Clonal Multidrug-Resistant Corynebacterium striatum Strains, Italy

Floriana Campanile, Edoardo Carretto, Daniela Barbarini, Annalisa Grigis, Marco Falcone, Antonio Goglio, Mario Venditti, and Stefania Stefani

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, cmx/A/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 immunocompromisation 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/nis_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 phtionic characteristics, resistance profiles, and similarities among genomes of multidrug-resistant (MDR) C. striatum strains.

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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 SmaI 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 \( \geq 3 \) classes of drugs; MICs required to inhibit growth of 90% (MIC\(_{90}\)) were penicillins \( \geq 256 \) mg/L, carbapenems \( \geq 256 \) mg/L, gentamicin 32 mg/L, levofloxacin 256 mg/L, tetracycline \( \geq 256 \) mg/L, lincosamides \( \geq 256 \) 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 (MIC\(_{90}\) 1 mg/L, quinupristin/dalfopristin (MIC\(_{90}\) 0.25 mg/L), and linezolid (MIC\(_{90}\) 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 SmaI 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). Macrorestriction 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 PFGE\(_{XbaI}\) (\( \approx 15 \) kb) and PFGE\(_{SmaI}\) (\( \approx 280 \) 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

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### Table 1. Primer conditions, PCR products, and related sequences confirmed by BLAST analysis of 36 strains of multidrug-resistant *Corynebacterium striatum*, Italy, 2005–2007*

<table>
<thead>
<tr>
<th>Primer</th>
<th>Related Resistance</th>
<th>Sequence (5' → 3')</th>
<th>Temperature, °C</th>
<th>Size, bp</th>
<th>BLAST from–to, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>ermX up</td>
<td>Erthromycin and clindamycin</td>
<td>AACCATGATTGTTTTCTGAAACG ACCAGGAACGGGTCGCCC</td>
<td>57</td>
<td>566</td>
<td>2,285–2,850</td>
</tr>
<tr>
<td>tetA up</td>
<td>Tetracycline, oxytetracycline, and oxacillin</td>
<td>TTAGCGTTCCGCGCACTTGG AACTGGTGCCCTTGACGGTC</td>
<td>58</td>
<td>1,829</td>
<td>5,496–7,324</td>
</tr>
<tr>
<td>cmx B down</td>
<td>Cloramphenicol (2 identical subunits)</td>
<td>AGTCCGATAGGGTGCTGCG GCTCCGATATTCAATGCTGCG</td>
<td>57</td>
<td>879</td>
<td>16,031–16,909</td>
</tr>
<tr>
<td>cmxA down</td>
<td>Aminoglycoside GCTCCGATATTCAATGCTGCG</td>
<td>58</td>
<td>879</td>
<td>36,078–36,956</td>
<td></td>
</tr>
<tr>
<td>aphA1 up</td>
<td>Replicase</td>
<td>GGCAGAAGTCTGGATACGGCTG AGACTAAGTGGGCAGGGCAT</td>
<td>57</td>
<td>480</td>
<td>41,859–42,338</td>
</tr>
<tr>
<td>aphA1 down</td>
<td></td>
<td>CGTCCTGGAATTTTGTCTGCG</td>
<td>57</td>
<td>480</td>
<td>32,523–33,397</td>
</tr>
<tr>
<td>repB up</td>
<td></td>
<td>CTGCTGTAATAGACCCCGGT</td>
<td>57</td>
<td>875</td>
<td></td>
</tr>
</tbody>
</table>

*BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis of each gene with pTP10 sequence (GenBank accession no. AF024666) showed nucleotide identities >99%.
Clonal Multidrug-Resistant C. *striatum* strains

Table 2. Clinical diagnoses for 36 patients with *Corynebacterium striatum* infection, Italy, 2005–2007*

<table>
<thead>
<tr>
<th>Specimens</th>
<th>No. isolates</th>
<th>From ICU</th>
<th>From non-ICU wards</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL fluid, pleural fluid, blood, tracheal aspirate</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>Ventilator-associated pneumonia</td>
</tr>
<tr>
<td>BAL fluid</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>Ventilator-associated tracheobronchitis</td>
</tr>
<tr>
<td>BAL fluid, lung biopsy</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>Blood, CVC tip</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>CVC-related bacteremia</td>
</tr>
<tr>
<td>CVC tip</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>CVC exit-site cellulites</td>
</tr>
<tr>
<td>Blood, surgical wound</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>Ventilator-associated respiratory tract colonization</td>
</tr>
<tr>
<td>Tracheal aspirate</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CVC tip</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>CVC-exit site colonization</td>
</tr>
<tr>
<td>Urine</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>Urinary tract catheter colonization</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>26</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

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

Acknowledgments

We are indebted to Antony Brigdewood for the language revision of the manuscript.

This work was supported by grants from European Union Drug Resistance Spread 2 project contract no. 018705 to S.S. and from Fondazione IRCCS Policlinico San Matteo, Pavia, to E.C. (Ricerca Finalizzata 2006) and to P.M. (Ricerca Corrente 1998–2006: “Sorveglianza delle infezioni ospedaliere: tipizzazione epidemiologica di microrganismi patogeni mediante metodiche molecolari”).

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


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 PFG marker). A) XbaI (A1and A2 profiles); B) SwaI (a1 and a2 profiles).

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