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Corynebacterium glucuronolyticum causing genitourinary tract infection: Case report and review of the literature

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ABSTRACT

Corynebacterium species are increasingly recognized as opportunistic pathogens. A growing number of taxonomic studies has yielded a description of numerous new *Corynebacterium* species, such as those related to the urogenital tract, with *Corynebacterium glucuronolyticum* found to be rarely involved in genitourinary tract infections, particularly in male individuals.

In this report, we describe a urethritis case caused by *C. glucuronolyticum* in a 37-year-old, apparently healthy male, who complained mild pain in the lower abdomen, with several urinary symptoms. While urethral and semen specimens did not yield positive results for microbiological evaluation, cultures of urine samples revealed the monomicrobial growth on blood-containing media of tiny colonies after 24 h of incubation, clearly evident only after 48 h of incubation under CO_2-enriched atmosphere. Colonies were identified as *C. glucuronolyticum* both by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) and 16S rRNA gene sequencing. Oral ciprofloxacin gradually led to clinical improvement and, finally, to a complete recovery, in accordance with microbiological findings. In spite of its infrequent detection, *C. glucuronolyticum* might be a potential urogenital pathogen in males more commonly that what believed, perhaps due to slow growth leading to underrecognition; we suggest therefore to consider the organism in the differential diagnostics of bacterial diseases of the urinary tract.

Introduction

Corynebacterium species, also referred as "Gram-positive rods", "diphtheroids", or "coryneform bacteria", are Gram-positive bacteria belonging to the genus *Corynebacterium*, characterized by a high G + C content, and comprising a collection of aerobically growing, non-partially-acid-fast, non-sporogenous, irregular rod-shaped microorganisms [1].

Corynebacterium species are increasingly being recognized as causing opportunistic diseases under certain predisposing clinical conditions [2]. The most important pathogen of this genus remains *Corynebacterium diphtheriae*, the causative agent of diphtheria, that has essentially disappeared from developed countries after implementation of universal vaccination [3]. Corynebacteria have been also involved in zoonotic infections, as is the case of *Corynebacterium pseudotuberculosis*, and *Corynebacterium ulcerans*, historically thought to cause disease in patients who consumed contaminated milk or were close to farm or companion animals [3,4].

A growing number of taxonomic studies have yielded a description of numerous new *Corynebacterium* species, such as those related to the urogenital tract and, in this context, the names *Corynebacterium glucuronolyticum* and *Corynebacterium seminale* were proposed in 1995 by two independent studies focusing on isolates mainly recovered from human genitourinary tract [5,6]. It has been later reported that the presumed two species were indeed the same one [2], as confirmed by genotypic analyses [7,8]. Because of earlier description of *C. glucuronolyticum* [5], nomenclature priority has been assigned to this species, with *C. seminale* being today a synonym.

Case report

A 37-year-old, apparently healthy male without any known immune deficit presented with lower abdomen mild pain along

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with urinary symptoms including hesitancy, painful difficulty urinating, and frequency, suggestive of prostatitis or urethritis. Kidney and pelvic ultrasonography did not show, however, any pathologic alterations either in the mentioned gland or in other districts of the urinary tract. These findings, together with the clinical syndrome referred, were therefore suggestive of urethritis. Urethral swabs, semen, and urine samples were collected and sent to laboratory for microbiological evaluation. Pending results, the patient received empiric ciprofloxacin orally, 500 mg every 12 h, for 10 days. Urethral sampling was performed by inserting and rotating a dry rayon swab with an aluminum shaft (Copan Italia, Brescia, Italy) 2 cm into the urethra. A *Chlamydia* Direct IF (ID) Assay (bioMérieux, Mercy l'Etoile, France) for identification of *Chlamydia trachomatis* by the direct fluorescent antibody technique and a *Mycoplasma* Mycofast US Kit (ELITech France, Signe, France) for *Ureaplasma* and *Mycoplasma* species gave negative results. Semen specimen examined by wet-mount microscopy did not show the presence of *Trichomonas vaginalis*. Both urethral swab and semen specimens inoculated on Columbia Agar with 5% defibrinated sheep blood and PVX chocolate agar (Liofilchem, Roseto degli Abruzzi, Italy) and incubated at 37 °C aerobically and under 5% CO_2 atmosphere were negative. Urine sediment analysis provided instead evidence for bacteriuria, presence of urinary esterase, as well as leukocyturia (570 cells/μL).

Urine samples streaked on Columbia Blood Agar, Columbia Blood Agar supplemented with colistin and nalidixic acid, and MacConkey agar (Liofilchem) yielded a significant growth ($>1 \times 10^5$ CFU/mL), as a pure culture, on blood-containing media of an organism producing, after 24 h of incubation, tiny, white-yellow, non-hemolytic colonies of about 1.5 mm of diameter-sized, that grew better after 48 h of incubation under CO_2-enriched atmosphere. Gram staining revealed Gram-positive bacilli with a distinctive 'club-shaped' arrangement. The colonies were catalase-positive, identified as *C. glucuronolyticum* by MALDI-TOF MS using Bruker Biotyper software 2.0 (Bruker Daltonics, Germany), with an excellent score to the species level (score of 2.2). Identification was confirmed by sequencing of a 16S rRNA gene 900-bp amplicon, analyzed using BLAST (see http://blast.ncbi.nlm.nih.gov/Blast.cgi), with a BLAST 100% homology with *C. glucuronolyticum* strain V17 2011556 (GenBank accession no. KF926050.1). Agar disk diffusion test performed according to the EUCAST 2015 guidelines (www.eucast.org, last update 26 January 2015) showed susceptibility to ciprofloxacin, penicillin, vancomycin, linezolid, rifampicin, but resistance to tetracycline, gentamicin, erythromycin, and clindamycin. Accordingly, the abovementioned empirically administered fluoroquinolone led to complete clinical resolution.

Two months later, the patient was still asymptomatic, and a follow-up urine sample yielded 50,000 CFU/mL *C. glucuronolyticum*, with sediment analysis documenting a significant decrease of leukocytes number (50 cells/μL). The patient did not receive any further treatment and, four months later, a third urine sample was finally negative for both bacterial growth and sediment leukocyte observation.

Discussion

A growing number of infections caused by coryneform bacteria have been documented in the past years, mainly due to the increased number of immunocompromised patients, and to a deeper attention given to both the pathogenic potential and taxonomy of this bacterial genus. *C. glucuronolyticum* is a rare species isolated from male patients with genitourinary tract infections, probably being part of the normal male genitourinary microbiota, while its presence in females is uncertain. Uncommonly, *C. glucuronolyticum* has been also found in blood and peritoneal fluid [5,9], and, recently, it has been recognized among the most common agents of monomicrobial paucisymptomatic bacterial prostatitis, along with coagulase-negative *Staphylococcus* spp., and *Escherichia coli* [10]. Finally, it has been found that *C. glucuronolyticum* is not associated exclusively with humans, but also with animals [7,11].

In this report we documented an episode of urethritis caused by *C. glucuronolyticum*, thus further confirming the pathogenic role of this species in the genitourinary tract infections, mainly in otherwise healthy males, in agreement with the more recently published literature [12,13].

Although *C. glucuronolyticum* is reported to be a non-lipophilic microorganism, lipophilic *Corynebacterium* species have been described to grow poorly in broth or as tiny pinpoint colonies onto standard agar plates after 24 h-incubation [14]. In this case the strain grew as tiny colonies after 24 h-incubation under 5% CO_2 atmosphere, being more clearly observed only after 48 h of incubation.

Identification of coryneforms to the species level is often problematic [15]. It should be always performed when they grow as pure culture from clinical specimens and when they represent the most abundant organisms in samples collected from physiologically sterile sites. Establishing an association between *Corynebacterium* species and disease is strictly dependent on a correct identification to the species level [16]. In fact, an accurate species identification of this group of bacteria is worthwhile to ascribe potential pathogenic role to species that were previously thought to be mere innocent bystanders, and to discriminate infective agents from harmless colonizers. Therefore, the methods used for identification have to be appropriate and must reflect taxonomic changes observed among coryneform bacteria.

Most coryneform taxa can be identified to the species level mainly through three different identification approaches, (i) phenotypic methods, mostly relying on biochemical tests; (ii) proteomic-based analysis, meaning the use of MALDI-TOF technology; (iii) sequence-based identification methods [17,18]. It is also necessary to emphasize the importance of Gram staining for the preliminary identification of coryneform bacteria [14]. As regards biochemical tests, the key reactions that are used to differentiate coryneforms are catalase activity, fermentative or oxidative carbohydrate utilization, urea production, esculin hydrolysis, and the CAMP reaction, the latter being obtained by inoculating a studied isolate perpendicularly to a β-hemolysin-producing *Staphylococcus* strain [14,19]. Motility and establishing whether the isolate is lipophilic are also helpful [14]. MALDI-TOF represents a revolutionary technology, that rapidly became a routinely used tool in many microbiology laboratories, whereby specific bacterial proteins are ionized and detected by a mass spectrometer; then the generated spectrum is analyzed, and its pattern compared to entries found in a database, thus giving rise to a score matching species-specific profile.

There are limited studies evaluating the use of MALDI-TOF for *Corynebacterium* spp. identification, although findings are comforting [20–22]. Molecular genetic techniques for species identification of *Corynebacterium* strains include 16S rRNA gene and *rpoB* gene sequencing [14,17]. In this report, *C. glucuronolyticum* was correctly and consistently identified to the species level by using both MALDI TOF and 16S rRNA gene sequencing, thus confirming the former as a rapid and valuable system for identification of this species.

C. glucuronolyticum isolates have been frequently shown to be tetracycline-resistant and may also exhibit resistance to macrolides and lincosamides [23]. *C. glucuronolyticum* strains resistant to ciprofloxacin have been recently described [13]. Our strain resulted to be resistant, *in vitro*, to tetracycline, macrolides, and lincosamides, but susceptible to all other antibiotics tested and, particularly, the fluoroquinolone proven to be effective to treat the

infectious process described. Although *C. glucuronolyticum* is infrequently isolated, we strongly recommend that it should be considered as a potential cause of urogenital infection in male patients, and therefore always included in the etiologic diagnostic algorithm. In particular, the slow growth this species may display on agar media should be taken into account, as emphasized here, to reach a reliable diagnosis.

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