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Case Report

Anaerobic urinary tract infection caused by *Veillonella parvula* identified using cystine-lactose-electrolyte deficient media and matrix-assisted laser desorption ionization-time of flight mass spectrometry

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ABSTRACT

We report a case of *Veillonella parvula* causing a urinary tract infection. The organism was isolated from urine using cystine-lactose-electrolyte deficient media and identified using matrix-assisted laser desorption ionization-time of flight mass spectrometry confirmed by 16s RNA. This case highlights important clinical and microbiological considerations for urinary tract infections.

Case report

A 47-year-old female presented to her general practitioner on January 14, 2013, with increasing confusion and weakness since being discharged from hospital that day and a urine culture was sent. Co-morbidities included organic brain disease secondary to a stroke ten years prior, possible past seizures, bipolar and borderline personality disorder, gastroesophageal reflux disease, dyslipidemia, hypertension, hypothyroidism and a past history of substance abuse.

She was admitted to hospital on December 1, 2012 due to confusion caused by high levels of valproate. During her admission she developed urinary retention and was catheterized. Subsequently, she developed multiple urinary tract infections (UTI) (Table 1). After her confusion improved, she developed fever and leukocytosis 10 days into admission that was attributed to a bowel micro-perforation indicated by a small amount of extraluminal air on CT abdomen. A cystogram done to rule out enterovesicular fistula was normal. Her fever and WBC count resolved 2 days after treatment with vancomycin, ciprofloxacin and metronidazole, which were continued for ten days. She required intensive rehabilitation and continued to require intermittent catheterization up until discharge on January 14th, 2013.

Before discharge, a urine culture was submitted to our laboratory on January 9th. As per routine protocols, the urine was submitted in a sterile container and dipped at the point of collection using the Uricult® Trio dip slide culture method with MacConkey medium, a β-glucuronidase-producing *Escherichia coli* detection medium, and CLED medium. Pure 10^8 cfu/ml gram-negative cocci, grew only on the CLED media after 24 h of incubation and the organism failed to grow for identification by routine aerobic culture methods. At the direction of the medical microbiologist, the isolate was further plated onto other types of media to rule out other possible uropathogens, including

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Table 1
Urine culture results using Uricult® Trio.

	Date	Source	Organisms	Urine microscopy	Antibiotic dates	Antibiotics
First admission	Dec 1	MSU	No growth	Not done	None	
	Dec 4	In and out	*E. coli* 10^8 *E. faecalis* 10^6	RBC + Bacteria	Dec 4–9	Ciprofloxacin
	Dec 5	In and out	*E. faecalis* 10^6	RBC, WBC, Bacteria	Dec 9–20	Vancomycin[a]
	Dec 11	Unknown	Gram-negative bacilli 10^6	RBC, WBC, Bacteria	None	
	Dec 12	Unknown	Yeast 10^7	RBC, WBC, Bacteria	None	
	Dec 13	Indwelling	*E. faecalis* 10^8 Yeast 10^8	RBC + WBC	None	
	Dec 14	Unknown	*C. glabrata*	1–5 RBC >50 WBC Bacteria	Dec 17–23	Fluconazole
	Dec 23	Unknown	*C. glabrata*	WBC + Bacteria	Dec 17–23	Fluconazole
	Dec 24	Intermittent	*E. coli* 10^8	WBC + Bacteria	Dec 25–31	Ciprofloxacin
	Jan 1	Catheter NS	No growth	WBC + Bacteria	None	
	Jan 1	Catheter NS	No growth	WBC + Bacteria	None	
	Jan 5	Unknown	No growth	Not done	None	
	Jan 9	Unknown	Gram-negative cocci 10^8	WBC + Bacteria	None	
	Jan 12	In and out	*V. parvula* 10^8	Not done		
Outpatient	Jan 14	In and out	*V. parvula* 10^8	Not done	Jan 14–20	Metronidazole
Second admission	Jan 20	MSU	Lactobacillus 10^8 Other Gram positive 10^6	11–25 WBC 1–5 RBC Bacteria	Jan 20–24	Ceftriaxone
	Jan 20	MSU	Mixed 10^7	RBC WBC		
Outpatient	Jan 30	In and out	NG	11–25 WBC 1–5 RBC Bacteria		

Colony forming units per mL are denoted next to the organism name and were determined based on the manufacturer's estimates of the number of colonies growing on the slide that correspond to the colony forming units per mL. For urine microscopy, most had too many cells to perform accurate cell counts. When cell count was performed, numbers denoted are per high-powered field microscopy.
MSU, midstream urine; In-and-out, catheter was aseptically inserted to collect urine and removed afterwards. Catheter NS, not specified if urine was collected from an indwelling catheter or from an in-and-out; WBC, white blood cells; RBC, red blood cells.
[a] Continued in addition to ciprofloxacin and metronidazole for 10 days to treat query microperforation and *E. faecalis* in the urine.

Haemophilus and *Neisseria* species, but no growth was observed (Table 2). Considering the possibility of an anaerobic UTI, the January 12 and 14 isolates were cultured anaerobically. Anaerobic gram-negative cocci grew in 48 h under these conditions (Table 2).

Suspecting an anaerobic UTI, the medical microbiologist notified the general practitioner about the possibility and advised appropriate antimicrobial coverage with metronidazole. The patient was treated with metronidazole 500 mg twice daily starting January 14, but was readmitted in the hospital on January 20 due to her persistent delirium. While waiting for antimicrobial susceptibility results on the anaerobic gram-negative cocci, the infectious diseases service started empiric coverage for a UTI with intravenous ceftriaxone 2 g daily. Head CT and MRI, both revealed a subacute right-sided pontine infarct not present on her last CT head (December 3rd), which was associated with no new focal neurological exam findings and no significant findings on stroke work up.

Table 2
Growth of the *V. parvula* isolate on different media & atmospheres.

Culture step	Media	Atmosphere	Growth
1	CLED[a]	Ambient (UricultTrio®)	Gram-negative cocci
1	MacConkey[a]	Ambient (Uricult Trio®)	No growth
1	Beta-gluconuronidase[a]	Ambient (Uricult Trio®)	No growth
2	Columbia Blood Agar[b]	Ambient + 5% CO_2	No growth
2	Chocolate Agar[b]	Ambient + 5% CO_2	No growth
2	Martin Lewis Agar[b]	Ambient + 5% CO_2	No growth
2	Fastiduous Organism Broth[b]	Ambient + 5% CO_2	No growth
2	Trypticase Soy Broth[c]	Ambient + 5% CO_2	No growth
3	Thioglycollate Broth[c]	5% CO_2, 8% H_2, 87% N_2	Gram-negative cocci
3	Fastidious Anaerobic Agar[b]	5% CO_2, 8% H_2, 87% N_2	Gram-negative cocci

All cultures incubated for 24–48 h. "Culture step" is the order in which cultures were performed.
[a] Orion Diagnostica, Oy, Finland.
[b] Oxoid Ltd., Ontario, Canada.
[c] Dalynn Biologicals, Calgary, Alberta, Canada.

Using the Vitek® MS (bioMérieux, Marcy L'Etoile, France), the isolate was identified as *Veillonella parvula* with 99.9% certainty. Molecular confirmation using 16s rRNA sequencing was later done with primers 16S-F (5′-AGAGTTTGATCATGGCTCAG-3′) and 16S-R (5′-GGACTACCAGGGTATCTAAT-3′) [1]. A 624 bp product confirmed the identity with 99% sequence homology based on the GenBank database using the Basic Local Alignment Search Tool algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

The isolate was sensitive to ceftriaxone, tetracycline, imipenem, meropenem, ertapenem, penicillin, and metronidazole (Clinical Laboratory Standards Institute breakpoints) and was tested by E-test (bioMérieux) on *Brucella* agar with laked blood (Dalynn Biologicals) [2].

Discussion

Veillonella species are conventionally part of the normal oral and genital flora [3]. Only one other publication describes a *Veillonella* spp. UTI, which is a report of pyelonephritis with bacteremia in a pregnant woman caused by an unspecified species of *Veillonella* [4]. In this case, *V. parvula* was isolated as a pure culture in three separate in and out catheter urine collections at 10^8 cfu/ml and subsequent urine cultures after treatment no longer grew *V. parvula* nor any other uropathogen. With treatment

of the UTI, the patient's delirium improved within 10 days of antibiotic therapy. This was despite the presence of a subacute (based on imaging characteristics) pontine infarct that could have occurred at any point between head CTs on December 3rd and January 20th. Often, an episode of delirium may persist for weeks, months, or even years especially if the underlying inciting etiology causes permanent physio-neurological damage, as in the case of an infarct [5]. The reversibility of delirium, at the same time, is often directly dependent on whether its underlying cause can be promptly diagnosed and is treatable [5]. Due to the rapid improvement of this patient's delirium following appropriate antimicrobial therapy, her initial cognitive decline was postulated to be most likely due to an UTI caused by *V. parvula* and not to the infarct. This case exemplifies the difficulty in diagnosing an UTI in patients presenting with atypical symptoms (i.e. confusion/delirium), which is typically seen in the elderly population.

It is more common to find anaerobes associated with abscesses of the urinary tract than with UTIs [6]. When urine is cultured anaerobically, the isolation of anaerobic bacteria is rare (<1%) and the patients are often asymptomatic [7]. There are however, case reports demonstrating the ability of anaerobic bacteria to cause cystitis and pyelonephritis, especially in patients such as ours with a recent history of catheterization and/or instrumentation (i.e. cystoscopy) [8–10].

Due to the rare occurrence of UTIs caused by anaerobes, microbiology laboratories have discontinued routine urine culture for them; consequently, clinicians must be aware that laboratories may not culture anaerobes unless specifically requested. In this case, the urine dip slide method with CLED media allowed for isolation of the *V. parvula.* Clinically significant anaerobes including *Veillonella* can be aerotolerant [11], therefore we hypothesize that because the dip slide is incubated in a sealed container, the oxygen content is low enough to permit growth of *V. parvula*. Additional nutrients such as cysteine found in CLED media may have also provided additional growth support.

We report on *V. parvula*'s role in UTIs and illustrate how *V. parvula* can be reliably identified in the clinical laboratory. Our case highlights several clinical and laboratory pitfalls for consideration including the need for consultation with the medical microbiologist to investigate for anaerobic and fastidious organisms when a patient presents with a clinical picture consistent with recurrent or persistent UTIs.

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