Fatal Case of Bacteremia Caused by an Atypical Strain of *Corynebacterium mucifaciens*

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Corynebacterium **species have often been considered normal skin flora or contaminants; however, in recent years they have been increasingly implicated in serious infections. Moreover, many new species have been discovered and old species renamed, especially after molecular biology techniques were introduced. *Corynebacterium mucifaciens* is mainly isolated from blood and from other normally-sterile body fluids; it forms slightly yellow, mucoid colonies on blood agar. We report a fatal case of bacteremia due to an atypical strain of *C. mucifaciens*. This strain had atypical colony morphology; analysis of the 16S rRNA gene was used to define the species.**

Key Words: Bacteremia, blood infection, *Corynebacterium*, *Corynebacterium mucifaciens.*

Determining the clinical significance of corynebacteria isolated from clinical specimens is often challenging for clinical microbiologists. For many years, these organisms were disregarded as skin contaminants and no further attempt to identify them at the species level was made. However, they have increasingly been recognized as important human pathogens, often acting as opportunistic pathogens in immunocompromised or severely-ill patients. Identification to the species level is recommended if more than one blood culture bottle tests positive, the patient has symptoms compatible with bacteremia, and no other pathogenic organisms are present [1]. *Corynebacterium mucifaciens* is a newly-described species that typically grows on blood agar plates, forming slightly yellow and mucoid colonies [2]. Identification can be made with standard biochemical tests or with commercial identification systems, such as API Coryne. However, differentiation from closely-related species is better accomplished with molecular biology techniques, such as sequencing of the 16S rRNA gene.

Antimicrobial susceptibility tests for *Corynebacterium* species lack standardization, and empirical therapy is often used to treat patients known to be infected by *Corynebacterium* spp. With few exceptions, such as *C. jeikeium,* all *Corynebacterium* spp. show good clinical response to penicillin or vancomycin [3]. In the case of *C. mucifaciens,* beta-lactam antibiotics and aminoglycosides appear to have good activity [1].

We report isolation of an atypical strain of *C. mucifaciens* recovered from multiple blood cultures made from an elderly female patient.

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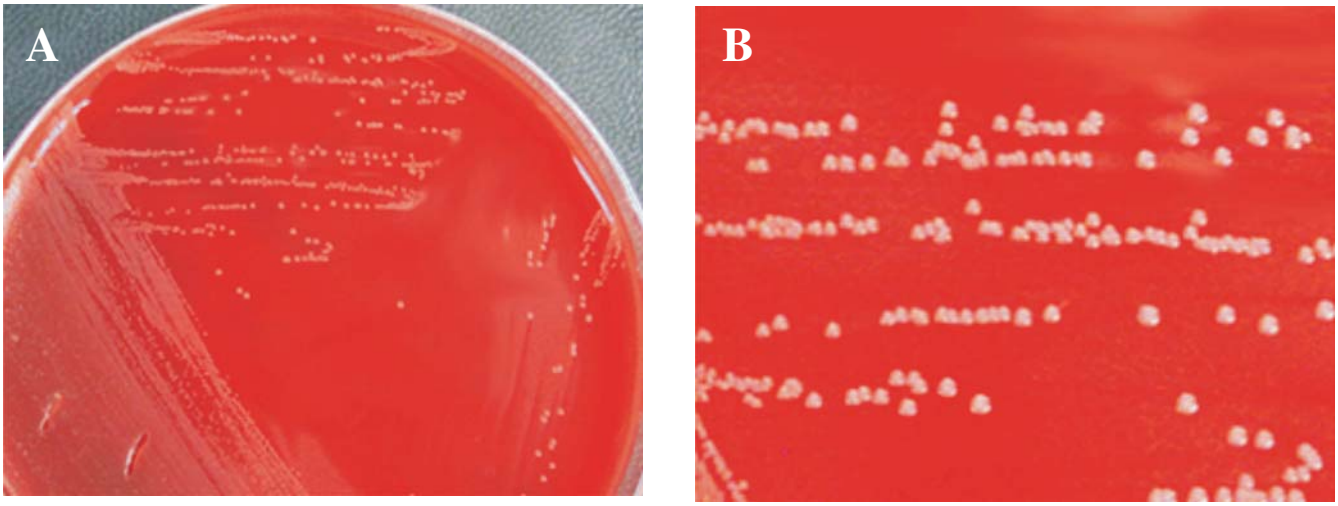
Case Report

An 81-year-old woman was admitted to the hospital after a bad fall. Her past clinical history included: diabetes, ischemic cardiopathy, pulmonary fibrosis and an esophagic tumor that had been treated with radiotherapy a couple of years before. Laboratory findings revealed a white-blood-cell count of 15,500/mm^3, with 85% neutrophils (7% bands); the platelet count was 277,000/mm^3, with no marked alteration in the erythrocytic series. Blood glucose was 213 mg/dL. Three blood samples from three separate venipunctures were submitted to the clinical microbiology laboratory for aerobic culture and were continuously monitored for any bacterial growth, using the BactAlert instrument (Organon-Teknika). All samples became positive after three days of incubation, and a Gram stain revealed Gram-positive, non-spore-forming diphtheroid rods. The patient died before complete identification of the organism was available.

The organism was subcultured on both chocolate and plain sheep blood agar (bioMérieux), revealing approximately 1-mm diameter whitish non-mucoid colonies after 24h incubation. Colonies were about the same size after 72h incubation (Figure 1). Catalase reaction tested positive. The first attempt to identify the organism was made using API-Coryne (BioMeriéux) strips, following the manufacturer's instructions. The numerical code (2100104) suggested *C. mucifaciens* [1,4].

In disagreement with published information on *C. mucifaciens,* our strain produced smooth-white colonies (Figure 1), instead of yellow and mucoid colonies [2]. This prompted us to check our initial identification with 16S rRNA gene sequence analysis. DNA was obtained using a modified guanidine-isothiocyanate method [5], and the 16S rRNA gene (ca. 1500 bp) was amplified using primers 285 (5' – GAGAGTTTGATCCTGGCTCAG – 3') and 261 (5'-AAGGAGGTGATCCAGCCGCA - 3') [6] and a GeneAmp™ PCR reagent kit (Applied Biosystems). PCR product was cleaned using shrimp alkaline phosphatase and Exonuclease I (USB) and sequenced using primer 806-F (5' –

Figure 1. A. Small white colonies of *Corynebacterium mucifaciens* on sheep blood agar after 72 h of incubation. 1. B. higher magnification of colonies from the same plate.



ATTAGATACCCTGGTAGTCC – 3') and the Big Dye terminator DNA Sequencing kit (Applied Biosystems), as described elsewhere [7]. A partial sequence (562 bp, corresponding to nucleotides 765 to 1326) was aligned and compared with other available 16S rRNA gene sequences using the bioinformatics bacterial identification tool (BIBI) program [8]. There was 100% homology with *C. mucifaciens* NML 97-0160 (GenBank accession no. AF537601), followed by two other sequences of *C. mucifaciens* (GenBank accession no. AF537600 and Y11200, respectively) with 99.1% and 98.0% similarity, respectively.

Strain NML 97-0160 is a blood isolate; it was described in GenBank as "closest to *C. mucifaciens"*, with colonies that are smooth, not mucoid or yellow, and urease positive [2]. Colony description is consistent with the characteristics of our isolate; however, our strain was unable to hydrolyze urea.

Discussion

Identification of *Corynebacterium* to the species level is problematic. Recently, several species were renamed and some were even moved to other genera. Correct identification of these organisms would help us better understand the pathogenic potential of each *Corynebacterium* spp. and of related species.

Identification can be attempted by API Coryne; however, the database provided by the manufacturer is not complete, and some strains may be misidentified due to overlapping numerical codes. Additional numerical codes for recently-described species are often found in published papers [4] or in microbiology manuals [1], which should be consulted whenever an unusual *Corynebacterium* strain is isolated from a clinical sample. We suggest that results obtained using API Coryne should be carefully checked and compared with other microbiological information, such as colony morphology, color, and reference texts. Discrepancies must be checked, and other tests should be performed to assure the correct identification of the species. Preferably, DNA amplification and sequencing of target genes, such as 16S rRNA, should be done to confirm discrepant results obtained with biochemical and commercial identification tests.

The increasing number of different species of *Corynebacterium* being reported also has implications for the treatment of infections due to these organisms. Weiss et al. [3] suggest that disk diffusion tests, following interpretation using streptococcus criteria, would be useful for assessing the activity of penicillin against *Corynebacterium* spp.

Corynebacterium mucifaciens is often isolated from blood or other normally-sterile body fluids [1,4]. In our case, we isolated it from a severely-ill patient who had several complicating factors. The patient died before results from blood cultures were available to guide antibiotic therapy.

In summary, we describe the isolation of a *C. mucifaciens* strain from blood cultures, which was correctly identified by API Coryne when the numerical code was checked in the reference literature [1,4]. However, this particular strain showed colony morphology and a partial 16S rRNA sequence that were different from what is reported for reference strains [2]. The best match for the partial 16S rRNA sequence was strain NML 97-0160, described as "closest to *C. mucifaciens"*; but unlike our strain, it was urea positive. Since aberrant strains are likely to exist and may complicate the interpretation of

phenotypic tests, molecular methods, such as analysis of 16S rRNA gene sequences can provide a viable alternative for bacterial identification, especially for bacterial strains isolated from relevant clinical samples.

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