Phenotypic and molecular characterization of resistance to macrolides and Lincosamides in Corynebacterium striatum clinical strains isolated from Tunisia

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Abstract

Objectives: In this study we investigated the susceptibility profiles against macrolides and lincosamides of 85 C. striatum strains isolated at a clinical centre in Sousse (Tunisia).

Methods: The strains were identified by the routine biochemical assays and then confirmed by Vitek-Maldi-Tof-MS. MIC’s of erythromycin and clindamycin were determined using the microdilution method. The detection of erm(X), erm(B), msr(A), mph(A) and mef(A-E) resistance genes was performed by PCR. The strains were typed by PFGE using XbaI.

Results: Sixty-nine (81.17%) strains were resistant to erythromycin, 58 (67.44%) strains were resistant to clindamycin. There was a high correlation between the resistance to erythromycin and clindamycin and the presence of erm(X) gene in 85.50% and 89.65% respectively. The erm(B) gene was detected in 21(24.70%) strains whereas, no others genes were detected in our strains collection. By PFGE, the 85 strains belonged to 18 different clones.

Conclusion: erm(X) is implicated in macrolide resistance for almost all the Corynebacterium strains analyzed in our study. Other resistance genes like erm(B) must also be implicated in this resistance, although its presence seems to be unusual in previously reported studies.

Key words: Corynebacterium striatum, macrolide, Lincosamide, resistance, erm genes, PFGE

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Introduction

Corynebacterium striatum is an ubiquitous microorganism of the human skin and mucous membranes. However, it is a significant emerging opportunistic pathogen causing serious infections in immunocompromised and hospitalized patients and isolated as the etiological factor of various infections such as pneumonia vertebral osteomyelitis septicemia and endocarditis [1-4]. Different studies showed high resistance rates against several antimicrobials and many outbreaks of multidrug resistant C. striatum have been recently reported [5,6]. Macrolides are frequently used in infections that can be caused by these microorganisms and are an alternative to b-lactams and tetracycline. They act by binding to the 50S subunits of bacterial ribosomes and interfere with protein synthesis by inhibiting the elongation of peptide chains [7]. Macrolide resistance in C. striatum is of particular interest because it is clearly an acquired phenotype and is common in clinical isolates. In C. striatum, resistance to macrolides was previously shown to be mainly due to methylase enzymes that are codified by erm genes.

Other resistance mechanisms such as mutations in 23S rRNA domain V, modifications of proteins L4 and L22, efflux pump systems, and hydrolytic enzymes have been reported in different genera [8,9]. Several studies reported high levels of resistance to macrolides among Corynebacterium species [10,11,12]. However, in Tunisia, no data are available. Therefore, we investigated the susceptibility profiles against macrolides and lincosamides of 85 C. striatum strains isolated at a clinical centre in Sousse (Tunisia).

Materials and methods

Bacterial strains

We tested 85 C. striatum strains isolated from different specimens of patients presented at the laboratory of microbiology at the tertiary Hospital F. Hached Sousse, Tunisia, between January 2007 and December 2012. In this study, one isolate per patient was used. Strains were identified using a phenotypic method with the API Coryne system (bioMerieux) according to the manufacturer's instructions and by comparison with the API WEB database. Strains were also identified using a MALDI-TOF/ VITEK MS mass spectrometry system (bioMerieux). Obtained spectra were analyzed with the VITEK MS database.

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Antimicrobial susceptibility testing

In the screening tests for all investigated strains, inducible type of MLSB resistance was tested. Antibiotic discs (Biorad) of erythromycin (E - 15 mg) and clindamycin (Clin-2 mg) were placed 15–20mm from each other on the on cation-adjusted Mueller-Hinton agar with 5% sheep blood and inoculums of 1.0 McFarland. The plates were incubated for 48 hours at 35°C. The appearance of a flattening zone around the clindamycin confirmed the presence of inducible resistance. In the case that approved breakpoints for Corynebacterium spp were absent, criteria for MLSB antibiotics for Staphylococcus spp were used. The susceptibility to erythromycin and clindamycin was also investigated using microlodulation method in cation adjusted Muller-Hinton broth in accordance with guidelines of the Clinical and Laboratory Standards Institute (CLSI). The minimum inhibitory concentration (MIC) was estimated according to the CLSI guidelines for Corynebacterium spp. In the absence of approved breakpoints for Corynebacterium spp, breakpoints reported for Staphylococcus spp were used. C. striatum (ATCC 6940), C. jeikeium (ATCC 43734), E. coli (ATCC 25922) and S. pneumoniae (ATCC 49619) and S. aureus (ATCC 29213) strains were used as controls. The strains were grown on blood agar plates during 24–48 hours at 37°C in normal atmosphere and checked for purity.

Nucleic acid extraction and PCR

Few colonies were suspended in 200 ml of Instagene matrix (Biorad). The bacterial suspension was incubated during 15 minutes at 56°C followed by 8 minutes at 100°C and subsequently centrifuged at 12,000 rpm for 3 minutes. Supernatant was used for PCR studies. PCR was performed using commercial KAPA2G Fast HotStart Ready Mix (2X). The primers used for detection of the erm(X) gene were Cerm 1 (5'-GAC ACG GCC GTC ACG AGC AT-3') and Cerm 2 (5'-GGG GGC GAG CTA CC-3'); for the ermA gene: Erm A-1 (5'-TCT AAA AAG CAT GTA AAA GAA-3') and Erm A-2 (5'-CTT CTA GGA TAG TTT ATT AAT AGT-3'); for the ermB gene: Erm B-1 (5'-GAA AAG GTA CTG CTA AAA AT-3') and Erm B-2 (5'-AGT AAC GGT ACT TAA ATT GTT TAC-3'); and for the mep(A-E) gene: Mep A-E-1 (5'-AGT ATC ATT CAC TAG TGC-3') and Mep A-2 (5'-TTC TTC TGG TAG TAC AAG TGG-3') and for the mph(A) gene: MphA-1 (5'-AAC TGT ACG CAC TCG-3') and MphA-2 (5'-GCT GGT CTT CGT TAC C-3'); and for the msrA gene: MsrA-1 (5'-GCA CTA ATT GGG GTT AAT GGG) and MsrA-2 (5'-GTC TAT AAG TGC TCT ACT GTG-3'). The amplicons were detected by electrophoresis in 2% agarose. The bacterial DNA of all 85 strains was amplified with primers for detection of the erm(X) gene was detected in 26.68% and 20.68% of the strains, and in 12 strains both erm(B) and erm(A) genes were present. The amplification product of the erm(X) gene was present. The amplification product of the erm(X) gene was detected in 21 C. striatum strains, and in 12 strains both erm(X) and erm(B) amplicons were detected. There was a high correlation between the resistance to erythromycin and clindamycin and the presence of erm(X) gene in 85.50% and 89.65% respectively (Figure 1). Whereas, the erm(B) gene was detected in 26.68% and 20.68% of the strains resistant to erythromycin and clindamycin respectively (Figure 1). No amplification products of other tested genes were detected in any of the studied strains (Table 3). Although they showed resistance to macrolide and clindamycin, no amplification products were detected in 3 strains.

PFGE analysis indicated that the 85 C. striatum isolates represented 18 distinct strain types (data not shown), with the forty ERY-susceptible strains being distinct from the ERY resistant strain types. Ten strains resistant to erythromycin, isolated from the same ward in the same period, belonged to the same clone.

Discussion

The genus Corynebacterium includes a high number of species that are usually isolated from human skin as saprophytes. However, these microorganisms have also been reported as infectious agents in a broad group of patients and have showed broad-spectrum resistance.

Results

A total of 85 C. striatum strains were isolated from different specimens (Table 1). The API Coryne often provided ambiguous results and could not differentiate between C. striatum and other related species such as C. amycolatum, C. aurimucosum, C. minutissimum and C. simulans. However, in the MALDI-TOF-MS method the strains were identified with cut off ≥299.99. Screening test for sensitivity to erythromycin and clindamycin was performed using the disc diffusion method. MIC determinations for erythromycin and clindamycin with microdilution method confirmed the results of the disc diffusion method.

Resistance to examined antibiotics was observed in approximately 80% of the strains, ten of which appeared to be resistant only to one antibiotic: seven were resistant to clindamycin, while three were resistant to erythromycin. Fifty-three strains showed resistance to both antibiotics (Table 2). The shape of the growth inhibition zones indicated the presence of a constitutive MLSB resistance type.

The bacterial DNA of all 85 strains was amplified with primers for resistance genes to macrolide and lincosamide antibiotics. In 62 out of 85, the erm(X) gene was present. The amplification product of erm(B) gene was detected in 21 C. striatum strains, and in 12 strains both erm(X) and erm(B) amplicons were detected. There was a high correlation between the resistance to erythromycin and clindamycin and the presence of erm(X) gene in 85.50% and 89.65% respectively (Figure 1). Whereas, the erm(B) gene was detected in 26.68% and 20.68% of the strains resistant to erythromycin and clindamycin respectively (Figure 1). No amplification products of other tested genes were detected in any of the studied strains (Table 3). Although they showed resistance to macrolide and clindamycin, no amplification products were detected in 3 strains.

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### Table 1. Isolated strains of C. striatum (n = 85) from various infection sites

| Source              | Number of species | Percentage % |
|---------------------|-------------------|--------------|
| Wound swab          | 20                | 23.53%       |
| blood               | 3                 | 3.53%        |
| Eye conjunctival swab| 7                 | 8.23%        |
| Ear swab            | 15                | 17.68%       |
| Sputum              | 5                 | 5.89%        |
| Urine               | 9                 | 10.58%       |
| Vaginal swab        | 8                 | 9.41%        |
| Tracheostomy secretion| 6               | 7.06%        |
| Catheter injection site| 4               | 4.70%        |
| Spem                | 3                 | 3.53%        |
| Medical devices     | 5                 | 5.89%        |

### Table 2. Susceptibility of 85 C. striatum strains studied to MLS antibiotics

| Antibiotics     | Susceptibility, number of strains (%) |
|-----------------|--------------------------------------|
| Erythromycin    | 0.06-64 | 14 | 2 | R       |
| Clindamycin     | 0.06-64 | 7  | 20| 58      |

S: Susceptible, R: Resistant, I: Intermediate
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### Table 3. Antibiotic susceptibility patterns of C. striatum resistant isolates and their genotypes

| E and Cln susceptibility phenotype (MIC) | No. of strains (%) | Genes | mefeA-E, mphA, msrA |
|-----------------------------------------|--------------------|-------|---------------------|
| E\(^a\) Cln\(^a\)                      | 53 (62.35%)        | 50    | 12                  |
| E\(^a\) Cln\(^b\)                      | 7 (8.23%)          | 6     | 1                   |
| E\(^a\) Cln\(^a\)                      | 3 (3.52%)          | 1     | 0                   |
| E\(^a\) Cln\(^b\)                      | 2 (2.35%)          | 1     | 0                   |
| E\(^a\) Cln\(^a\)                      | 9 (10.58%)         | 3     | 5                   |
| E\(^a\) Cln\(^b\)                      | 1 (12.94%)         | 1     | 3                   |
| Total                                   | 85 (100%)          | 62    | 21                  |

\(^a\) Cln\(^a\), erythromycin and clindamycin-resistant strains.
\(^b\) Cln\(^b\), erythromycin-resistant and clindamycin-susceptible strains.
\(^c\) Cln\(^c\), erythromycin-susceptible and clindamycin-resistant strains.
\(^d\) Cln\(^d\), erythromycin-resistant and clindamycin-intermediate susceptibility strains.
\(^e\) Cln\(^e\), erythromycin-intermediate susceptibility and clindamycin-resistant strains.
\(^f\) Cln\(^f\), erythromycin-susceptible and clindamycin-intermediate susceptibility strains.

Besides, there were 12 strains that carried the erm(X) and erm(B) genes simultaneously, a phenomenon previously detected in C. urealyticum and in Staphylococcus where the presence of several erm genes is very common [23].

In our study, no amplification products of the mefe(A-E) gene were detected, although this gene has been previously reported in the genus Corynebacterium [22]. Other efflux pump genes such as msr have also been reported in this genus [24], but they were not detected in our report. PFGE analysis showed that the strains tested were genetically unrelated. The 10 resistant strains belonging to the same clone and isolated from the same ward in the same period suggested a clonal outbreak nosocomial infection.

### Conclusion

According to our data erm(X) is implicated in macrolide resistance for almost all the C. striatum strains analyzed in our study. Other resistance genes like erm(B) must also be implicated in this resistance, although its presence seems to be unusual in previously reported studies.

### Competing interests

None declared.

### Ethical approval

Not required.

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