Ciprofloxacin-resistant *Corynebacterium glucuronolyticum* as a cause of male urethritis syndrome

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Introduction: *Corynebacterium glucuronolyticum* is a non-lipophilic coryneform species and a rare isolate from male patients with genitourinary tract infections. This organism is typically associated with urethritis or prostatitis syndrome, although it can also be isolated from other sites.

Case Presentation: We describe a case of urethritis in a 24-year-old Croatian male caused by a strain of *C. glucuronolyticum* showing resistance to ciprofloxacin in an agar dilution susceptibility test procedure. The same drug was used as an empirical treatment before microbiological evaluation was conducted. Upon identification of the organism, the patient was successfully treated with doxycycline, which resulted in complete regression of symptoms and lack of growth of the organism in culture.

Conclusion: Although uncommon detection of this organism hinders research efforts, *C. glucuronolyticum* should always be regarded as a possible cause of urogenital infections in male patients. In addition, antibiotic susceptibility testing should be performed due to possible resistance.

Keywords: agar dilution; antimicrobial susceptibility; ciprofloxacin; *Corynebacterium glucuronolyticum*; prostatitis; urethritis.

Introduction

Gram-positive bacteria in the genus *Corynebacterium* belong to the class Actinobacteria and are characterized with a high G+C content (Ventura et al., 2007). They comprise a collection of morphologically similar, non-sporing and often irregularly shaped aerobic microorganisms. Species in this genus are increasingly recognized as opportunistic pathogens, and a growing number of taxonomic studies have yielded a myriad of new species. Among them, species related to the urogenital tract are well represented. Phylogenetic analysis and rRNA gene sequencing of isolates from patients with prostatitis syndrome have revealed a distinct group within the genus *Corynebacterium* (Tanner et al., 1999). This group was believed to encompass two previously named species: *Corynebacterium seminale* and *Corynebacterium glucuronolyticum*. These names were proposed in 1995 by two independent research groups for strains isolated primarily from the human urogenital tract (Funke et al., 1995; Riegel et al., 1995). However, Funke et al. (1997) subsequently reported that the two species were in fact the same, and Tanner et al. (1999) demonstrated that 16S rRNA sequences of strains of *C. glucuronolyticum* and *C. seminale* isolated from patients with prostatitis had 96–97 % DNA homology. Although there are certain metabolic differences between the two (*C. seminale* strains do not hydrolyse esculin, whilst *C. glucuronolyticum* has this ability), Devriese et al. (2000) used genotypic characterization to show that they are actually the same species. Thus, nomenclatural precedence should be given to the name *C. glucuronolyticum*, as it was published first (Funke et al., 1995), and *C. seminale* can be considered a synonym for *C. glucuronolyticum*.

Case report

A 24-year-old, otherwise healthy Caucasian male without any known immune deficit developed urethritis syndrome...
characterized by urethral discharge, dysuria, urethral itching, mental erythema and mild pain in the lower abdomen. He was prescribed ciprofloxacin (CIP) empirically by his physician, but the oral treatment at a dose of 500 mg every 12 h, administered for a total of 10 days, yielded no improvement in his health status. As all the symptoms persisted, microbiological evaluation ensued.

A discharge specimen, urethral swabs and a semen specimen were taken for the diagnostic work-up. A Gram-stained smear of the urethral discharge revealed the presence of many polymorphonuclear leukocytes per oil immersion field (magnification ×1000) in five non-adjacent, randomly chosen fields on a smear. Gram-positive coryneform rods were also visible. Urethral swab specimens were collected by inserting and rotating a dry rayon swab with an aluminum shaft (Copan) 2 cm into the urethra. A VIDAS Enzyme Linked Fluorescent Assay (bioMérieux) for Chlamydia trachomatis and a Mycoplasma Duo kit (Bio-Rad) for Ureaplasma species and Mycoplasma hominis gave negative results. Wet-mount microscopy of semen specimen did not show the presence of Trichomonas vaginalis.

Cultivation of urethral swab and semen specimens on Blood Agar Base No. 2 (Oxoid) with 7% defibrinated sheep blood and chocolate agar at 36.7 °C in an aerobic atmosphere supplemented with CO₂ revealed prolific growth of white to slightly yellow, non-haemolytic colonies in pure culture. No other isolates were recovered after a 5 day incubation period. A Gram-stained smear of colonies showed Gram-positive bacilli in a distinctive ‘Chinese letters’ arrangement. A CAMP-test was positive, which was demonstrated as a CAMP reaction with staphylococcal β-haemolysin after the plate was incubated at 36.7 °C for 24 h. The microorganism was finally identified as C. glucuronolyticum using API Coryne (bioMérieux) with 99.9% certainty (biocode 3201705). The isolate was catalase, nitrate, urease, pyrazinamidase and β-glucuronidase positive and produced acid from glucose, ribose, xylose and sucrose.

As the empirically prescribed therapy was ineffective, susceptibility testing of CIP and other antimicrobial drugs was performed using an agar dilution method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2013). Because there are no CLSI-determined breakpoints for susceptibility testing of Corynebacterium species or other coryneform bacteria, Staphylococcus aureus ATCC 25923 was used as a control strain. The isolate was found to be resistant to CIP (MIC 32 μg ml⁻¹) and susceptible to penicillin (MIC 0.5 μg ml⁻¹), amoxicillin-clavulanic acid (MIC 0.06 μg ml⁻¹), cefazolin (MIC 0.25 μg ml⁻¹), cefuroxime (MIC 0.125 μg ml⁻¹), piperacillin-tazobactam (MIC 0.5 μg ml⁻¹), imipenem (MIC ≤0.06 μg ml⁻¹), gentamicin (MIC 0.25 μg ml⁻¹) and doxycycline (MIC 0.06 μg ml⁻¹). The patient was treated with doxycycline at 100 mg orally twice a day for 7 days, resulting in a complete regression of symptoms and the absence of bacterial growth upon culture of a urethral sample and semen specimen.

Discussion

C. glucuronolyticum is a non-lipophilic coryneform and a rare isolate from male patients with genitourinary tract infections, namely non-gonococcal urethritis and prostatitis (Winn et al., 2006). Although CIP-resistant C. glucuronolyticum strains have been described previously, this is the first clinical report of such a strain causing male urethritis syndrome in Croatia. Furthermore, the documented MIC value in this case was higher than in previously characterized strains with MICs for CIP not exceeding 16 μg ml⁻¹ (Funke et al., 1996). A similar case report with the same causative agent was described recently in Spain, but that specific isolate was susceptible to all tested antimicrobial drugs (Galan-Sanchez et al., 2011).

Although highly characteristic for the male genitourinary tract, C. glucuronolyticum has also been isolated from other sites. From 17 strains isolated by Funke et al. (1995), the majority (n=14) were from male patients with urethritis or prostatitis, two were from patients with infertility and one was from a blood culture of a patient with fever of unknown origin. C. glucuronolyticum was also found in blood and peritoneal fluid by Bernard et al. (2002), as well as in a woman with vaginosis by Devrieze et al. (2000). A recent article by Novo-Veleiro et al. (2013) emphasizes the importance of C. glucuronolyticum in paucisymptomatic monomicrobial infectious prostatitis as a cause of fever without an apparent origin.

C. glucuronolyticum is not found exclusively in humans; it is also associated with other mammalian hosts. It has been isolated in the semen of boars and in vaginal and uterine secretions of sows (Devrieze et al., 2000). In a study by Takahashi et al. (1997), the nucleotide sequence of Japanese swine isolate SC8 was found to be similar to that of C. glucuronolyticum, with approximately 0.01–0.02 evolutionary distances. As it resides in the same anatomical region as in humans (primarily in the urogenital tract), the pig could serve as a suitable animal model in future studies, although the clinical importance of C. glucuronolyticum in pigs and other suids is still unknown.

In Croatia, CIP is the most commonly administered drug in empirical therapy for urinary tract infection in males (Škert et al., 2009) and is often prescribed by urologists for empirical treatment of urethritis and prostatitis. As microbiological evaluation followed unsuccessful therapy with this fluoroquinolone drug, eventual concomitant infections cannot be ruled out. In addition, although the unavailability of nucleic acid amplification methods and lack of commercially available diagnostic tests precluded testing for Mycoplasma genitalium, a well-documented cause of acute and chronic urethritis in men, it is well known that this mycoplasma does not respond to treatment with doxycycline and tetracyclines (Falk et al.,
2003; Taylor-Robinson, 2008). Therefore, the possibility of infection with M. genitalium can be excluded indirectly.

Given the persistence of symptoms despite the initial fluoroquinolone therapy, isolation of the microorganism in pure culture and regression of symptoms after specific therapy based on antimicrobial susceptibility testing, C. glucuronolyticum can be considered as the probable cause of this patient’s urethritis. It is also unknown whether the treatment with CIP acted as a selective force that drove the development of resistance in this case. Hence, although infrequent detection of this organism hinders research efforts, C. glucuronolyticum should always be regarded as a possible cause of urogenital infection in male patients.

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References


