First report of infection with *Pseudomonas citronellolis*: a case of urosepsis

G. Williams

Pathology and Laboratory Medicine Service, VA Central Western Massachusetts, Leeds, MA 01053, USA

Abstract

This is the first case report of infection with the environmental bacterium *Pseudomonas citronellolis*, presented here as a urinary tract and bloodstream infection that occurred shortly after a transrectal ultrasound-guided prostate biopsy.

Keywords: Bacterial identification methods, ciprofloxacin resistant, environmental bacterium, prostate biopsy, *Pseudomonas citronellolis*, urosepsis
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Corresponding author: G. Williams, Pathology and Laboratory Medicine Service, Veterans Affairs Medical Center, Central Western Massachusetts, 421 North Main Street, Leeds, MA 01053, USA.
E-mail: glynne.williams@va.gov

Case report

A 71-year-old male presented to the urgent care clinic at the Veterans Affairs Central Western Massachusetts Medical Center with complaints of dysuria, intermittent bloody urine, lower abdominal pain and chills for 4 days. His temperature was measured at 39.6°C. The patient had a history that included kidney stones, horseshoe kidney, nephrolithiasis, diabetes mellitus, and elevated prostate-specific antigen levels. He had undergone a transrectal ultrasound-guided prostate biopsy 12 days earlier. Symptoms had started 8 days after the biopsy. He had been given perioperative prophylaxis with ciprofloxacin 500 mg tablets twice daily for 3 days. The biopsy had proved negative for malignancy.

Clean-catch urine and blood specimens were collected, including two sets of blood cultures. Laboratory results were notable for a complete blood count that included a white blood cell count of 20.6 x 10^3/μL (normal 4.5–11 x 10^3/μL) and an absolute neutrophil count of 17.7 x 10^3/μL (normal 1.8–6.5 x 10^3/μL). The urinalysis was positive for leukocyte esterase and blood. Microscopic examination showed >50 white and red blood cells/high-power field (HPF) (normal 0–5 cells/HPF).

It was decided to transfer the patient to a larger Veterans Affairs facility for treatment. In preparation for transport he received a ciprofloxacin/dextrose solution (400 mg intravenously). Upon arrival at the receiving hospital the patient was started empirically on piperacillin–tazobactam and ciprofloxacin IV.

At our laboratory the urine was inoculated using a 1-μL loop onto sheep-blood and MacConkey agars (BD, Sparks, MD, USA) and incubated in ambient air at 35°C. The blood cultures were analysed in a BacT/Alert instrument (BioMérieux, Durham, NC, USA). After overnight incubation, oxidase-positive colonies grew on plates in numbers that indicated >10^5 colony-forming units (CFU)/mL urine. The blood cultures were positive for Gram-negative rods in the aerobic bottles of both sets. Upon subculture on sheep blood and MacConkey agars, the colonies had the same morphology as those on the urine culture plates, as expected. The oxidase-positive colonies were large, flat with irregular edges, light grey, and at first appeared to be *Pseudomonas aeruginosa*.

The urine isolate was designated NH-138 and the blood isolates NH-125 and NH-126. The three isolates were tested for identification and antibiotic susceptibility with the MicroScan AutoSCAN System (Beckman Coulter, Brea, CA, USA). The results came back as *Pseudomonas 'fluorescens/putida'* with a 95.8% probability. The MicroScan AutoSCAN does not differentiate between *Pseudomonas fluorescens* and *Pseudomonas putida*. Along with the identification, the isolates showed susceptibility to piperacillin–tazobactam, ceftriaxone, ceftazidimine, tobramycin, gentamicin, amikacin and tetracycline. The isolates were resistant to aztreonam, ciprofloxacin and trimethoprim–sulfamethoxazole.

The Clinical and Laboratory Standards Institute (CLSI) document M100 under the section "*Minimum Inhibitory Concentration Interpretative Standards (μg/mL) for Other Non-Enterobacteriaceae*" was used for antibiotic reporting purposes [1]. When the results were released, the patient's treatment was changed to ceftazidime 1 g intravenously every 8 h. After 14 days of treatment he was discharged from the hospital.

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To help verify the MicroScan AutoSCAN results, NH-126 was sent to a large commercial reference laboratory where it was identified biochemically as *Pseudomonas fluorescens*. NH-126 was also sent to a nearby community hospital laboratory where it was identified as *Pseudomonas fluorescens* using a Vitek 2 system. Their report also showed ciprofloxacin resistance.

Further investigation was warranted because the MicroScan identification included a positive nitrite reaction. Only 5% of *Pseudomonas 'fluorescens/putida'* strains are positive for nitrite in the MicroScan system (personal communication, Beckman Coulter). NH-126 was tested for growth at 42°C on trypticase soy agar slant along with strains of *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas fluorescens* derived from the American Type Culture Collection (ATCC). The clinical isolate grew readily at 42°C, as did *Pseudomonas aeruginosa*. *Pseudomonas putida* and *Pseudomonas fluorescens* did not grow, suggesting that the earlier identifications had been erroneous [2].

Isolate NH-126 was sent for identification to reference labs: MIDI Labs in Newark, Delaware, USA, for matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), and the Culture Collection, University of Göteborg, Sweden (CCUG).

The CCUG reported an identification of *Pseudomonas citronellolis*. NH-126 was assigned number CCUG 59066. The identification was reported after extensive phenotypic testing that included cellular fatty acid methyl ester (CFA-FAME) analysis and comparison to their collection of non-fermenters followed by CCUG software analysis.

At MIDI Labs the isolate was analysed by MALDI-TOF MS (Bruker Biotyper Microflex LT Bruker Daltronics, Billerica, MA, USA). Both Bruker and MIDI libraries were searched. The isolate was identified as *P. citronellolis* with a score of 2.423 (>2.0 species level). The source of the entry was DSM 50332 THAM, the type strain for *Pseudomonas citronellolis*. The next closest match was *Pseudomonas citronellolis* 993700254 LBK, with a score of 2.079. The next result was *Pseudomonas jinjuensis* with score of 1.841.

For final confirmation full 16s rRNA gene sequencing was performed at MIDI Labs followed by Sherlock DNA (MIDI) analysis. The closest match of the 1498 base pairs was the *Pseudomonas citronellolis* type strain with 99.7% sequence homology (Sherlock DNA software (MIDI), www.midi-inc.com). The sequence was submitted to GenBank and was assigned accession number MK404228.

For comparison, the type strain of *Pseudomonas citronellolis* was obtained. ATCC 13674^T and isolate NH-126 were tested using the MicroScan, and both were identified as *Pseudomonas 'fluorescens/putida'* with essentially the same probabilities and susceptibilities, with one noticeable difference: ATCC 13674^T was ciprofloxacin-susceptible. It was resistant to aztreonam and trimethoprim-sulfamethoxazole, as was NH-126.

ATCC 13674^T (DSM50332) originated from soil under pine trees in northern Virginia, USA, when research was being done concerning the degradation of isoprenoid compounds, such as citronellol, by bacteria. The new species in the genus *Pseudomonas* was proposed in 1960 [3]. There was a later emended description of *Pseudomonas citronellolis* [4].

A literature search did not reveal any published reports of human infection. Isolates from human sources can be found on the CCUG website (www.ccug.se, 2018) and include one isolate from a human appendix, two from human blood, and one from a rectal sample. Previous reports of *Pseudomonas citronellolis* susceptibilities were not found for comparison. There have been publications presenting *Pseudomonas citronellolis* as a possible agent for bioremediation [5,6].

Discussion

It is unusual for an environmental bacterium such as *Pseudomonas citronellolis* to cause an infection, in this case involving a prostate biopsy. The CCUG collection contains a rectal isolate. Possibly in this instance the patient's rectum became colonized by *Pseudomonas citronellolis* due to some contact with soil.

P. citronellolis has likely been overlooked in the past due to its identification as *P. putida* or *P. fluorescens* by systems that use phenotypic methods, such as Vitek or MicroScan. With the rapidly expanding use of MALDI-TOF MS in clinical microbiology laboratories it is possible that more human isolates of *P. citronellolis* will be detected.

In conclusion, a case of *P. citronellolis* infection has been presented here. Its presence in both urine and blood suggests it can act as a pathogen.

Transparency declaration

The author declares no conflict of interest.

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