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CASE REPORT

A microbial game of whack-a-mole: clinical case series of the urethral uncloaking phenomenon caused by *Corynebacterium glucuronolyticum* in men treated for *Chlamydia trachomatis* urethritis

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Abstract
Corynebacterium glucuronolyticum is a rare and neglected, but increasingly recognized bacterial agent of infectious urethritis and other genitourinary syndromes in men. This is the first description of its proclivity to resurface as a cause of sustained urethritis in male patients previously treated for *Chlamydia trachomatis*, which represents a phenomenon that has to be differentiated from a simple post-treatment overgrowth of a colonizing agent.

Keywords Urethritis · Men · *Chlamydia trachomatis* · *Corynebacterium glucuronolyticum*

Introduction

Coryneform bacteria (often referred to as 'diphteroids') found in genitourinary tract are still generally regarded as saprophytes; however, their pathogenicity (even in immunocompetent individuals) may have been underestimated and underappreciated. *Corynebacterium glucuronolyticum* (*C. glucuronolyticum*) is a recognized and potentially pathogenic non-lipophilic coryneform bacterial species occasionally implicated in human infections [1–5]. Due to certain nomenclature precedence issues (that were subsequently resolved with the use of 16S rRNA sequencing), its synonym *Corynebacterium seminale* is still encountered in the medical literature [6, 7].

Although this bacterial species may be found in different sites, published studies reflect its specificity for male genitourinary tract. Different research groups interrogated its role in non-gonococcal urethritis (NGU) [1–3] and prostatitis [4–6], while the adverse influence of *C. glucuronolyticum* infection on semen parameters has also been investigated [7, 8]. In this case, series report previously unidentified urethral uncloaking phenomenon is described, where initially occult and undetected *C. glucuronolyticum* appears as the sole cause of lingering urethritis in men previously treated for *Chlamydia trachomatis* (*C. trachomatis*) urethritis—further establishing the protean pathogenic potential of this species in genitourinary tract infections of otherwise healthy male individuals.

Clinical case series description

Three Caucasian male patients that visited the outpatient clinic in Zagreb, Croatia within the observed 4-year period presented with signs and symptoms suggestive of urethritis syndrome. A 28-year-old (Patient 1) exhibited dysuria coupled with a light-green purulent urethral discharge; a 36-year-old (Patient 2) presented with and a clear urethral discharge, urethral itching, testicular tenderness and mild pain in the lower abdomen; a 21-year-old (Patient 3) presented with dysuria, severe burning sensation during urination, scanty cloudy discharge and meatal erythema. All of them reported heterosexual intercourse preceding these symptoms and were HIV negative.

Microscopic analysis of the first-void urine sediment and a Gram-stained smear of the urethral discharge revealed the presence of more than 10 polymorphonuclear leukocytes per high-power field and more than 2 polymorphonuclear leukocytes per oil immersion field, respectively. A thorough microbiological evaluation of

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urethral swabs, semen and urine specimens ensued with the use of molecular and culture methods. From the clinical perspective, a rectal pain was suspected in Patient 2 as well, thus digital rectal prostate exam was pursued, but was uneventful.

In all three patients, a sole infection with *C. trachomatis* was confirmed with the use of the Hybrid Capture 2 technology (HC2) (Digene, Gaithersburg, MD, USA)—a chemiluminescent signal amplification assay that employs an RNA probe to target DNA, subsequently detecting the resulting RNA:DNA hybrid with antibodies. The presence of other sexually transmitted causes of urethritis (such as *Neisseria gonorrheae, Mycoplasma genitalium* and other genital mycoplasmas, *Trichomonas vaginalis*) and aerobic urogenital pathogens were excluded by molecular and culture methods, and the prolonged cultivation time was also pursued to foster the growth of coryneform and fastidious bacteria.

In accordance with the findings, all three patients were treated with oral doxycycline in two divided doses (i.e., 100 mg every 12 h) for 7 days, and then were reevaluated at therapy completion. In Patient 1, urethral discharge vanished, but mild dysuria persisted; in Patient 2, urethral discharge disappeared (although it could have been elicited after milking the urethra) alongside abdominal/testicular pain, but urethral discomfort persisted; in Patient 3 cloudy discharge and burning sensations disappeared, but dysuria was still present. As microscopic analysis of the first-void urine sediment again revealed an increased number of polymorphonuclear leukocytes, a complete microbiological evaluation was repeated.

This time, chlamydia testing yielded negative results, and the only isolate detected in all three cases was revealed in cultures of the urethral swabs using Blood Agar Base Number 2 (Oxoid) with 7% defibrinated sheep blood and chocolate agar at 36.7 °C in an aerobic atmosphere supplemented with CO_2. There was an abundant growth of non-hemolytic, white to slightly yellow colonies in monoculture that were absent during the initial work-up, showing a positive CAMP test (i.e., the enhanced hemolysis feature) the following day. Gram-stained smeared colonies demonstrated Gram-positive bacilli in 'Chinese letters' formation, highly suggestive of coryneform bacteria, which were visible even on the direct Gram-stain from the follow-up urethral swab.

The isolates from Patient 1, 2 and 3 were finally identified as *C. glucuronolyticum* by the use of analytical profile index biotyping strip system API Coryne (bioMerieux, Marcy-l'Etoile, France) with 99.9% (biocode 2200725), 99.8% (biocode 3200325) and 99.9% (biocode 3201705) certainty, respectively. All three isolates were additionally confirmed by matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry (MALDI-TOF MS) (MicroflexTM Maldi Bio-typer MS, Bruker-Daltonik, Fremont, CA, USA; software used: MALDI BIOTYPER version 3.1., Build 65).

Antimicrobial susceptibility was performed on all *C. glucuronlyticum* isolates using agar disc diffusion or Kirby–Bauer method, in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines and breakpoint tables. All isolates were susceptible to penicillin G, vancomycin, gentamicin and rifampicin; however, isolate from Patient 2 was resistant to ciprofloxacin, isolate from patient 3 was resistant to clindamycin, and all three isolates were resistant to tetracycline.

Due to aforementioned tetracycline resistance in agar disc diffusion, broth dilution susceptibility test was additionally performed for doxycycline (according to the Clinical and Laboratory Standards Institute guidelines or CLSI) that revealed increased minimal inhibitory concentrations (MIC) interpreted as resistant (MICs for Patient 1, 2 and 3 were 32, 64 and 32 µg/mL, respectively). After obtaining susceptibility testing results, a repeated patient treatment was administered according to the *C. glucuronolyticum* respective antibiograms, which resulted in complete resolution of symptoms. Microbiological clearance was demonstrated 1 week, 1 month and 2 months post treatment.

Discussion

This study confirms the role of *C. glucuronolyticum* in male urethritis syndrome, and further refines its potential to resurface after successful treatment of some other pathogenic causes of urethritis (in this case *C. trachomatis*) in an uncloaking fashion. It is evident from the antimicrobial susceptibility testing results that all three strains of *C. glucuronolyticum* were resistant to the first-line treatment administered for chlamydial infection (i.e., doxycycline), and indeed some other studies already described the trend of this species towards increased resistance to fluoroquinolones, tetracyclines and lincosamides [1, 2, 7, 8].

However, the pertinent question is why *C. glucuronolyticum* was not identified as a co-infecting agent alongside *C. trachomatis* in the first place (although a full spectrum of microbiolocal methods has been used), as it is hard to envision a competitive relationship between these two vastly different bacterial agents. The answer may be hidden in the intricate nature of the local immune system. It is known that human α-defensin 5 (HD5) is upregulated in the human urethra during the infection with *C. trachomatis* [9], which is far more efficient in clearing the infection with extracellular pathogens than intracellularly located chlamydia [9]; thus it may adversely affect the population of *C. glucuronolyticum* indirectly. As *C. trachomatis* can also alter the local cytokine profile, further research is definitely warranted to adequately address this issue.

In any case, a refinement of the clinical terminology describing specific clinical entities of urethritis and its

Table 1 Proposed classification of clinical urogenital syndromes in men caused by *Corynebacterium glucuronolyticum*

Anatomic locale	Clinical syndrome	Description	Reference
Urogenital region	Colonization	Putative organism isolated from different urogenital specimens without any signs or symptoms of the infection, as well as without any adverse effects on semen parameters	[8, 11]
	Asymptomatic infection	Putative organism isolated from urogenital specimens in patients without any symptoms, but with increased number of polymorphonuclear leukocytes. There is also a possibility of adverse influence on semen parameters	[7, 8]
Urethra	Acute symptomatic urethritis	Frank urethritis presenting with dysuria, urethral discharge and/or urethral irritation/itching. An increased number of polymorphonuclear leukocytes is also evident at microscopy	[1–3]
	Uncloaking urethritis	Sustained urethritis predominantly presenting with mild dysuria and scarce urethral discharge after the successful treatment of urethral infection with *Chlamydia trachomatis* (or potentially other bacterial causes of frank urethritis). An increased number of polymorphonuclear leukocytes is also evident at microscopy	This study
Urinary bladder	Simple cystitis	A combination of urinary frequency, urgency, dysuria, cloudy urine, and/or low-grade fever	[12]
	Encrusted cystitis	Encrustration of the urinary bladder mucosa with related chronic inflammation	[10]
Prostate gland	Paucisymptomatic prostatitis	Prolonged fever (> 38 °C) without an apparent origin, minor genitourinary alterations (urethral stinging and/or urinary frequency and/or organoleptic alterations of the semen), and positive Meares–Stamey four-glass test	[5]
	Chronic bacterial prostatitis	Irritative voiding symptoms, recurrent urinary tract infection, testicular/low back/perineal pain, and positive Meares–Stamey four-glass test	[4]

related manifestations may follow. Persistent urethritis (no substantial improvement within 1 week after initiating treatment for NGU) and recurrent urethritis (occurring within 6 weeks of an antecedent episode of NGU) are frequently used definitions that usually entail infections with the same causative agent. However, 'uncloaking urethritis' may be introduced to the medical literature to refer to the urethral uncloaking phenomenon and pathogen switch described in this paper, of course, if adequately supported by further studies and described for some other combinations of microorganisms as well.

Moreover, although *C. glucuronolyticum* is a rare isolate, it should be considered as a potential cause of genitourinary infections in male patients, and thereby incorporated in the diagnostic algorithm. To facilitate this effort, this paper also proposes the first classification of clinical urogenital syndromes in men caused by *C. glucuronolyticum* (Table 1), as hitherto no such endeavors were pursued. The classification is completely based on the available literature and can serve as a primer for approaching patients—especially those with certain distinctive clinical entities such as encrusted cystitis [10], paucisymptomatic prostatitis [5] or uncloacking urethritis. The latter phenomenon (described here) implies the need for thorough microbiological evaluation, even during control testing, as simple post-treatment colonization or overgrowth usually does not present with the symptoms of urethritis, nor respond so successfully to the specific treatment.

Nonetheless, the exact instances when the typical commensals (such as corynebacteria) of the male genitourinary tract may act as pathogens signify a good question without a good answer, as nicely stated by Türk et al. [6]. A first step towards establishing whether there is a pathogenic role of certain *Corynebacterium* species and to discriminate potentially infective ones from harmless colonizers strictly depends on accurate identification to the species level. Biochemical methods are still commonly employed for that purpose, but there is an increasing need for MALDI-TOF technology or sequence-based molecular identification methods. And as these technologies are still not omnipresent in many institutions dealing with the infections of genitourinary tract, clinical savviness and astute diagnostic reasoning (such as fulfilled urethritis criteria like in the presented case) is needed when confronted with corynebacteria in urogenital specimens.

Compliance with ethical standards

Conflict of interest The author declares that there is no competing interest.

Ethical approval The study describes clinical and diagnostic procedure of a specific case. All procedures performed were in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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