JOURNAL OF CLINICAL MICROBIOLOGY, June 2006, p. 2242–2243 Vol. 44, No. 6
0095-1137/06/$08.00+0 doi:10.1128/JCM.02473-05

Isolation of *Corynebacterium xerosis* from Animal Clinical Specimens

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Received 30 November 2005/Returned for modification 13 February 2006/Accepted 26 March 2006

This article describes the first identification of *Corynebacterium xerosis* from animal clinical specimens, which was confirmed by microbiological and molecular genetic (16S rRNA gene sequencing) methods.

Corynebacterium xerosis is considered a commensal microorganism of human skin and mucous membranes. There are a limited number of reports associating *C. xerosis* with different human infections (1, 6), but it has been demonstrated that most of the *C. xerosis* isolates identified in the routine clinical laboratory represent *Corynebacterium amycolatum* strains (5, 6). Therefore, true human clinical isolates of *C. xerosis* are extremely rare (8), and there are no reports of its isolation from animals. We report the isolation and biochemical and genetic identification of eight strains of *C. xerosis* from different animal clinical specimens.

The eight isolates identified in this study were recovered from different clinical specimens routinely submitted for microbiological diagnosis to the Exopol laboratory in Zaragoza, Spain. Clinical specimens were collected under aseptic conditions and cultured aerobically on Columbia sheep blood agar (Oxoid España, Madrid, Spain) for 24 to 48 h at 37°C. For all clinical specimens, *C. xerosis* was recovered in moderate to high numbers in pure culture or as the predominant isolate from normally sterile body locations. Details of the clinical isolates are given in Table 1. Although only eight isolates were biochemically and genetically identified, coryneform bacteria similar to isolates St33874, St34960, St38671, and St49327 (similar colony morphology on sheep blood agar plates and similar Gram stain morphology) were also recovered from other organs from the same animal or different animals in the same disease episode. Thus, coryneform bacteria similar to isolate St33874 were also recovered in pure culture from the liver, spleen, and kidney of the same animal and from the liver of a second pig. A coryneform bacterium similar to isolate St34960 was also recovered in pure culture from the liver of the same animal. A coryneform bacterium similar to St38671 was also isolated from the joint of another pig; in this case, *Mycoplasma hyosinoviae* was also detected by immunocytochemistry. Coryneform bacteria similar to isolate St49327 were also recovered in pure culture from the liver and kidney of the same animal.

Preliminary identification of the clinical isolates as *Corynebacterium* spp. was performed following standard procedures (4, 10). Further biochemical identification was achieved by using the commercial API Coryne (version 2.0) system (bioMérieux España S.A., Madrid, Spain) according to the manufacturer's instructions. The lipophilic requirement was determined by growing the isolates on brain heart infusion agar supplemented with 1% Tween 80 and comparing them to isolates grown on brain heart infusion agar lacking lipid supplementation. Antimicrobial susceptibility was tested by the disk diffusion method on Mueller-Hinton agar (bioMérieux España S.A.) according to the protocol of the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) (9). The 16S rRNA genes of the eight isolates were amplified by PCR and further sequenced to determine their genotypic identities (11). The determined sequences consisted of about 1,400 nucleotides and were compared with the sequences of other gram-positive and catalase-positive species available in the GenBank database by using the FASTA program (http://www.ebi.ac.uk/fasta33).

The eight isolates grew on sheep blood agar, forming small (1 to 2 mm in diameter after 48 h of incubation), dry, and rough colonies with a yellowish pigment. They consisted of gram-positive, nonmotile, non-spore-forming, catalase-positive, rod-shaped organisms that were tentatively identified as *Corynebacterium* spp. The isolates were nonhemolytic and nonlipophilic and gave two numerical profiles with the API Coryne system, i.e., 2110325 (St36404) and 3110325 (St33874, St34960, St36130, St38671, St49845, St49327, and St47126), that correspond to a doubtful discrimination between *Corynebacterium striatum* and *C. amycolatum*. The API Coryne database does not include *C. xerosis*, which can create misidentification when using this commercial identification system (5). The clinical isolates were able to produce acid from maltose and exhibited strong α-glucosidase activity, which are among the few biochemical test results that can differentiate *C. xerosis* from *C. striatum* and *C. amycolatum* (5, 6). Clinical isolates were susceptible to penicillin, ampicillin, amoxicillin-clavulanic acid, doxycycline, ceftiofur, cephalexin, gentamicin, oxytetracycline, and enrofloxacin, which also agrees with previous data about the antimicrobial susceptibility of *C. xerosis*, whereas strains of *C. amycolatum* are typically resistant to most of these antibiotics (5).

Sequencing of the 16S rRNA gene is a very useful tool for the identification of unusual clinical isolates or those which cannot be identified easily by conventional phenotypic methods (2, 7, 12). The 16S rRNA gene analysis revealed that all isolates were genotypically identical, displaying 100% and

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TABLE 1. Details of clinical isolates included in this study

Isolate	Yr of isolation	Animal origin	Organ	Clinical history of animal	Primary isolation
St33874	2002	Pig	Lung	Suspected erysipelas	Predominant[a]
St34960	2002	Pig	Kidney	Respiratory problems	Pure culture
St36404	2003	Goat	Liver	Suspected paratuberculosis	Pure culture
St36130	2003	Pig	Skin	Subcutaneous abscess	Pure culture
St38671	2003	Pig	Joint	Arthritis	Pure culture
St49485	2005	Pig	Joint	Subcutaneous abscess	Pure culture
St49327	2005	Pig	Blood	Sudden death	Pure culture
St47126	2005	Pig	Joint	Arthritic abscess	Pure culture

[a] Recovered as the predominant isolate, together with *Streptococcus suis*. *Mycoplasma hyopneumoniae* was also detected by immunocytochemistry.

99.8% sequence similarity with *C. xerosis* (accession numbers AF145257 and X84446, respectively). The overall phenotypic characterization results for the clinical isolates were consistent with those described for this species (5, 6), and they support the genetic identification of the clinical isolates as *C. xerosis*. Strain St36130 has been deposited in the Spanish Type Culture Collection as strain CECT 7156.

Although only a few species of *Corynebacterium* are traditionally considered well-recognized animal pathogens (10), the genus *Corynebacterium* has been subjected during the last decade to a substantial taxonomic revision with the description of a plethora of new species from human and animal sources (4, 6), some of which have a pathogenic role (3). This report represents the first identification of *C. xerosis* from animal clinical specimens, which was confirmed by molecular genetic methods. Although most isolates of *C. xerosis* were isolated in pure culture from normally sterile organs (Table 1), which may be indicative of clinical significance, in a few cases it was isolated from other organs of the same animal or different animals in the same disease episode, together with other veterinary pathogens, such as *Streptococcus suis*, *Mycoplasma hyopneumoniae*, or *M. hyosinoviae*, which makes it difficult to draw conclusions about the possible pathogenic character of *C. xerosis* for animals. Nevertheless, the report of the identification of *C. xerosis* from clinical samples of animals may be useful for veterinary clinical microbiologists to improve the knowledge of its distribution and its possible association with disease in animals.

Nucleotide sequence accession number. The 16S rRNA gene nucleotide sequence for strain St36130 has been deposited in the GenBank/EMBL data library under accession number AM233487.

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