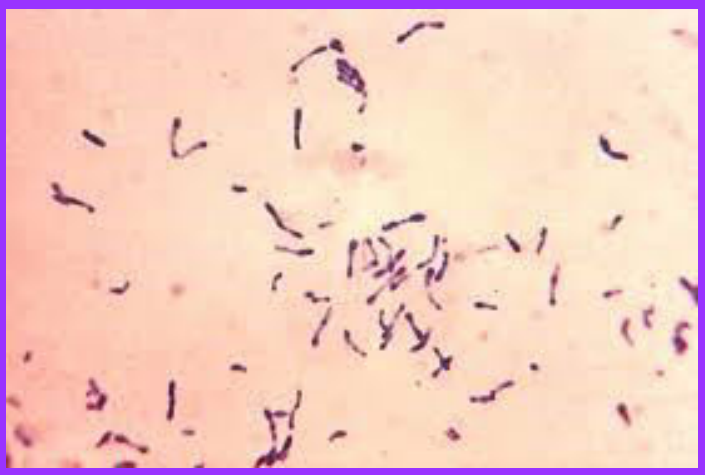

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# Implication of *ermCX* gene of *Corynebacterium striatum* in macrolide resistance in Beijing, China

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## Abstract

To study the resistance mechanism of *Corynebacterium striatum* clinical isolates to macrolide antibiotic, 57 strains of *C. striatum* clinical isolates were detected by susceptibility testing, PCR amplification and DNA sequencing. The MICs of erythromycin and azithromycin were measured. The *Staphylococcus aureus* (ATCC 29213) strain was used as control strain for susceptibility testing. A novel pair of specific primers for *C. striatum* was firstly designed. Out of the 57 strains of *C. striatum* isolates there were 9 strains of *ermCX-carrying* isolates screened. The amplified *ermCX* gene of *C. striatum* was characterized. High MICs (≥256 mg/L) of erythromycin and azithromycin was showed in 9 strains which carried *ermCX* gene. Thus, *ermCX* gene plays an important role in resistant mechanism of *C. striatum* in China to macrolide antibiotic. The *ermCX* gene DNA sequences of two strains (one *C. striatum* from blood and one *C. striatum* from pleural fluid) were submitted to GenBank database.

## Introduction

*Corynebacterium striatum* is frequently found among clnical isolates of corynebacteria in China and usually regarded as a kind of contaminant in clinical laboratory. However, infections caused by *C. striatum* emerged increasingly such as pneumonia (Tarr et al., 2003) vertebral osteomyelitis (Fernández-Ayala et al., 2001) septicemia (Martín et al., 2003) and endocarditis (de Arriba et al., 2002). It has increasingly been recognized as opportunistic pathogen even pathogen (Campanile et al., 2009). The rising importance in the clinical infection of *C. striatum* makes it necessary to know its susceptibility to the clinical antibiotic. Because of the abusage of antibiotic, an alarming rate of antibiotic resistance among *C. striatum* was developing. Soriano et al. (1998) reported the $MIC_{50}$ (MIC at which 50% of the isolates tested are inhibited) and the $MIC_{90}$ (MIC at which 90% of the isolates tested are inhibited) of erythromycin and azithromycin (>128 mg/L) for 25 strains of *C. striatum* clinical isolates. A multidrug-resistant strain of *C. striatum* only susceptible to rifampin and vancomycin was reported in 2003 (Tarr et al., 2003). In 2006 Otsuka Y described the emergence of multidrug-resistant *Corynebacterium* striatum as a nosocomial pathogen in long-term hospitalized patients with underlying diseases (Otsuka et al., 2006). Bacteriemia caused by a multidrug-resistant strain is fatal to immunocompromised crowd. All these reminds us to concern the resistance for *C. striatum* to antibiotic. Macrolide antibiotic such as erythromycin and azithromycin was widely applied in clinical treatment. It is very significant for controlling resistance to study macrolide antibiotic resistance mechanism. Therefore, the main aim of this study was to learn the mechanisms of resistance to macrolide antibiotic in *C. Striatum.*

## Materials and Methods

### *Bacterial strains and identification*

57 strains of *C. striatum* were isolated in the Department of Clinical Laboratory Medicine, People's Hospital of Peking University and China-Japan Friendship Hospital

between June 2000 and June 2006. All of the strains were identified by the API Coryne system (bioMe´rieux, Marcy l'E´toile, France). In our previous study (Li and Zhang, 2007), the standard according to which the partial strains were chosen and the analysis of susceptibility testing of these strains were mentioned.

### *Antimicrobial agents*

Erythromycin and azithromycin were from National Institute For The Control of Pharmaceutical and Biological (Beijing, China).

### *MIC determinations*

The MICs of erythromycin and azithromycin were determined against 57 strains of *C. striatum* by a standard agar dilution method (Clinical and Laboratory Standards Institute, 2010). Briefly, inocula of approximately $10^4$ CFU per spot were applied to the surface of Mueller-Hinton agar (Oxoid, Basingstoke, UK) supplemented with 5% defibrinated sheep blood containing doubling dilutions of antibiotics from 0.00098 to 512 mg/L. Plates were incubated aerobically at 35°C for 24 or 48 hours, as required, to determine the MIC endpoint. There are no specific guidelines by Clinical and Laboratory Standards Institute for the susceptibility testing of corynebacteria (Clinical and Laboratory Standards Institute, 2010). Any criteria defined for *C. striatum* is unsuitable. All strains of *C. striatum* were chosen to seek for *ermCX* gene. *Staphylococcus aureus* (ATCC 29213) was used as control.

### *Amplification and sequence of ermCX gene*

To amplify the *ermCX* gene of *C. striatum*, a couple of primers for *ermCX* were firstly used. Based on the sequence of gene *ermCX* on *C. striatum* R-plasmid pTP10 a novel pair of specific primers for *C. striatum* was designed by Primer 5 software: E1 (CGGGTTTGGTGTAGATGGTGAG) and E2 (CGTTTCGGCAGATACGCTTTC). The PCRs were performed as follows. One colony was resuspended in nucleic acid extracted tube (Lot: 200612004. Beijing CapitalBio Co., Ltd), 50 µL of lysate buffer was added and shaken for 5 min, followed by incubation for 5 min at 95°C. Afterward, as genomic DNA (about 5-50 ng), 3 µL of the supernatant of the tube were added to 20 µL final volume PCR reactions containing $MgCl_2$ 2.0 mM, 200 µM each of dATPs, dCTPs, dGTPs and dTTPs, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1 U Taq polymerase. 10 pmol of each primer were added too. Oligonucleotides were synthesized by Beijing SBS Genetech Co., Ltd (Beijing, China). The PCR is conducted under the condition of a pre-denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 62°C for 1 min and 72°C for 1 min, and a final extension step was performed at 72°C for 5 min. Amplified products were electrophoresised in 1.2% agarose and stained with 0.5 µg/mL ethidium bromide and photographed with GENIUS Image System. PCR product of corresponding size of DNA was purified with gel purification and sequenced by BGI LifeTech Co., Ltd. (Beijing, China). Afterward, the corresponding size of DNA sequence was blasted on http://www.ncbi.nlm.nih.gov/.

**Table I**

**MICs of erythromycin and azithromycin for 57 strains of *C. striatum* (unit: mg/L)**

| Antibacterial agent | MICs of 57 strains of *C. striatum* | | | MICs of control |
|---|---|---|---|---|
| | Range | $MIC_{50}$ | $MIC_{90}$ | ATCC 29213 |
| Erythromycin | 32 - 512 | 512 | 512 | 0.5 |
| Azithromycin | 16 - 512 | 256 | 512 | 2.0 |

## Results

The results of the tests in which the MICs of erythromycin and azithromycin for the clinical isolates of *C.striatum* were determined are presented in Table I. The MICs for control strain of *Staphylococcus aureus* (ATCC 29213) was within normal range (Table I). There were high-level resistance to erythromycin and azithromycin. $MIC_{50}$ and $MIC_{90}$ of erythromycin were both 512 mg/L. Azithromycin was little better than erythromycin (MIC at which 50% of the isolates tested are inhibited of 256 mg/L). $MIC_{90}$ of azithromycin remains 512 mg/L. MICs of erythromycin ranging between 32 and 512 mg/L. The range of MICs of azithromycin was broader (16 to 512 mg/L).

Among the 57 strains of *C. striatum* isolates, 9 strains with *ermCX* gene were screened out，the detection rate was 15.8% (9/57). Amplified products were sequenced and blasted with GenBank database. It showed 100% identity and 100% similarity to the *C. striatum* R-plasmid pTP10 at position 48741 to 49501, just was *ermCX* gene. The *ermCX* gene DNA sequences of two strains (one *C.striatum* isolated from blood and one *C.striatum* from pleural fluid) were submitted to GenBank database under Accession No. EF608448-608449 (Table II). High MICs (≥256 mg/L) of erythromycin and azithromycin was showed in 9 strains which carried *ermCX* gene (Table II).

## Discussion

Erythromycin and azithromycin are clinically important broad-spectrum antibiotic that belongs to the macrolide class. However the application of macrolide antibiotic was influenced due to the resistance. It is very significant for controlling resistance to study macrolide antibiotic resistance mechanism. The macrolide

**Table II**

**Analysis of 9 strains of ermCX-carrying C. striatum (unit: mg/L)**

| No. | Strains | Specimens | MICs (mg/L) | | Genbank accession number |
|---|---|---|---|---|---|
| | | | Erythromycin | Azithromycin | |
| 1 | *C. striatum* | Bronchofibroscope secretion | 512 | 512 | |
| 2 | *C. striatum* | Bronchofibroscope secretion | 512 | 512 | |
| 3 | *C. striatum* | Bronchofibroscope secretion | 512 | 512 | |
| 4 | *C. striatum* | Bronchofibroscope secretion | 256 | 256 | |
| 5 | *C. striatum* | Blood | 512 | 512 | EF608448 |
| 6 | *C. striatum* | Bronchofibroscope secretion | 512 | 512 | |
| 7 | *C. striatum* | Pleural fluid | 512 | 512 | EF608449 |
| 8 | *C. striatum* | Bronchofibroscope secretion | 512 | 512 | |
| 9 | *C. striatum* | Bronchofibroscope secretion | 256 | 256 | |

resistance determinants and genetic elements mainly carried the mef gene and erm gene. An important resistance mechanism to macrolide antibiotic was to modify or reconstitute the site in 23 S rRNA on the large ribosomal subunit (50S) close to the peptidyl transferase center. This kind of resistance was conferred by four kinds of mechanism: The mutation in the base of the large ribosomal subunit on 23 S rRNA; the mutation in protein; the presence of resistant short peptide and the presence of erm methyltransferases. The target for erm methyltransferases is at nucleotide A2058 in 23S rRNA, and erm methylation occurs before the rRNA has been assembled into 50S ribosomal particles. Erm methyltransferases are a clinically prevalent group of enzymes that confer resistance to the therapeutically important macrolide, lincosamide, and streptogramin B ($MLS_B$) antibiotics, it was encoded by *erm* dimethyltransferase genes which was founded in many pathogenic bacteria. Erm methyltransferases occur in a phylogenetically wide range of bacteria and differ in whether they add one or two methyl groups to the A2058 target (Douthwaite et al., 2008). Clinical isolates of *Streptococcus pneumoniae* with constitutive *erm*(B) and *Streptococcus pyogenes* with constitutive *erm*(A) subtype

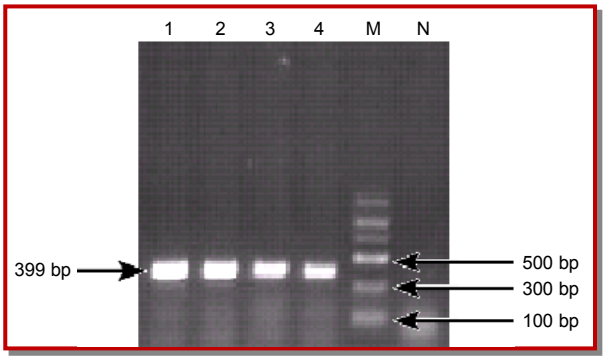


Figure 1: Agarose gel electrophoresis of the products of PCR. Lanes 1-4, samples; M, molecular weight markers (Beijing SBS Genetech Co., Ltd, China); N, represents the negative control without DNA template

(TR) are resistant to macrolides (Douthwaite et al., 2005). Staphylococcus aureus encoded methyltransferases by *ermA*、*ermB*、*ermC* and *ermF*. Tauch et al. reported that the erythromycin resistance gene, *ermCX,* is located on a 4524-bp composite transposable element, Tn5432 in the 50-kb R-plasmid pTP10 from the clinical isolate *Corynebacterium xerosis* M82B (Tauch et al., 1995). Nothing else has been published on *ermCX* about *C. striatum,* no report about the resistance caused by *ermCX gene* in clinical isolates. Based on the sequence of gene *ermCX* on *C. striatum* R-plasmid pTP10 at position 48741 to 49501, a novel pair of specific primers for *C. striatum* was firstly designed by Primer 5 software in the present study. Amplified products were sequenced and blasted with GenBank database. It showed 100% identical and 100% similarity to those of *C. striatum* R-plasmid pTP10 at position 48741 to 49501. The *ermCX* gene DNA sequences of two strains of *C. striatum* (one from blood and one from pleural fluid) were submitted to GenBank database under Accession No. EF608448-608449 (Table II).

There were high-level resistance to erythromycin and azithromycin among the 57 strains of *C. striatum*. $MIC_{50}$ and $MIC_{90}$ of erythromycin were both 512 mg/L. azithromycin was little better than erythromycin (MIC at which 50% of the isolates tested are inhibited of 256 mg/L). $MIC_{90}$ of azithromycin remains 512 mg/L. It showed the serious resistance of macrolide antibiotic in *C. striatum* in Beijing, China. 9 strains of *ermCX*-carrying *C. striatum* showed high MICs, with MICs of erythromycin and azithromycin 512 mg/L except two 256 mg/L. It implicated that the *ermCX* gene was associated with the high-level resistance of macrolide antibiotic and played an important role in resistance of *C. striatum* to macrolide antibiotic. It could be *ermCX* gene that encodes the methyltransferases of *C. striatum*, thus the methyltransferases lead to the resistance to macrolide antibiotic. 9 strains with *ermCX* gene were screened out from 57 strains of *C. striatum*. The detec-

tion rate was 15.8%. Because that there are no specific guidelines by CLSI Standards for the susceptibility testing of corynebacteria, the proportion of *ermCX*-carring strains in those strains which is resistant to macrolide antibiotic couldn't be analyzed. It was inferred that there could be other resistant gene or mechanism in those strains without *ermCX* gene that showed high level resistance to macrolide antibiotic.

Erm genes are not only common but also circulate among species of this bacterial genus (Milagro et al., 2001). In conclusion, as a family of erm genes which lead to the resistance to macrolide antibiotic, an important resistance gene of *C. striatum*, e*rmCX* should be concerned. It is also necessary for hospital to take measures to control the resistance.

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