Isolation of *Streptococcus urinalis* from a human blood culture

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*Streptococcus urinalis* was isolated from a blood culture of a 60-year-old man with a history of urethral stricture. This species has been recently described as a new member of the pyogenic subgroup of streptococci that cause urinary tract infections.

A 60-year-old man with a 20-year history of ischaemic cardiomyopathy and urethral stricture presented to our emergency department with acute fever and shivering, which had started some hours before. Upon examination, his vital signs were significant with a body temperature of 40.1 °C, blood pressure of 100/60 mm Hg and an elevated heart rate of 90 beats min⁻¹. He complained of having had murky urine and dysuria for 2 days but denied having a cough, chest pain, sore throat, vomiting, diarrhoea or headaches. Physical examination gave the profile of a patient in a remarkably acute and poor general condition. A faint 2/6 systolic ejection murmur was heard with a point of maximal intensity (PMI) at Erb’s point. No oral or dental lesions were seen and there were no peripheral signs indicative of infective endocarditis or heart failure. Pulmonary examination was unremarkable. No crepitus or induration of the neck was notified. There was a slight abdominal tension in the suprapubic area but the patient denied pain in the loin region. Initial laboratory findings of the blood analysis included slight leukopenia (3000 m⁶l⁻¹), nitrite (+, positive) and protein (+, 1 µg ml⁻¹). The presumptive diagnosis at the emergency department was a complicated urinary tract infection (UTI) with suspected urosepsis. The subcultured non-haemolytic streptococci showed chains, indicative of streptococci, were detected in 6 days after admittance remained sterile. Gram-positive cocci in chains, indicative of streptococci, were detected in the initial blood cultures by using microscopic analysis. The subcultured non-haemolytic streptococci showed a positive reaction for pyroglyutamic acid arylamidase (PYR) and leucine aminopeptidase (LAP). The biochemical profile (3003 0111 100) in the rapid ID32 system (bioMérieux) revealed a 99.9 % ID-value for *Aerococcus viridans*, although

However, by the next morning the CRP level had increased up to 225 mg l⁻¹ and the white blood cell count had increased to 15 300 µl⁻¹. Microbiological examination of midstream urine samples and blood cultures was then initiated.

Further examinations of the patient did not reveal any potential origin of the presumptive sepsis other than the urogenital tract. In particular, pulmonary infiltrates were ruled out by a thorax X-ray and a diagnosis of endocarditis was excluded by transoesophageal echocardiography. An abdominal ultrasound examination revealed normal kidneys, whereas the urinary bladder was enlarged, with a thickened muscle wall and a residual urine volume of more than 500 ml.

A urological consultation confirmed a long-distance stricture of the urethra without any hints of another underlying disease. On the third day of the in-patient’s stay, a suprapubic catheter was put in place and antibiotic treatment was continued for 14 days. A transurethral urethrotomy was performed in the infection-free interval and the patient was discharged 2 days later in a good clinical condition.

Microbiological investigations revealed one set of positive aerobic (BPA) and anaerobic (BPN) blood culture bottles (BacT/ALERT, bioMérieux) after 1 day of incubation, whereas the urine culture (Uricult, Roche Diagnostics) did not show significant bacteriuria after 48 h of incubation at 37 °C in ambient air. Further blood cultures drawn 4 and 6 days after admittance remained sterile. Gram-positive cocci in chains, indicative of streptococci, were detected in the initial blood cultures by using microscopic analysis. The subcultured non-haemolytic streptococci showed a positive reaction for pyroglyutamic acid arylamidase (PYR) and leucine aminopeptidase (LAP). The biochemical profile (3003 0111 100) in the rapid ID32 system (bioMérieux) revealed a 99.9 % ID-value for *Aerococcus viridans*, although
the low t-value of 0.64 pointed toward a misidentification due to a number of atypical biochemical reactions, including a positive arginine dihydrolase test and a negative mannose fermentation test. MALDI-TOF mass fingerprinting (Microflex, Bruker Daltonics) of the isolate revealed the presence of *Streptococcus urinalis* with a log (score) value of 2.609 when compared to the fingerprint of *S. urinalis* DSM 16839\(^T\) in the Biotype 3.0 database.

For definitive species identification, sequence analysis of the 16S rRNA gene was performed (GenBank accession no. JQ307003), which revealed a 100% identity with that of *S. urinalis* DSM 16839\(^T\) (position 1–1476, accession no. DQ303194.1). The isolate was also deposited in the DSMZ as strain DSM 25036.

Susceptibility testing using the disk diffusion test revealed susceptibility against penicillin, amoxicillin, cefuroxime, erythromycin and clindamycin. Susceptibility testing using Etest strips (bioMérieux) revealed an MIC of 0.125 mg l\(^{-1}\) for both ciprofloxacin and cefotaxime.

To address the problem of poor or absent growth of streptococci on dip-slide urine cultures we extended the experiment of Jokipii & Jokipii (1979), which showed that *Streptococcus agalactiae* does not grow as easily recognizable colonies on cysteine lactose electrolyte deficient (CLED) agar. Uricult dip-slides (Roche Diagnostics) were immersed in a suspension of different streptococcal species that are typically found in urine of patients with UTIs. Inocula of 10\(^5\) and 10\(^7\) c.f.u. ml\(^{-1}\) of *S. urinalis* (CCUG 41590\(^T\) and DSM 25036), *S. agalactiae* (CCUG 22013) and *Streptococcus pneumoniae* (ATCC BAA-340) were prepared in physiological saline and tested. Even a prolonged incubation of the inoculated dip-slides (up to 48 h at 37 °C in a humidified 5% CO\(_2\) atmosphere) did not reveal any visible growth of colonies on the CLED agar of the Uricult dip-slides, with the exception of *S. agalactiae*, where tiny colonies could be seen when using a loop for both inocula tested.

**Discussion**

UTIs are among the most common bacterial infections, predominantly affecting women. In women, UTIs are often uncomplicated in otherwise healthy individuals, whereas in men, UTIs are often complicated and associated with underlying urological diseases (Raynor & Carson, 2011). In the case of urinary stricture disease in men, a strong association with UTIs has been described in ~42% of the patients studied (Anger et al., 2010). Urethral strictures pose a significant risk factor for further complications, for example incontinence (Anger et al., 2010). As with all other anatomical, functional, or structural alterations of the urinary tract, urethral strictures predispose patients to complicated nosocomial UTIs with a high risk of bacteriuria and sepsis.

*Escherichia coli* is the most common bacterial isolate in both complicated and uncomplicated UTIs; however, the bacterial spectrum of uncomplicated UTIs is more heterogeneous and comprises a wide range of Gram-positive species as well as Gram-negative pathogens (Wagenlehner & Naber, 2006). A comparison of four different studies in four different regions of the world revealed that 70–80% of complicated UTIs are caused by Gram-negative pathogens, whereas Gram-positive bacteria only account for about 15–30% (Wagenlehner & Naber, 2006). Nevertheless, rarely encountered Gram-positive bacteria can cause serious UTIs complicated by urosepsis, for example, *Actinobaculum schaudii*, *Actinobaculum urinale*, *Aerococcus sanguinicola*, *Aerococcus urinae* (Cattoir et al., 2010; Hall et al., 2003; Sturm et al., 2006), *Aerococcus urinaehominis* (Lawson et al., 2001) and *S. urinalis* (Collins et al., 2000). However, diagnostic dip-slides are designed to enable common uropathogens to be cultured, such as enteric Gram-negative rods, whereas the growth of more fastidious micro-organisms, such as species of *Actinobaculum*, *Aerococcus* and *Streptococcus*, might not be sufficiently supported. Thus, streptococci do not even rank among the ten most commonly reported uropathogens causing complicated UTIs, a notion that could be significantly biased (Foxman, 2010; Wagenlehner & Naber, 2006).

Among the streptococci, *S. agalactiae* is a relatively common cause of asymptomatic bacteriuria in pregnant women, whereas cystitis due to *S. agalactiae* appears most frequently in elderly and immunocompromised patients (Ulett et al., 2009). Both a history of UTIs (Muñoz et al., 1992) and increased age (Ulett et al., 2009) are significant risk factors for UTIs caused by *S. agalactiae*, whereas anatomical abnormalities of the urinary tract do not increase the risk of *S. agalactiae* UTI.

Another streptococcal species that is not commonly considered an uropathogen is *S. pneumoniae* (Burckhardt & Zimmermann, 2011). It has been suggested that this species plays a role in UTIs of children, again, mostly in patients with an underlying medical history of recurrent UTIs or genito-urinary abnormalities (Burckhardt & Zimmermann, 2011).

The fact that growth of *S. agalactiae* is not well supported on dip-slide urine cultures was reported previously (Jokipii & Jokipii, 1979) with growth of the colonies being almost invisible even after prolonged incubation time. We repeated this experiment with different streptococcal isolates and our results support the conclusion that *S. urinalis* does not grow on the Uricult slides and that its growth on other culture media can be easily overlooked when only incubated for 24 h, even when read by trained personnel. Therefore, the absence of growth of *S. urinalis* on the Uricult slides in the present case is not implausible.

The first isolation of *S. urinalis* was from a patient suffering from cystitis and abdominal pain (Collins et al., 2000). Although the species description was based on this single strain, distinct taxonomic characteristics, such as a 16S rRNA gene sequence divergence of 2.5% from *S. pyogenes* and *S. canis*, led to the description of *S. urinalis* as a new
species (Collins et al., 2000). To the best of our knowledge, this case report is the second report of \textit{S. urinalis} isolated from human urine. Phylogenetically, \textit{S. urinalis} is considered as a member of the ‘pyogenic subgroup’ of streptococci (just like \textit{S. agalactiae}), with \textit{S. pyogenes} and \textit{S. canis} as the nearest phylogenetic relatives (Collins et al., 2000). In contrast to its closest relatives, which display strong \( \beta - \)haemolytic activity, \textit{S. urinalis} does not synthesize any streptococcal Lancefield-group antigen.

The species description refers to facultative anaerobic colonies that routinely grow on conventional culture media without growth enhancement and are non-haemolytic, as found for the present isolate (Collins et al., 2000). Key reactions have identified this strain as being a member of the genus \textit{Streptococcus}, such as the production of pyroglutamic acid arylamidase (PYR) and leucine aminopeptidase (LAP). The biochemical profile revealed by the Rapid ID32 \textit{Strep} test (bioMérieux) was in agreement with the species description of \textit{S. urinalis} (Collins et al., 2000). The ID32 \textit{Strep} code (3003 0111 100) for the present isolate (DSM 25036) was identical to that of the \textit{S. urinalis}-type strain (CCUG 41590\(^T\)), including the ID- and \( t \)-values. Therefore, this code could be taken as a preliminary indication of the presence of \textit{S. urinalis}. Final identification of this rare \textit{Streptococcus} species was rapidly achieved by using MALDI-TOF MS fingerprinting. The threshold value achieved for the present isolate was \( >2.0 \), which is considered acceptable for determining correct species-level identification, as stated by the manufacturer.

The first \textit{S. urinalis} isolate was described by Collins et al. (2000) as showing no notable resistance to bacitracin or vancomycin; this was also true for the present isolate, which did not show any remarkable resistance patterns either.

The present case illustrates how the application of molecular techniques, such as DNA–DNA reassociation or 16S rDNA gene sequencing (Facklam, 2002), to the classification and taxonomic analysis of Gram-positive catalase-negative cocci, can uncover novel pathogens that must be taken into account in the clinical diagnostic laboratory, as well as by clinicians. Although \textit{S. urinalis} was not cultured from urine but from blood culture in the present case, it most probably represents a case of urosepsis. This diagnosis is supported by the identification of pathogens in the urine analysis and furthermore by the clinical symptoms, taking into account the urethral stricture as a predisposing risk factor. Since streptococci do not readily grow on dip-slides with CLED and MacConkey agar, in cases of uncertain UTI aetiology it is strongly recommended that cultures on blood agar plates be incubated for up to 48 h in a 5 % \( \text{CO}_2 \) atmosphere and that urine cultures be performed on selective media. Furthermore, we suggest that single micro-organisms should be identified to the species level in cases of significant bacteriuria (\( >10^5 \) c.f.u. \( \text{ml}^{-1} \)), particularly in cases where patients have underlying risk factors for UTI. This task can be facilitated by application of MS fingerprinting. Systems including a database for all rarely occurring Gram-positive uropathogenic bacteria mentioned above are essential for further elucidation of their aetiological relevance.

References


