Epidemiological investigation of a nosocomial outbreak of multidrug-resistant Corynebacterium striatum at one Belgian university hospital

1) Department of Clinical Microbiology, UCL Saint-Luc University Hospital, Brussels, 2) Department of Clinical Microbiology, UCL Mont-Godinne University Hospital, Yvoir, 3) Department of Clinical Microbiology, ULB Erasme University Hospital and 4) Microbiology Unit, Faculty of Medicine, Catholic University of Louvain, Brussels, Belgium

Abstract

During an 8-month period, 24 Corynebacterium striatum isolates recovered from lower respiratory tract specimens of 10 hospitalized patients were characterized. The organisms were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and by 16S rRNA gene sequencing. The cluster of C. striatum exclusively affected patients who had been admitted to an intensive care unit and/or subsequently transferred to one medium-size respiratory care unit. Prolonged duration of hospitalization, advanced stage of chronic obstructive pulmonary disease, recent administration of antibiotics and exposure to an invasive diagnostic procedure were the most commonly found risk factors in these patients. Seven patients were colonized and three infected. All strains displayed a similar broad spectrum resistance to antimicrobial agents, remaining susceptible to vancomycin only. Typing analysis by MALDI-TOF MS and by semi-automated repetitive sequence-based PCR (DiversiLab typing) showed that all outbreak-associated C. striatum isolates clustered together in one single type while they differed markedly from epidemiologically unrelated C. striatum isolates. Pulsed-field gel electrophoresis (PFGE) profiles revealed three distinct PFGE types among the C. striatum isolates associated with the outbreak while all external strains except one belonged to a distinct type. We conclude that C. striatum is an opportunistic nosocomial pathogen in long-term hospitalized patients and can be at the origin of major outbreaks. The routine use of MALDI-TOF MS greatly facilitated the recognition/identification of this organism in clinical samples and this technique could also offer the potential to be used as an easy and rapid epidemiological typing tool for outbreak investigation.

Keywords: Corynebacterium striatum, epidemiology, MALDI-TOF MS, nosocomial outbreak, typing method

Original Submission: 7 December 2012; Revised Submission: 17 February 2013; Accepted: 18 February 2013
Editor: F. Allerberger
Article published online: 26 February 2013
Clin Microbiol Infect 2014; 20: 44–50
10.1111/1469-0691.12197

Corresponding author: A. Verroken, Cliniques Universitaires St Luc, Laboratoire de microbiologie – Tour Franklin, Avenue Hippocrate 10, 1200 Bruxelles, Belgium
E-mail: alexia.verroken@uclouvain.be

Introduction

The genus Corynebacterium refers to gram-positive bacteria that form part of the normal microbiota of human skin and mucous membranes and that are also widely distributed in the environment [1,2]. The significance and prevalence of coryneform bacteria are not always well established.

Corynebacterium striatum has been isolated as part of the normal human nasopharyngeal flora and from the skin. However, this organism has also been shown to be potentially pathogenic in patients with chronic diseases and in specific circumstances (e.g. following repeated exposure to broad-spectrum antibiotics, after the use of invasive medical procedures and in the presence of organic obstructive pathologies) [3–5]. Most reported C. striatum infections involved the respiratory tract and several outbreaks of nosocomial infections have been described [6–9].

At the Mont-Godinne University Hospital (Yvoir, Belgium) we recovered 24 C. striatum strains from respiratory samples of 10 patients hospitalized in the intensive care unit (ICU) and at one medical respiratory unit over an 8-month period.
To understand the possible outbreak sources and transmission routes we reviewed the medical records of all patients from whom \textit{C. striatum} was isolated over the study period. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used for strain identification and was challenged as an epidemiological typing tool through the interpretative reading of the strain spectra in the form of a generated dendrogram. Results were compared with semi-automated repetitive-sequence-based PCR (rep-PCR) (DiversiLab, bioMérieux, Marcy-l’Etoile, France) and PFGE (pulsed-field gel electrophoresis).

**Material and Methods**

**Patients**
We reviewed the medical records of all patients from whom \textit{C. striatum} isolates had been recovered from clinical specimens for the presence of putative risk factors for nosocomial acquisition. The study took place at UCL Mont-Godinne University Hospital (Yvoir, Belgium), a 430-bed teaching hospital with a laboratory mostly receiving samples from the hospital. The status of colonization or infection was assessed according to the CDC criteria for definition of infection [10].

**Bacterial isolates**
From January 2011 to August 2011, all routine specimens yielding the isolation of \textit{C. striatum} at culture and considered to be of potential clinical significance (i.e. isolation from biologically sterile fluids or as a pure or predominant culture growth from non-sterile clinical specimens) were collected but only the first strain for each patient was selected for the different analyses.

In parallel, five epidemiologically unrelated \textit{C. striatum} clinical isolates, recovered from respiratory samples, collected from other Belgian hospitals between 2000 and 2010 and sent for confirmation of identification to a reference laboratory (Professor G. Wauters, Microbiology Unit, Faculty of Medicine, Catholic University of Louvain, Brussels, Belgium) were also included in the analysis for the purpose of comparison.

All \textit{C. striatum} isolates were initially identified through the clinical laboratory routine workflow by MALDI-TOF MS and their identification was further confirmed by sequencing of the entire 16S rRNA gene according to a previously published method [11].

\textit{In vitro} susceptibility to ten antimicrobial agents (penicillin, ampicillin, erythromycin, clindamycin, gentamicin, tetracycline, ciprofloxacin, co-trimoxazole, rifampicin and vancomycin) was assessed by broth microdilution according to Clinical and Laboratory Standards Institute guidelines [12].

**MALDI-TOF MS**
MALDI-TOF MS measurements were realized on a microflex LT (Bruker Daltonik, Bremen, Germany). Spectra were recorded by the positive linear method in a mass range from 2000 to 20000 Da.

**Strain identification.** Single colonies were spotted on a steel target overlaid with 1 \(\mu\)l matrix solution dissolved in a basic organic solvent composed of 50% acetonitrile and 2.5% tri-fluor-acetic-acid. The acquired bacterial spectra with MALDI-TOF MS were analysed in the MALDI Biotyper 2.0 software database, leading to scored identification results. According to the specifications of the manufacturer, a high log score \(\geq 2\) was required for identification to species level.

**Dendrogram creation.** Approximately 10 colonies of each \textit{C. striatum} isolate were scraped from a 24-h culture on trypticase soy blood agar and added to 500 \(\mu\)l distilled water followed by an ethanol-formic acid extraction procedure. One \(\mu\)l of the final extraction product was spotted eight times onto a steel target and was overlaid with 1 \(\mu\)l of matrix solution. Each spot was measured three times with the MBT_FC.par flexControl method and the MBT-autoX.axe autoExecute method. The resulting 24 spectra were downloaded into the MALDI BioTyper software and assembled in order to generate a single mean spectrum accounting for the extracted strain. In order to appreciate the correlation between the organisms and visualize the clustering, a dendrogram was calculated.

**DiversiLab typing**
DNA extraction for all \textit{C. striatum} strains was achieved with the UltraClean microbial DNA isolation kit (MoBio, Carlsbad, CA, USA) followed by DNA quantification with a NanoDrop 2000/2000C spectrophotometer (Thermofisher, Friendings, ME, USA). The DiversiLab Bacterial fingerprint kit (Ref\# 2000/2000C) was used according to the manufacturer’s instructions to perform the rep-PCR amplification. PCR products were detected and sized on an Agilent 2100 bioanalyser (Agilent Technologies, Diegem, Belgium). Sample clustering was studied by comparing the strain patterns looking for band differences and the percentage similarity analysed. Isolates with identical strain patterns were considered indistinguishable if the similarity percentage was \(\geq 97\%\) [13].

**PFGE**
For PFGE analysis, \textit{C. striatum} DNA was extracted using lysozyme (20 mg/mL) and achromopeptidase (500 Units/mL). Macorestriction (XbaI) patterns were analysed using Dice coefficient with Bionumerics software (Applied Maths, Kortrijk, Belgium). The classification criteria for PFGE analysis
included type, designated by a capital letter and patterns showing 0-3 DNA fragment difference corresponding to a similarity cut-off of $\geq 80\%$ [14].

**Results**

**Bacterial isolates and medical records**

From January 2011 to August 2011, we recovered among all routine culture microbiological samples a total of 24 clinical isolates of *C. striatum*. All strains originated from lower respiratory tract specimens of 10 distinct hospitalized patients. The 24 strains were correctly identified to the species $\log$ (score) $\geq 2$ with MALDI-TOF MS. 16S rRNA gene sequences of the 10 analysed strains (one strain/patient) showed $\geq 99.6\%$ similarity with the sequence from *C. striatum* type strain NCTC 764 T. All *C. striatum* clinical isolates displayed an identical multidrug resistance pattern with susceptibility to vancomycin only. The five epidemiologically unrelated *C. striatum* strains showed a less resistant profile as all isolates were susceptible to penicillin, ampicillin, erythromycin, clindamycin and vancomycin while showing heterogenous profiles to the other antibiotics (data not shown).

The clinical characteristics of the patients are presented in Table 1. According to CDC criteria, seven patients were considered to be colonized and three infected. All affected patients had been hospitalized for prolonged periods before the first isolation of *C. striatum* (mean, 33 days; range, 5–90 days).

During the outbreak, at least one colonized or infected patient was hospitalized in the ICU or in the medical respiratory unit at the time by which a new carrier of *C. striatum* was detected (Fig. 1). Eight of the 10 patients had major co-morbidities and had been exposed to multiple previous courses of antibiotic treatment. Eight patients did undergo a bronchial fibroscopy and five patients were intubated. The medical care of eight patients required the use of invasive medical devices. Finally, nine patients were discharged from the hospital while one colonized patient died during his stay.

**Epidemiological analysis**

**Dendrogram with MALDI-TOF MS.** The dendrogram (Fig. 2) generated from the 15 registered *C. striatum* spectra pointed out a distinctive cluster assembling the 10 outbreak-related *C. striatum* strains and the external strains 4 and 5 with a maximum distance level of 100. The acquired spectra of three out of the five unrelated tested *C. striatum* external strains showed a distinct spectrum pattern.

**DiversiLab typing.** All 10 outbreak-related *C. striatum* isolates had the same repPCR patterns and had $\geq 97.7\%$ similarity.

---

**TABLE 1. Summary of the medical records of the 10 patients with positive *C. striatum* isolates in respiratory samples**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/gender</th>
<th>Underlying condition</th>
<th>Unit</th>
<th>Antibiotic treatment</th>
<th>Associated strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62/M</td>
<td>Lobectomy Lung, carcinoma</td>
<td>Intensive care</td>
<td>PTZ, CPM</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>2</td>
<td>59/F</td>
<td>Lung transplant, COPD</td>
<td>Medical unit</td>
<td>AZT</td>
<td>Colonization</td>
</tr>
<tr>
<td>3</td>
<td>91/F</td>
<td>Corticotherapy, Follicular lymphoma</td>
<td>Medical unit</td>
<td>PTZ</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>79/M</td>
<td>COPD</td>
<td>Medical unit</td>
<td>MRP + AMI</td>
<td>Colonization</td>
</tr>
<tr>
<td>5</td>
<td>88/M</td>
<td>COPD</td>
<td>Medical unit</td>
<td>LVX</td>
<td>Pneumoniae P. aeruginosa S. maltophilia</td>
</tr>
<tr>
<td>6</td>
<td>65/M</td>
<td>Lung transplanted COPD</td>
<td>Intensive care</td>
<td>CAZ</td>
<td>IV catheter</td>
</tr>
<tr>
<td>7</td>
<td>65/M</td>
<td>Lung transplanted COPD</td>
<td>Intensive care</td>
<td>AMC</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>8</td>
<td>73/F</td>
<td>Lung transplanted COPD</td>
<td>Intensive care</td>
<td>AMC</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>9</td>
<td>64/M</td>
<td>Lung transplanted COPD</td>
<td>Intensive care</td>
<td>AMC</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>10</td>
<td>75/F</td>
<td>COPD</td>
<td>Intensive care</td>
<td>AMC</td>
<td>P. aeruginosa</td>
</tr>
</tbody>
</table>

©2013 The Authors
Clinical Microbiology and Infection ©2013 European Society of Clinical Microbiology and Infectious Diseases, CMI, 20, 44–50
level between each other while the five epidemiologically non-related strains displayed different strain patterns, with similarity levels <97% between each other (Fig. 3).

**PFGE.** PFGE delineated the 10 *C. striatum* patient strains into three distinct PFGE types (Fig. 3). Type A comprised the outbreak-related *C. striatum* isolates from patients 1 to 7 and *C. striatum* external isolate 5. Type F comprised the *C. striatum* isolates from patients 8 and 9. Finally Type H was a singleton only including the *C. striatum* patient 10 strain.

The analysis of the four leftover *C. striatum* epidemiologically non related strains led to four singleton PFGE types (B, C, D and G).

**Discussion**

Over recent years Corynebacteria have been recognized as opportunistic pathogens able to cause various types of healthcare-associated infections in immunocompromised hosts [2]. It is therefore essential for each clinical microbiology laboratory to use techniques that identify correctly and rapidly these bacteria. Identification of Corynebacteria by conventional methods is suboptimal and it is likely that their true prevalence in clinical specimens either as colonizers or as pathogens is largely underestimated. MALDI-TOF MS has proven accuracy for rapid identification of toxigenic Corynebacterium...
species and non-diphtheria Corynebacterium, including \textit{C. striatum} [15–17].

In our setting, the repeated identification of \textit{C. striatum} in clinical respiratory samples of several hospitalized patients over a limited period of time raised the suspicion of an ongoing outbreak. While only one \textit{C. striatum} isolate had been recovered from a hospitalized patient in the preceding 8-month baseline period, we identified 24 clinical \textit{C. striatum} isolates in 10 distinct hospitalized patients over the 8 following months, corresponding to an attack rate of 10 to 1. Following this observation, we established the ‘outbreak case definition’ as each hospitalized patient with a clinical sample growing mainly or exclusively \textit{C. striatum} colonies. A retrospective investigation of the patients’ medical records was carried out to describe the outbreak population. Several risk factors were identified in our patients. The most frequently found underlying co-morbidity in our series was chronic obstructive pulmonary disease for eight of the ten patients. It is very likely that such long-lasting chronic disease along with the frequent use of respiratory equipment did contribute to the respiratory tract colonization and the infectious potential of this organism in immunocompromised patients. Previous exposure to antibiotic treatment observed in eight patients also most likely contributed to the overgrowth of this multidrug-resistant organism through selective pressure.

Upon the case definition and the subsequent analysis of risk factors, an epidemic curve (date not shown) and a spatial/temporal distribution (Fig. 1) were drawn, aiming to understand the routes of transmission and sources of the \textit{C. striatum} outbreak. Figure 1 highlights the particularity of this outbreak, which was ongoing simultaneously in two units despite the fact that the medical and ICU wards were geographically separated and that each unit was staffed with distinct medical and nursing teams. The colonized/infected patients probably constituted the reservoirs of transmission because all had a very long hospital stay and at least one colonized patient was always present in one of the two affected units during the outbreak period at the time by which a new case of \textit{C. striatum} was recognized. We considered as the most probable scenario that patient #2 who had been nursed in contact with the index case (patient #1) in the ICU in January 2011 and who was subsequently transferred to the respiratory medical unit, was at the origin of the dissemination of this strain between the two units. The healthcare staff in both units are strongly suspected to be the vector of cross-transmission of the \textit{C. striatum} strain between patients. In a previous report, Brandenburg et al. reported the 12-month persistence of a single \textit{C. striatum} epidemic strain in patients in a surgical ICU, suggesting that asymptomatically colonized patients may constitute the main reservoir of \textit{C. striatum} and that patient-to-patient transmission probably occurs via the hands of the personnel [7]. Our study hereby highlights the protracted and silent pattern of a \textit{C. striatum} outbreak. Based on all these elements, an alert was issued by the infection control team to reinforce hand hygiene practice and barrier precautions.

In parallel, a laboratory investigation of the outbreak-related \textit{C. striatum} strains was performed. Several molecular typing methods (DNA fingerprinting and genotyping) have been used to demonstrate an epidemiological link between the isolates in previously described \textit{C. striatum} outbreaks [6–8]. These methods nevertheless require highly
qualified staff and expensive dedicated material and they usually require several days or weeks before results are obtained and a feedback is given to the units. MALDI-TOF MS has proven to be reliable for identification of bacteria but as yet there have been only few studies that have assessed the value of this technique as an epidemiological typing tool in the setting of a nosocomial outbreak [18]. In our study we aimed to set up an easy and fast subtyping technique accessible to any MALDI-TOF MS user. The technique was based on an ethanol-formic acid strain extraction method (routine test for difficult strains) and a dendrogram cluster analysis only requiring the Maldi BioTyper Software without any additional statistical tool. We determined the diversity of the C. striatum isolates collected in the setting of our local outbreak and compared these strains with epidemiologically unrelated isolates collected from specimens in several hospitals. All outbreak-related strains clustered in a single clone both by MALDI-TOF MS dendrogram and with the DiversiLab typing method. Yet, two epidemiologically unrelated isolates (external C. striatum strains #4 and #5 also) clustered within the outbreak cluster in the dendrogram while they corresponded to a different clone type with DiversiLab. PFGE analysis subdivided the ten outbreak-associated C. striatum strains into three clusters (A, F and H). Cluster A included the first seven patient strains and C. striatum external strain #5, an observation also made with the MALDI-TOF MS dendrogram. Interestingly, by PFGE the strains were found to be grouped into three different types according to chronological sequence (time) of isolation (i.e. cluster A included patient strains #1 to #7, cluster F strains #8 and #9, and finally cluster H strain #10). As the duration of this outbreak had spanned over an 8-month period, it may well be possible that the C. striatum outbreak strain had undergone some chromosomal changes that might have been detected by PFGE analysis, considered as a highly sensitive molecular epidemiological tool. In conclusion, typing results by MALDI TOF MS were subsequently confirmed by intergenic rep PCR typing analysis but both methods appeared slightly less discriminant than PFGE for recognition and differentiation of certain clonal lineages. Based on a diversity analysis of C. striatum strains by MALDI-TOF MS and by a multigenic sequencing approach, Gomila et al. similarly suggested that MALDI-TOF MS could be an efficient tool for discrimination of bacterial strains below the species level though not as discriminatory as the other molecular typing techniques [17].

A hospital outbreak is never a coincidence but a reflection of a failure in the infection control measures. In line with several previous reports, our study once more highlights the underappreciated community and nosocomial pathogen. Based on our experience, we believe that rapid confirmation of the spread of a single clone/type of a nosocomial pathogen within or across several hospital units by a locally available typing method such as MALDI-TOF MS may contribute to improving the management of an outbreak. This study is one of the first to document the potential usefulness of MALDI TOF MS as a subtyping tool for investigating nosocomial outbreaks. Further studies are, however, needed to confirm our observations.

**Transparency Declaration**

The authors declares no conflicts of interest.

**References**


