese patients of MAC disease, the result was that the MIC50 and MIC90 of moxifloxacin against *Mycobacterium intracellulare* was 2 μg/mL and 8 μg/mL respectively; the MIC50 and MIC90 of moxifloxacin against *Mycobacterium avium* was 2 μg/mL and 8 μg/mL respectively [11]. Li et al. have collected strains from 4 specialized tuberculosis hospitals in China, the drug sensitivity result showed that, the MIC50 and MIC90 of moxifloxacin against *Mycobacterium intracellulare* was 0.5 μg/mL and 1 μg/mL, and the drug resistance rate was 1.6%; the MIC50 and MIC90 of moxifloxacin against *Mycobacterium avium* was 0.5 μg/mL and 4 μg/mL, and the drug resistance rate was 10.8% [12]. According to Li et al. the drug resistance rate of moxifloxacin against *Mycobacterium avium* was significantly higher than that against *Mycobacterium intracellulare*, while our result showed that the sensitivity of moxifloxacin to *Mycobacterium avium* was higher than that to *Mycobacterium intracellulare*. In China, the reports on research applying micro-broth dilution method to detect the MIC of moxifloxacin against *Mycobacterium abscesses* are very rare. A Singapore study showed that the sensitivity of moxifloxacin to 124 strains of *Mycobacterium* abscesses was 3%, and the drug resistance rate was 93% [13]. The data from Taiwan showed that the drug resistance rate of moxifloxacin to 13 strains of *Mycobacterium* abscesses was 100%, and the MIC90 was 16 μg/mL (3 days) and 32 μg/mL (5 days) [14]. While our result indicated that the sensitivity of moxifloxacin to 26 strains of *Mycobacterium abscesses* was 11.5%. Currently, the use of moxifloxacin in the treatment of NTM infection is still lack of sufficient research data; however, in drug resistance test *in vitro*, it revealed a relatively strong antibacterial ability to *Mycobacterium avium* complex and weak antibacterial ability to *Mycobacterium abscesses*, and a better ability in removing MAC in animal models [15], which suggesting that moxifloxacin may have potential clinical value for the effective treatment of MAC infection. Certainly, this conclusion requires more in-depth study for confirmation.

In this study, the MIC50 and MIC90 of linezolid to *Mycobacterium intracellulare* were 8 μg/mL and 64 μg/mL respectively, with the drug resistance rate of 31.2%; while the MIC50 and MIC90 of linezolid against *Mycobacterium avium* were 8 μg/mL and 32 μg/mL respectively, with the drug resistance rate of 15.4%. Duan et al. showed that the MIC of linezolid to 76 strains of *Mycobacterium intracellulare* was 64 μg/ml, and the drug resistance rate was 44.7% [16]. Li et al. presented that the MIC50 and MIC90 of linezolid to *Mycobacterium intracellulare* were 8 μg/mL and 16 μg/mL respectively, with the drug resistance rate of 8.5%; while the MIC50 and MIC90 of linezolid to *Mycobacterium avium* were 16 μg/mL and 32 μg/mL respectively, with the drug resistance rate of 40% [12]. Huang et al. mentioned in their research that the MIC value of linezolid to 15 strains of *Mycobacterium* intracellulare was all less than 8 μg/mL [17]. The antibacterial activity of linezolid on NTM strains differed with different strains. In this study, the MIC50 and MIC90 of linezolid to *Mycobacterium abscesses* were 4 μg/mL and 16 μg/mL respectively, and the sensitivity rate was 88.5%, without non-sensitive strains. Similar to the result of this study, Chen et al. mentioned in their research report that the sensitivity of linezolid to *Mycobacterium abscesses* was 78.5% and the drug resistance rate was 3.5% [18]. The difference appeared in Singapore's research that the sensitivity of linezolid to 306 strains of *Mycobacterium abscess* was 42% and the drug resistance rate was 16% [13]. The reason of the difference in research results may be due to the differences of the strains' coming time, region and the sample size.

The result of this study suggests that, moxifloxacin and linezolid have relatively strong antibacterial ability on the clinical common *Mycobacterium intracellulare* and *Mycobacterium avium*, and have certain clinical value in the treatment of MAC infection. For *Mycobacterium abscesses*, linezolid has a relatively strong antibacterial ability, which could be used as a selective drug, while the use of moxifloxacin should be further studied. In summary, different species of NTM differed in their drug susceptibility profiles, therefore, it is necessary to identify and perform drug sensitivity test in the diagnosis and treatment of NTM diseases.

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