Clonal Multidrug-Resistant Corynebacterium striatum Strains, Italy

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We assessed the clinical relevance and performed molecular characterization of 36 multidrug-resistant strains of Corynebacterium striatum. Pulsed-field gel electrophoresis confirmed a single clone, possessing erm(X), tetA/B, cmxA/B, and aphA1 genes, but few related subclones. This strain is emerging as a pathogen in Italy.

Isolation of Corynebacterium spp. as the only organism from clinical specimens from patients, mostly with varying degrees of immunocompromise and severe infections, is increasing in Italy. Therefore, we evaluated the microbiologic characteristics, resistance profiles, and similarities among genomes of multidrug-resistant (MDR) C. striatum strains.

The Study

We evaluated 36 strains of MDR C. striatum, isolated from 3 hospitals in Italy during 2005–2007. Fourteen strains were from bronchoalveolar lavage (BAL) fluid, 3 from blood, 7 from central venous catheter tips, 5 from tracheal aspirates, 4 from wound specimens, 1 from BAL and pleural fluid, 1 from urine, and 1 from a lung biopsy specimen. To assess the clinical relevance of these strains, we used the Centers for Disease Control and Prevention 2004 definition for nosocomial infections (www.cdc.gov/ncidod/dhqp/pnss_pubs.html) (1) and tracked antimicrobial drug–resistance determinants.

We identified all strains as putative C. striatum by using the commercial system API 20 Coryne (bioMérieux, Marcy l’Etoile, France). C. striatum was differentiated from C. amycolatum by supplementary tests, i.e., tyrosine hydrolysis, N-acetylglucosamine assimilation, and phe-nylacetic acid assimilation (2); it was reconfirmed by sequencing the internal fragment of the 16S rRNA gene (3). The American Type Culture Collection (ATCC) 6940 C. striatum strain was included as phenotypic and molecular control. All strains were stored at –80°C until use.

MICs were determined by using microdilution in cation-adjusted Mueller-Hinton broth in accordance with guidelines of the Clinical and Laboratory Standards Institute (CLSI) (4). The following antimicrobial drugs were tested: tigecycline and piperacillin/tazobactam, oxacillin, gentamicin, kanamycin, levofloxacin, erythromycin, clindamycin, piperacillin, vancomycin, teicoplanin, tetracycline, moxifloxacin, imipenem, meropenem, quinupristin/dalfopristin, linezolid, and daptomycin. Etest strips (AB-BIODISK, Solna, Sweden) were used for vancomycin, teicoplanin, linezolid, and daptomycin. Daptomycin Etest was performed by using Muller-Hinton agar (Oxoid, Milan, Italy), supplemented to a final concentration of 50 mg/L calcium.

In the absence of approved breakpoints for Corynebacterium spp., we used those for a-hemolytic streptococci of the viridans group. Results were read after incubation at 37°C for 18–24 h. Susceptibility to daptomycin was defined as MIC ≤1 mg/L (5); CLSI guideline MIC breakpoints were used for all other drugs tested (4).

To further characterize the C. striatum isolates, we used 2 DNA fingerprinting techniques: automated ribotyping (Riboprinter Microbial Characterization System; DuPont Qualicon, Wilmington, DE, USA) with EcoRI as restriction enzyme and pulsed-field gel electrophoresis (PFGE) macrorestriction analysis with 2 enzymes (XbaI and Sfall; New England Biolabs, Beverly, MA, USA). We used 4 enzymes (XbaI, Sfall, SfiI, and Pael) to test 10 random strains, but because XbaI and Sfall enzyme-restriction patterns gave a better resolution for low and high molecular weight fragments, respectively, we used only these 2 restriction enzymes to type all 36 strains.

Whole genomic DNA chromosomal extraction, macrorestriction digestion, and PFGE (CHEF-DR II apparatus; Bio-Rad, Hercules, CA, USA) were performed as previously reported (6). Macrorestriction fragments were separated on 1% (wt/vol) ultrapure agarose gels (Sigma Aldrich, St. Louis, MO, USA) at 6 V/cm, for 21 h at 14°C with pulse times of 0.1–5 s, to separate XbaI fragments, and for 23 h with pulse times of 1–70 s, to separate Sfall fragments. Lambda DNA concatemers (New England BioLabs) were used as molecular size markers. Similarities among macrorestriction patterns were identified according to established criteria (7).

The sequence of pTP10 (GenBank accession no. AF024666) (8) was used to design the primers for erm(X), tetA and tetB, cmx, aphA1, and repB genes. The VectorNTI program (Invitrogen, www.invitrogen.com) was used...
for this purpose. The presence of pTP10 was confirmed first by amplification and sequencing of the resistance determinants and the replication gene (repB) and then by XbaI and SwaI PFGE hybridizations, performed with the specific probes (erm(X), tetAB, cmx, and aphA1), following a protocol previously described (9). The PCR amplifications were performed in a Techne TC412 thermal cycler (Barloworld Scientific, Staffordshire, UK). All primers and the related probe regions used in hybridization experiments are shown in Table 1.

All C. striatum isolates were recovered from hospitalized patients who had undergone surgery or been admitted to intensive care units (Table 2). We documented 19 cases of infections and discarded 17 as contaminants. The isolates that were considered causes of infections were responsible for 8 cases of ventilator-associated pneumonia (including 1 with associated pleural empyema), 2 cases of pneumonia, 1 case of catheter-related sepsis, 2 cases of ventilator-associated tracheobronchitis, and 6 cases of wound infections.

The 36 strains showed an MDR phenotype, including resistance to ≥3 classes of drugs; MICs required to inhibit growth of 90% (MIC90) were penicillins ≥256 mg/L, carbapenems ≥256 mg/L, gentamicin 32 mg/L, levofloxacin 256 mg/L, tetracycline ≥256 mg/L, lincosamides ≥256 mg/L, and erythromycin 32 mg/L. C. striatum strains were susceptible to only the most recent drugs used for treatment of infections with gram-positive organisms, such as glycopeptides and tigecycline (MIC90 0.05 mg/L), quinupristin/dalfopristin (MIC90 0.25 mg/L), and linezolid (MIC90 2 mg/L). A discrepancy was found when susceptibility testing using a disk-diffusion method was performed on different strains; the inhibition zone of erythromycin was always in the intermediate range, even if MICs for this drug were in the low-resistance range.

Ribotyping gave a unique profile for all strains in this study. PFGE enabled us to discriminate the right number of macrorestriction fragments (5,10,11) for pattern comparison.

Analyses of SwaI digestion patterns showed that of the 36 strains, only 1 clone had 3 different subtypes (30 strains subtype a1, 4 strains a2, and 2 strains a3). Macrogenetic analysis with XbaI showed almost comparable results (27 strains A1, 7 strains A2, and 2 strains A3) (Figure). This genotyping method and the enzymes used were defined as appropriate, comparing PFGE patterns of our clinical isolates with C. striatum ATCC 6940 type strain, which was different with respect to the epidemic strains. This result demonstrates that single MDR C. striatum clones had been selected and were circulating in the 3 hospitals.

Further, the molecular characterization of some of the resistance genes in the 36 C. striatum isolates demonstrated the presence of erm(X), codifying for the resistance to erythromycin and clindamycin; tetA, and tetB, codifying for the resistance to tetracycline, oxytetracycline, and oxacillin; and cmx and aphA1, responsible for resistance to aminoglycosides and chloramphenicol, respectively. The presence of pTP10 carrying all these determinants was confirmed by amplification and sequencing of these genes and the replication gene of the plasmid, together with hybridization experiments demonstrating that all resistance determinants were localized in the same hybridization band generated by each probe onto PFGE280 kb membranes (Figure).

**Conclusions**

We report isolation of MDR C. striatum from clinical specimens responsible for cases of pneumonia, catheter-related bacteremia, and wound infections. Infections sustained from this species are strongly associated with devices, not only tubes or catheters (91%) but also sternal surgical wound wires.

The MDR phenotype of these strains was immediately observed and was responsible for the alarm that led to the subsequent in-depth examination of these strains. Their clonal nature, as demonstrated in our study, is of particular concern. Further, the MDR phenotype correlated to the
presence of the pTP10 plasmid, which demonstrates that these MDR microorganisms acquired not only the capability to cause infections but also increased resistance and the ability to spread by virtue of their clonal nature. The only drugs still active against these MDR strains are glycopeptides, linezolid, quinupristin/dalfopristin, daptomycin, and tigecycline. To avoid using drugs that appear active in vitro but that could be ineffective in vivo, clinicians should be aware of the circulation of these MDR strains.

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Dr Campanile is a researcher at the Department of Microbiology, University of Catania. She is involved in the fields of antimicrobial drug resistance, molecular typing, evolutionary relationships among strains of diverse sources, and horizontal exchange of antimicrobial drug resistance determinants by mobile genetic elements.

References

<p>| Table 2. Clinical diagnoses for 36 patients with Corynebacterium striatum infection, Italy, 2005–2007* |</p>
<table>
<thead>
<tr>
<th>Specimens</th>
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<th>From non-ICU wards</th>
<th>Diagnosis</th>
</tr>
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<tr>
<td>BAL fluid, pleural fluid, blood, tracheal aspirate</td>
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<td>1</td>
<td>Ventilator-associated pneumonia</td>
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<td>BAL fluid</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>Ventilator-associated tracheobronchitis</td>
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<td>2</td>
<td>Pneumonia</td>
</tr>
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<td>0</td>
<td>CVC-related bacteremia</td>
</tr>
<tr>
<td>CVC tip</td>
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<td>1</td>
<td>0</td>
<td>CVC exit-site cellulites</td>
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<tr>
<td>Blood, surgical wound</td>
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<td>1</td>
<td>4</td>
<td>Sternal wound cellulites and infections</td>
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<td>10</td>
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</table>

*ICU, intensive care unit; BAL, bronchoalveolar lavage; CVC, central venous catheter.

Figure. Pulsed-field gel electrophoresis (PFGE) patterns of Corynebacterium striatum and their representative hybridizations obtained with probes corresponding to the resistance genes erm(X), tetA-tetB, cmx, and aphA1 (m, lambda ladder PFGE marker). A) XbaI (A1and A2 profiles); B) SwaI (a1 and a2 profiles).


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