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Respiratory infection by *Corynebacterium striatum*: epidemiological and clinical determinants

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Abstract

The increasing prevalence of advanced chronic respiratory disease, with frequent exposure to broad-spectrum antibiotics for repeated and prolonged hospitalizations, favours the emergence of nosocomial respiratory infection by Gram-positive bacteria, such as outbreaks of *Corynebacterium striatum*. There is little evidence about patterns of respiratory infection, transmission and adaptive ability of this pathogen. Seventy-two *C. striatum* isolates from 51 advanced respiratory patients, mainly chronic obstructive pulmonary disease, were studied during 38 months. Patients were 74.8 ± 8.6 years old and 81.9% were men, who had required an average of 2.2 hospitalizations and 63.5 days in the hospital in the previous year. Of 49 isolates from 42 patients we were able to identify 12 clones by multilocus sequence analysis (MLSA), nine phenotypic variants and 22 antibiotic susceptibility patterns, and we determined their clinical and epidemiological determinants. MLSA allows identification of the existence of nosocomial outbreaks by transmission of the same or different clones, the persistence of the same clone in the environment or in patient airways for months. The study showed the high variability and adaptive capacity of the isolates, the antibiotic multidrug-resistance in all of them, and their contribution to a high morbidity and mortality (41%) during the study period.

Keywords: Advanced chronic obstructive pulmonary disease, *Corynebacterium striatum*, epidemiology, genotype, multidrug-resistance, respiratory infection

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Introduction

Corynebacterium species are found colonizing skin, other tissues, and in the environment [1,2]; they are considered normal flora and not potentially pathogenic. But in 1997 Funke *et al.* [3] described a massive increase in the number of publications related to this genus, which were attributed to the increase of immunocompromised patients susceptible, to improved microbiological diagnoses, and to a precise taxonomic classification that allows the correct identification of different species with different clinical expression.

In the last decades, in addition to *Corynebacterium diphtheriae*, the pathogenicity among *Corynebacterium* spp. has been reported associated with *Corynebacterium amycolatum* [4,5], *Corynebacterium jeikeium*, *Corynebacterium macginleyi*, *Corynebacterium urealyticum*, *Corynebacterium pseudodiphtheriticum* and, less frequently with *Corynebacterium xerosis* [4,6–8]. *Corynebacterium striatum* has been reported colonizing prostheses, catheter tips, and ventilator and feeding tubes, and it has been also identified as causative in cases of endocarditis, sepsis and bacteraemia [9–11].

Until 1993 there were only three individual case reports of confirmed respiratory infections by *C. striatum* [12–14]. Since then, numerous individual cases and various nosocomial infectious outbreaks of *C. striatum* have been reported [15–21] mostly in patients with chronic diseases requiring frequent and prolonged hospitalizations with repeated exposure to antibiotics against Gram-negative bacteria, organic obstructive disorders, or exposed to invasive procedures that disrupted the skin barrier. Most reported *C. striatum* infections have been found in respiratory samples, the vast majority of strains being multidrug-resistant.

The study of nosocomial infections and outbreaks is fundamental to have reliable methods for the identification and typing of the bacteria responsible. It allows further efforts to prevent and control these events, and to reduce individual and population burden. There are several available fast and affordable methods that allow a good identification of species and useful for the identification of an outbreak, but understanding the mechanisms and transmission circumstances during an outbreak requires a molecular study of the isolates. Several studies have tried to accomplish this objective [16,19,21,22], but none of them employed a methodology for identification and typing of strains until the study of Gomila *et al.* [23]. In that study, isolates of *C. striatum* were analysed using different approaches, ribotyping, phenotype, matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy, and multilocus sequence analysis (MLSA). They demonstrated that isolates of *C. striatum* were best identified using gene-based molecular methods.

The main aim of this prospective study is to explore the epidemiological and pathological circumstances of a group of patients susceptible to be infected by *C. striatum*, managed in a respiratory ward. We determined the genetic identity of *C. striatum* strains isolated in cultures of biological samples obtained during infectious respiratory exacerbations. We aimed to study the clinical and environmental determinants, the transmission mechanisms between patients, their adaptive ability and variability in phenotype and susceptibility to antibiotics, its ability to persist in the environment and respiratory airways, and its impact on morbidity and mortality in this group of patients.

Material and Methods

Corynebacterium striatum culture collection

The Hospital Joan March, in Bunyola, Mallorca, Spain, is a secondary healthcare centre that hosts a convalescence and rehabilitation department, with a 26-bed ward. It aims to deliver care to patients with severe, chronic respiratory disease referred from tertiary-care hospitals within its catchment area.

Seventy-one *C. striatum* isolates from cultures of respiratory samples, and one from a dermal ulcer, from 51 patients with advanced respiratory disease seen in the hospital, were obtained during 38 months (from May 2006 to June 2009), and we explored their clinical and epidemiological determinants. Forty-nine of these *C. striatum* isolates, from 42 patients, could be recovered with the aim of analysing their antibiotic susceptibility, phenotype and genotype.

All respiratory samples were obtained during respiratory infectious exacerbations that fulfilled GOLD criteria [24], and according to the criteria of Anthonisen *et al.* [25] were of potential infectious origin. Specimens were cultured on Columbia agar with 5% sheep blood (bioMérieux, Marcy l'Etoile, France). Before processing, all samples were Gram-stained so as to discard the samples not representative of the lower respiratory tract and/or contaminated with microbiota from the upper respiratory tract according to the Murray and Washington criteria [26]. The clinical relevance of *C. striatum* isolation from respiratory samples was based on their correct identification, their abundance, their isolation as a single microorganism or their predominance when found in association, and the repetition of positivity [1]. The processing and incubation of plates was performed following routine laboratory conditions. All isolates were stored at −80°C for future study.

Phenotypic and antibiotic susceptibility, and molecular analysis

All information about phenotype, genotype and antibiotic susceptibility was consistent with our previous work on 49 recovered isolates of the total 72 [23], and they were labelled with alphabetical codes. Coding is described in Table S1 (see the Supporting information). From the molecular analysis only the internally transcribed spacer 1 (ITS1) region and the *gyrA* and *rpoB* genes were used, as they were the most useful for discriminating between strains due to their variability.

Isolates versus temperature and humidity

Given that *C. striatum* is an environmental bacterium, temperature and humidity were recorded on the date of obtaining each isolate. Information was obtained from the official weather station in the enclosed area of the hospital. For this environmental analysis, the 43 isolates of *C. striatum* from a previous outbreak that occurred in our hospital between January 2004 and June 2005 were also considered [20].

Statistical analysis

Statistical analyses were performed using the SPSS v.15.0 software. All data were quality controlled and comprehensive