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Microbiological and clinical features of *Corynebacterium urealyticum*: urinary tract stones and genomics as the Rosetta Stone

F. Soriano[1] *and A. Tauch*[2]

[1]Department of Medical Microbiology and Antimicrobial Chemotherapy, Fundación Jiménez Díaz, Madrid, Spain and [2]Institut für Genomforschung und Systembiologie, Centrum für Biotechnologie, Universität Bielefeld, Bielefeld, Germany

ABSTRACT

Corynebacterium urealyticum, formerly known as coryneform CDC group D2, was first recognized to be involved in human infections 30 years ago. It is a slow-growing, lipophilic, asaccharolytic and usually multidrug-resistant organism with potent urease activity. Its cell wall peptidoglycan, menaquinone, mycolic and cellular fatty acid composition is consistent with that of the genus *Corynebacterium*. DNA–DNA hybridization studies and 16S rDNA sequencing analysis have been used to determine the degree of relatedness of *C. urealyticum* to other corynebacterial species. The genome of the type strain consists of a circular chromosome with a size of 2 369 219 bp and a mean G + C content of 64.2%, and analysis of its genome explains the bacterium's lifestyle. *C. urealyticum* is a common skin colonizer of hospitalized elderly individuals who are receiving broad-spectrum antibiotics. It is an opportunistic pathogen causing mainly acute cystitis, pyelonephritis, encrusted cystitis, and encrusted pyelitis. More infrequently, it causes other infections, but mainly in patients with urological diseases. Infections are more common in males than in females, and treatment requires administration of antibiotics active against the organism *in vitro*, mainly glycopeptides, as well as surgical intervention, the latter mostly in cases of chronic infection. Mortality directly associated with infection by this organism is not frequent, but encrusted pyelitis in kidney-recipient patients may cause graft loss. The outcome of infection by this organism is reasonably good if the microbiological diagnosis is made and patients are treated appropriately.

Keywords *Corynebacterium urealyticum*, epidemiology, genome, infections, lifestyle, pathogenicity, susceptibility, treatment

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HISTORY

Isolation of urease-positive coryneforms from urine samples in cases of encrusted cystitis was reported in 1945 [1] and later [2], but such corynebacteria were not well characterized.

A non-spore-forming, aerobic and Gram-positive bacillus with strong urease activity was described by King in 1972 as coryneform CDC group D2 [3]. In 1979, Jacobs and Perlino described a case of pneumonia in a debilitated patient from whom such an organism was isolated [4]. This organism was implicated for the first time in urinary tract infections (UTIs) in a report published in 1985 [5] concerning four patients with alkaline encrusted cystitis. In the same year, eight cases of UTI caused by this organism were also reported [6], and 2 years later, a report concerning 43 patients with asymptomatic bacteriuria, cystitis, acute pyelonephritis with bacteraemia, encrusted cystitis and wound infection was published [7]. These results were expanded and confirmed 3 years later in the largest series reported so far [8].

After different taxonomic studies, it was concluded that coryneform CDC group D2 was a true *Corynebacterium* species, different from all others known, and it was named *Corynebacterium urealyticum*, stressing its strong ability to split urea [9]. Recently, the complete genome sequence of

Corresponding author and reprint requests: F. Soriano, Department of Medical Microbiology and Antimicrobial Chemotherapy, Fundación Jiménez Díaz, Avenida de Reyes Católicos 2, 28023 Madrid, Spain
E-mail: fsoriano@fjd.es

C. urealyticum has been determined [10]. Interpretation of genome sequences requires a 'Rosetta Stone' to decode biological information in the finest detail, just as the deciphering of the ancient Egyptian hieroglyphics required the original Rosetta Stone. The combination of genomics and bioinformatics constitutes a discipline that has now been applied to understanding the genome of a stone-forming bacterial pathogen.

TAXONOMIC FEATURES

The taxonomic classification of *C. urealyticum* (CDC coryneform group D2) was originally based on biochemical characteristics [9,11,12]. The cell wall peptidoglycan of *C. urealyticum* contains *meso*-diaminopimelic acid, and arabinose and galactose are the major cell wall sugars. The major menaquinone is MK-9(H_2). Tuberculostearic acid and short-chain mycolic acids (C_{26}–C_{36}) are present [11,13]. Members of the species *C. urealyticum* are characterized by their potent ability to hydrolyze urea [5,14] and by their failure to produce acid from carbohydrates [9,14]. Moreover, DNA–DNA hybridization studies [9,12] and 16S rRNA sequence analysis [15,16] were used to determine the degree of relatedness of *C. urealyticum* to other corynebacterial species. On the basis of small-subunit rRNA sequence data, a close phylogenetic relationship between *C. urealyticum* and *C. jeikeium* was observed [15,16]. More recently, this analysis was complemented by using partial sequences of the RNA polymerase beta-subunit gene (*rpoB*) in phylogenetic studies of corynebacteria [17,18]. Both *C. urealyticum* and *C. jeikeium* are strict aerobes that exhibit lipid-requiring and multidrug-resistance phenotypes [19] and belong to a separate branch in the genus *Corynebacterium* [16,17]. However, *C. jeikeium* can be differentiated from *C. urealyticum* because it is non-urealytic and produces acid from glucose [19,20].

A recent study demonstrated that cellular fatty acid analysis is a powerful method for the reliable identification and differentiation of corynebacterial species [21]. The cellular fatty acid composition of *C. urealyticum* is consistent with that of the genus *Corynebacterium*, with the majority of cellular fatty acids being of the straight-chain, monounsaturated types [11,21]. In addition, small amounts of tuberculostearic acid were consistently detected in several studies [11,13,21]. A comparative study on the phospholipid composition of *C. urealyticum*, *C. jeikeium* and *C. amycolatum* strains revealed significant differences related to the acyl chains of the glycerol moiety of these compounds, and most notably, the presence of high levels of a 10-methyleneoctadecanoyl moiety in the detected inositol-containing phospholipids of *C. urealyticum* [22].

DNA–DNA hybridization studies for intraspecies comparison revealed tight hybridization groups of *C. urealyticum* strains that were distinct from validly established species [9,12]. The high levels of interstrain DNA homology were consistent with the criterion of a homogenous species. Ribotyping (rRNA gene restriction length polymorphism) of *C. urealyticum* strains from urine samples and skin infections was used for epidemiological tracking [23]. The *C. urealyticum* isolates were remarkably homogenous and could be assigned to eight clusters only, despite the deliberate use of strains from a variety of sources. Comparison by ribotyping of *C. urealyticum* strains from human-related and animal-related sources revealed predominant ribotypes for each group of isolates [24]. Some ribotypes were found only in strains from human-related sources, whereas others were obtained only from isolates from animal-related sources. Two isolates with different antimicrobial susceptibilities exhibited an identical ribotype, indicating that the genotypic profile is more stable than the antibiogram [24].

THE GENOME SEQUENCE OF *C. UREALYTICUM* DSM7109

Very recently, the complete genome sequence of the type strain *C. urealyticum* DSM7109 (NCTC 12011; ATCC 43042) [9] has been determined by a combination of ultrafast pyrosequencing and Sanger technology [10,25]. The genome of *C. urealyticum* DSM7109 consists of a circular chromosome with a size of 2 369 219 bp and a mean G + C content of 64.2%, which is close to the value of 62% that was determined previously by the thermal DNA denaturation method [9]. Genome annotation revealed 2024 protein-coding sequences, of which 78% were considered to be orthologues of genes in the *C. jeikeium* K411 genome. The close phylogenetic relationship between *C. urealyticum* and *C. jeikeium* is also reflected by the highly conserved order of

orthologous genes and the presence of only two breakpoints in the architecture of the chromosome [10].

Metabolic analysis of the genome sequence provided clear evidence that the lipid-requiring ('lipophilic') phenotype of *C. urealyticum* is attributable to the absence of a microbial type I fatty acid synthase gene and thus represents a fatty acid auxotrophy [10]. In addition, *C. urealyticum* is apparently unable to utilize sugars as carbon and energy sources because of the absence of characteristic sugar uptake systems and the absence of genes for anaplerotic functions. Accordingly, exogenous fatty acids are required not only to supplement the fatty acid auxotrophy, but also to serve as sources of carbon and energy for *C. urealyticum*. The utilization of exogenous fatty acids occurs via the β-oxidation pathway and a large repertoire of auxiliary genes involved in uptake and degradation of structurally diverse fatty acid compounds [10]. This comprehensive gene repertoire reflects the adaptation of *C. urealyticum* to those habitats on the human skin that provide an appropriate amount of exogenous fatty acids for growth.

The urease genes of *C. urealyticum* DSM7109 are organized in a cluster with the order *ureABCEFGD*, and seem to play the dominant role in the pathogenicity of this species [10]. A remarkable feature of the *C. urealyticum* DSM7109 genome sequence is the absence of genes for potential transcription regulators of the urease gene locus [26], suggesting that the lack of efficient transcriptional control contributes to the strong urease activity of *C. urealyticum* strains [10].

Adherence of *C. glutamicum* to host tissues may be mediated by a proteinaceous pilus that is covalently anchored to the cell wall and encoded by the structural genes *spaDEF* and the cognate sortase genes *srtBC* in *C. urealyticum* DSM7109 [10]. Alternatively, only the minor pilin SpaB might be covalently anchored to the cell wall in the absence of a pilus shaft to provide tight contact between the *C. urealyticum* cell and the host tissue [10,27,28].

Moreover, the *C. urealyticum* DSM7109 genome contains two genes (*surA* and *surB*) for cell surface proteins that display homologies to biofilm-associated proteins from *Staphylococcus epidermidis* [10]. In particular, the structural similarities and potential variations in the number of internal repeat units among different *C. urealyticum* strains suggested that these proteins contribute to an evasion of the immune system and to the formation of biofilms on medical devices, such as urinary catheters. The predicted surface proteins SurA and SurB may thus play important roles in UTIs in catheterized patients.

ANTIMICROBIAL SUSCEPTIBILITY

The susceptibility pattern of *C. urealyticum* with respect to antimicrobial agents varies among isolates obtained from different sources [24]. *C. urealyticum* strains from humans and human-related sources were more frequently resistant to ampicillin, gentamicin, norfloxacin, erythromycin and rifampin than were isolates from animals and animal-related sources. The higher rate of antibiotic resistance in strains from humans and human-related sources may be due to selective pressure of antibiotics in the human environment and especially the hospital setting [24,29]. The majority of *C. urealyticum* strains obtained from clinical samples typically display multiple resistance to antibiotics [19,30,31]. *C. urealyticum* is normally highly resistant to β-lactams and aminoglycosides, and variably susceptible to fluoroquinolones, macrolides, ketolides, rifampin, and tetracyclines [5,32–44]. *C. urealyticum* strains are, however, uniformly susceptible to the glycopeptides vancomycin and teicoplanin [5,31,35,37,39,41,43]. The psychiatric drug sertraline significantly enhanced the activity of ciprofloxacin and tetracycline against *C. urealyticum* [45].

C. urealyticum isolates were previously found to be susceptible to ofloxacin, norfloxacin, and ciprofloxacin [32,33,46], but more recent studies revealed an increased level of resistance to fluoroquinolones [35,36,41,43,47,48]. A recent study indicated that only 20.3% of the tested *C. urealyticum* isolates (64 strains) were susceptible to ciprofloxacin [43]. Newer fluoroquinolones, such as clinafloxacin, gemifloxacin, levofloxacin, and ofloxacin, are more effective *in vitro* than ciprofloxacin and norfloxacin, but may remain ineffective against high-level ciprofloxacin-resistant isolates [40,41,43,48]. Likewise, the ketolide telithromycin is more active *in vitro* than the macrolide erythromycin, but only against erythromycin-susceptible and erythromycin-intermediate isolates [39,40,43], whereas the ketolide cethromycin (ABT-773) is only poorly active

against *C. urealyticum* [42]. On the other hand, newer antibiotics, such as linezolid and quinupristin–dalfopristin, proved to be effective *in vitro* against *C. urealyticum* strains [39,43,44]. These antibiotics may be useful alternatives to glycopeptides in the treatment of *C. urealyticum* infections caused by multidrug-resistant strains.

An investigation of external factors contributing to antimicrobial resistance in *C. urealyticum* strains from hospitalized patients revealed that the use of antibiotics could favour the appearance of multiresistant strains, which were likely to be acquired directly from the bacterial flora present in the hospital environment [29]. The annotated genome sequence of *C. urealyticum* DSM7109 provided clear evidence that horizontal gene transfer is the main cause of multiple resistance to antibiotics [10], as the detected resistance genes are apparently part of (defective) mobile genetic elements (Table 1). Highly similar, or even identical, antibiotic resistance determinants are well known in other pathogenic and non-pathogenic corynebacteria [49–54], and are collectively present on the large multiresistance plasmid pTP10 from *C. striatum* M82B [50]. Functional analysis of the respective genes demonstrated that they confer high levels of resistance to aminoglycosides, macrolides, lincosamides, telithromycin, chloramphenicol and tetracyclines in corynebacteria [50,55,56] and cutaneous propionibacteria [57]. The repeated detection of the same set of mobile antibiotic resistance genes in several sequence analyses (Table 1) indicated that these determinants play a prominent role in conferring multidrug resistance in corynebacteria [49–54].

EPIDEMIOLOGY

C. urealyticum is the corynebacterium most frequently isolated from clinical specimens, provided that urine specimens are adequately processed. The incidence of *C. urealyticum* varies from 0.016% to 0.32% [7,46,58] among all urine samples sent for routine culture. The rates of detection may be increased by a factor of 3–30 if selective media are used, but even those rates would not not be clinically relevant [30,46,59–61].

C. urealyticum has been isolated from the skin of 25–37% of healthy elderly individuals, predominantly females [62]. This organism has also been detected in the air of hospital wards housing colonized and non-colonized patients but rarely in other dependencies [63]. A possible hospital outbreak of infection by *C. urealyticum*, affecting 15 elderly patients with either an intravesical catheter or a urinary collector, has been described [64].

PATHOGENICITY

C. urealyticum is frequently isolated from the groin in elderly patients receiving broad-spectrum antibiotics [62], and this circumstance may favour colonization of urinary catheters. It has also been shown that *C. urealyticum* adheres quite efficiently to urinary catheters [65], so this organism may infect the bladder mucosa, especially if there is an underlying disorder (trauma, tumour, or inflammation). Strong adherence to uroepithelial cells has been demonstrated [27], and in bladder biopsies the organism has been seen completely embedded within the mucosa [5,8].

Table 1. Antibiotic resistance genes detected in the complete genome sequence of *Corynebacterium urealyticum* DSM7109

Gene	Gene product or function	Mobile genetic element	Resistance spectrum determined in corynebacteria or propionibacteria	Gene(s) previously detected in
erm(X)	23S rRNA adenine *N*-6-methyltransferase	Tn*5432*-like	Azithromycin, clindamycin, erythromycin, josamycin, lincomycin, midecamycin, pristinamycin I_A, roxithromycin, spiramycin, telithromycin, tylosin	*C. striatum*, *C. jeikeium*, *C. diphtheriae*, cutaneous propionibacteria
aph(3′)-Ia	Aminoglycoside-3′-phosphotransferase	Tn*5715*-like	Kanamycin, lividomycin, neomycin, paromomycin, ribostamycin	*C. striatum*
strA–strB	Aminoglycoside-3″-phosphotransferase and aminoglycoside-6-phosphotransferase	Tn*5393*-like	Streptomycin[a]	*C. striatum*
cmx1/cmx2	Chloramphenicol exporter of the major facilitator superfamily	Tn*45*-like	Chloramphenicol	*C. striatum*, *C. jeikeium*, *C. glutamicum*
tetA–tetB	Tetracycline exporter of the ATP-binding cassette superfamily	Tn*3598*-like	Tetracycline, oxytetracycline	*C. striatum*

[a]Not verified experimentally.

C. urealyticum could also reach the renal pelvis through a nephrostomy catheter [7] or by an ascendant route [66].

Once the organism adheres to the urinary tract, it grows under the stimulation of the urea present in the urine. The urease leads to hyperammoniuria and alkalinization of the urine, which causes hypersaturation with struvite and calcium phosphate with consequent crystallization of struvite and apatite. These types of urinary stone have been experimentally reproduced *in vitro* [67,68] and *in vivo* [66,67]. In the same experimental model, it was shown that bladder instillation of *C. urealyticum* can produce both encrusted cystitis and pyelitis [66].

In addition to stone production and obstructive complications, urease is also considered to be responsible for an ammonia-induced cytotoxicity for the renal epithelium. Therefore options to neutralize such effect with acetohydroxamic acid (AHA) have been investigated. *In vitro* studies have shown that this drug is able to neutralize the urease of *C. urealyticum*, thereby preventing the formation of struvite crystals [68] and, as some antibiotics are less active at alkaline pH, compounds such as AHA may also act synergistically with antibiotics [33].

RISK FACTORS

Most UTIs caused by *C. urealyticum* occur mainly in immunocompromised patients with underlying urological disease, patients who have undergone urological manipulations, those with a history of previous UTIs, those hospitalized for long periods, and those treated with broad-spectrum antibiotics [7,8].

Immunosuppression and kidney transplantation are risk factors for developing pyelonephritis. Urological diseases, mainly of the bladder, prolonged vesical catheterization, and previous UTIs in patients with chronic debilitating conditions appear to be the most important risk factors for the development of encrusted cystitis. Bladders damaged by trauma, tumour or intravesical administration of cytotoxic drugs are frequently the main risk factors for developing encrusted cystitis [7,8].

Risk factors for encrusted pyelitis are kidney transplantation, reparative urological surgery after transplantation, and the use of a pig-tail ureteral catheter for more than 1 month [69].

UTIs, including pyelonephritis and encrusted cystitis, may occur at any age (with a mean age of 55–65 years). On the other hand, encrusted pyelitis usually occurs at an earlier age (with a mean age of 40–45 years). It is of interest that all UTIs caused by this organism are more frequent in males than in females. Infections outside the urinary tract occur at any age, predominantly also in males, and more than 50% of the cases occur in patients with urological diseases. The higher incidence of *C. urealyticum* infections in males than in females contrasts with the various rates of skin colonization by corynebacteria found in the two sexes [62].

CLINICAL INFECTIONS

Urinary tract infections

Bacteriuria due to *C. urealyticum*, if properly investigated, is very common, although true symptomatic infections occur in fewer than 60% of the cases [8]. UTIs can be acute or chronic, and treatments and outcome may differ.

Acute urinary tract infections.
The more common clinical picture is acute cystitis, although in many reported cases no differentiation between cystitis and pyelonephritis was made [6–8,14,30,46,58,64,69–71]. These patients suffer from dysuria or urethral discomfort (in catheterized patients), and frequency and urgency of urination. Less often, patients have suprapubic pain or macroscopic haematuria, and only occasionally is gritty material in the urine or low-grade fever recorded [7,8].

Pyelonephritis was diagnosed in 11 patients, with simultaneous isolation of the organism from urine and blood or from urine obtained through a perirenal catheter, as well as in seven other patients where the organism was isolated only from urine [8]. Nearly 80% of these patients were immunosuppressed. Three bacteraemic patients were seriously immunosuppressed (leukaemia in two cases and AIDS in one) and had no previous renal or urological diseases. Eight patients were renal transplant recipients and seven others had previous urological diseases and were older than 60 years [8]. In addition, one case of pyelonephritis was described in a 75-year-old male patient with bladder carcinoma who underwent cystectomy and nephrostomy [72], and one in a 49-year-

old male patient without urological disease or manipulation [73].

Chronic urinary tract infections.

One of the most frequent chronic infections caused by *C. urealyticum* is encrusted cystitis. This is a chronic inflammatory condition with localized ulcerative inflammation of the bladder, with deposits of ammonium magnesium phosphate on the surface and on the walls of the ulcer [74]. This disease is associated with infection by a urea-splitting organism in a bladder that already harbours an inflammatory or neoplasic lesion. Such inflammation produces a fibrotic and retractile bladder with reduced capacity, which may produce stenosis of the ureteral meatus, leading to dilatation of the upper urinary tract. This is not a life-threatening disease, but is a tremendously painful and disturbing condition. An expert urologist has stated: 'si la cystite incrustée n'est pas un arrêt de mort, c'est le supplice à perpétuité' [74]. It is thus not surprising that some patients with this condition become suicidal. Encrusted cystitis causes long-lasting symptoms in the lower urinary tract, with frequent relapses [1,2,5,7,8,30,46,58,69,75–90]. Immunosuppression is usually less frequent than in patients with acute infections, but most patients have experienced previous bladder diseases and have undergone urological manipulations. The patients usually suffer from dysuria, suprapubic pain, haematuria, elimination of gritty material or struvite stones with the urine, turbid urine, and, frequently, an ammonia-like odour of the urine. The urine is usually alkaline, with struvite crystals, pyuria, and haematuria [8]. Plain radiography can demonstrate bladder calcifications. Two-dimensional echography of the bladder can show hyperechogenic foci, and the uroscanner can show calcified plaques in the bladder. It is also possible to demonstrate, in some cases, ureterohydronephrosis [88,90] by echography or intravenous pyelography. Encrusted cystitis is usually confirmed by cystoscopy, which, at the same time, allows the surgical resection of the encrusted lesions. Bladder biopsies from patients with encrusted cystitis usually have an ulcerous necrotic appearance with crystals encrusted in the chorion and frequent microcolonies of *C. urealyticum* [5,7,8].

Another serious chronic infection is encrusted pyelitis [69,82,85,86,91–98], which has been mainly described in renal transplant recipients [69,82,85,86,93–95], but also in native kidneys of patients with urological diseases [95–97]. It is a severe event that can destroy the renal graft of transplanted patients [69,82,85,86,94,98]. The disease is characterized by the presence of struvite encrustations on the renal pelvis wall, observed when the pelvis is open during a urological intervention [69]. Plain radiography can demonstrate unusual calcifications in the kidneys and ureters. Computed tomography can show calcifications of the pelvis and ureters. Ultrasound examination of the kidneys may detect hyperechogenic material in the pelvis and also hydronephrosis. Intravenous pyelography can show calcifications, pyelocalicilial dilatation, and cortical hypotrophy. These patients present with UTI symptoms, pyuria, haematuria, and ammonium magnesium phosphate crystals in their urine [69]. The kidneys and pyelocalicilial cavities are usually dilated, with capsular adherences and necrotic areas being encrusted with multiple calcifications [69,82]. Patients with encrusted pyelitis may develop obstructive uropathy with deterioration of renal function, pyelonephritis, or renal abscesses [97]. Another severe complication is ureteric stricture, which also requires surgical repair [94,95]. Encrusted pyelitis may also be complicated by encrusted cystitis [69,82,86]. One debilitated patient developed encrustations of the entire upper (pyelitis and ureteritis) and lower (cystitis) urinary tract and died [84]; another patient developed shock and died [97].

Other infections

There are 11 well-documented cases of bacteraemia caused by *C. urealyticum* [8,69,73,99–101]. The mean age was 52.9 years, and there were seven males, two females and two patients of unknown gender; in seven cases, there were urological disorders [8,69,73,99]. Four patients had leukaemia [73,100,101] and one had AIDS [8].

Three patients had endocarditis, two on native valves [75,102] and one on a prosthetic valve [103]. One native valve endocarditis occurred in a drug addict and the other in a patient who developed encrusted cystitis caused by *C. urealyticum* and died [75]. The third patient had a valvular prosthesis and an aortic–coronary bypass, developed endocarditis unrelated to urinary tract disorders, and died.

Twelve other kinds of infection have been described: four wound infections [100], two localized renal infections [104,105], and one each of peritonitis [14], pericarditis [106], encrusted urethritis [107], osteomyelitis [108], pneumonia [4], and soft tissue infection [109]. Six of the patients had urological disorders [14,100,104,105,107] and two were neutropenic, with breast cancer and acute leukaemia [100,109]. It is of interest that there were urological disorders [8,14,69,73,75,99,100,104,105,107] in 14 of the 26 above-mentioned infections (54%).

MICROBIOLOGICAL DIAGNOSIS

C. urealyticum can be isolated after culture in several media and mainly on blood agar plates. Growth in pure culture, or as predominant flora, especially if seen with polymorphonuclear cells in Gram-stained samples, is of great value.

Most *C. urealyticum* isolates are missed in routine urine cultures because the organism does not grow after 'overnight' incubation. Therefore, incubation should last longer than 24 h when patients are symptomatic or have alkaline urine or struvite crystals in their urine sediment.

The organism grows on blood agar as pinpoint colonies after 48 h of incubation at 35–37°C. Colonies are whitish, opaque, smooth, convex, circular, entire, and non-haemolytic [3,5,9,19]. At the time of writing, *C. urealyticum* is the only known *Corynebacterium* species that is lipophilic, asaccharolytic, and strongly urease-positive [19]. The final identification can be easily performed by phenotypic studies [5,19,36,37], using home-made media or commercial systems [110].

In a case of encrusted cystitis with routine urine culture being negative, DNA of *C. urealyticum* was detected by PCR [83]. A culture-independent, 16S rRNA gene-based approach has been used to identify this organism in clinical specimens. Amplicons of the 16S rRNA gene encompassing the V6–V8 regions have been analyzed with denaturing HPLC, and the identities of distinct peak profiles were confirmed by sequencing individually collected peak products [111]. The RNA polymerase beta-subunit-encoding gene (*rpoB*) can also be used for identification of *Corynebacterium* species [17]. Finally, another molecular technique useful for difficult-to-identify organisms, including coryneforms, is rRNA gene sequencing, because of the universal distribution of, and the presence of, species-specific variable regions in the genes [112,113].

TREATMENT AND OUTCOME

In a large published series, 96% of the patients receiving an adequate antibiotic were cured or improved, whereas only 35% (mostly with acute cystitis) improved with an antibiotic considered not to be active against *C. urealyticum in vitro* [8]. Glycopeptides [6–8,69,71,73], mainly vancomycin, tetracyclines [6,7,58,72], and fluoroquinolones [7,8,58], have been used with great success in many patients with UTIs. In some acute UTIs, AHA has been concurrently administered [8,71].

Most cases of encrusted cystitis have required surgical resection of the bladder encrustations, as well as prolonged (more than 1 month) antimicrobial treatment. Antibiotics successfully used in this condition have been glycopeptides [7,8,69, 76–79,83,85,89], tetracyclines [5,7,8,58,83], and fluoroquinolones [7,8,58,77,80,90]. Teicoplanin has been successfully used in two patients with encrusted cystitis caused by *C. urealyticum*, even without cystoscopic resection of the stones, which were spontaneously eliminated [79]. AHA has been concurrently used in several patients with encrusted cystitis [8,77,78]. Oral acidification of the urine has also been recommended as an adjuvant therapy [5,90].

The outcome in patients with encrusted pyelitis receiving adequate treatment, especially with glycopeptides [69,85,93–96], has been favourable. However, a non-transplanted 70-year-old patient treated for bladder carcinoma developed encrusted pyelitis and pyelonephritis and, while receiving vancomycin combined with fusidic acid, ureteral catheterization and nephrostomy, suffered septic shock and died [97]. AHA has also been used in this condition [93,95,96]. Ureterostomy and graft removal have also been necessary for the management of similar conditions [69,82,85,86,94,98]. Five renal transplant recipients developed encrusted pyelitis (four cases) and encrusted cystitis (one case), and all were successfully treated with vancomycin, but all patients required a derivative procedure or a surgical resection of the encrustations to improve [69]. Six patients were treated with vancomycin, percutaneous acidification of the renal collecting system for chemolysis [93,95,96] and, in some cases,

administration of AHA. With this conservative treatment, it has been possible to dissolve encrustations within 2 months [95], with preservation of renal function and without renal loss.

In one case of endocarditis due to *Corynebacterium* group D2, a young drug addict was successfully treated with replacement of the aortic and mitral valves in conjunction with a 4-week course of vancomycin [102]. In other cases of endocarditis, one patient did not receive adequate treatment and died [75], and one, although receiving apparently adequate treatment, died shortly after the surgical procedure [103].

Nine bacteraemic patients were treated with an antibiotic active *in vitro* against *C. urealyticum* [8,69,70,101], mainly vancomycin, and one by a partial nephrectomy [99]. One neutropenic patient with acute myeloblastic leukaemia and catheter-related bacteraemia was cured after a course of vancomycin in conjunction with catheter removal, but this also coincided with an increase in neutrophil counts [101]. Another neutropenic patient developed septicaemia caused by *C. urealyticum* and *Candida albicans* and died [100]. All other patients recovered.

Among 12 patients with other infections, ten were successfully treated with vancomycin [14,100,105–109], with or without surgery, and one neutropenic patient recovered, with a coinciding increase in polymorphonuclear cells [109]. One patient with pneumonia caused by a penicillin-sensitive strain was successfully treated with this antibiotic [4], and in the remaining case [104] a ureterocalycostomy with lower-pole resection was necessary for cure. All patients recovered uneventfully, and no mortality directly related to the infection was observed.

Development of resistance during treatment has been observed with beta-lactam antibiotics [71], fluoroquinolones [8,71,81], macrolides [8,81], rifampin, tetracycline, and gentamicin [8].

CONCLUSIONS

The complete genome sequence of *C. urealyticum* has been determined by a combination of pyrosequencing and Sanger technology. The chromosome of the type strain has a size of 2.37 Mbp, containing 2024 predicted coding sequences, and revealed a detailed picture of the lifestyle of this opportunistic pathogen, including potential mechanisms of multidrug resistance. *C. urealyticum* is not a rare cause of infection, especially in patients with urological diseases and in immunocompromised patients. For diagnosing human infections caused by *C. urealyticum*, strong collaboration between microbiologists, infectious diseases specialists and urologists is necessary. Clinical specimens must be correctly taken, transported, and processed. Urine specimens should be incubated for longer than 24 h (48–72 h) when patients present symptoms of or have alkaline urine or struvite crystals in their urine sediment. The resistance of this organism to multiple antibiotics dictates that empirical therapy with a glycopeptide antibiotic should be used until the results of susceptibility testing are available. It appears that successful therapy for chronic infection due to *C. urealyticum* often requires removal of an infected foreign body or surgical intervention. The prognosis for patients with infections caused by *C. urealyticum* appears to be reasonably good if the microbiological diagnosis is made and medical and surgical intervention is timely.

TRANSPARENCY DECLARATION

The authors declare that they have no conflicts of interest.

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