Case Report

First report of Wautersiella falsenii genomovar 2 isolated from the respiratory tract of an immunosuppressed man

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ABSTRACT

Wautersiella falsenii is a Gram-negative, non-motile rod, which grows aerobically on common isolation media and is the only acknowledged species among the genus Wautersiella. Two genomovars, namely 1 and 2, phenotypically indistinguishable but genotypically different, are described. To date, few case reports detailing the clinical disease associated with W. falsenii have been reported, all describing localized infection. To our knowledge, this study reports the first isolation of W. falsenii genomovar 2 from a respiratory sample of an immunosuppressed man. Our hypothesis is that the patient was harboring W. falsenii genomovar 2 and both the immunosuppression and the antimicrobial treatments provided a chance for this organism to emerge. The clinical significance of this result is yet to be evaluated. Although infection with W. falsenii remains rare, this bacterium should not be underestimated mainly because of its natural resistance to many available antimicrobials.

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Introduction

Wautersiella falsenii is a Gram-negative, non-motile rod, positive for urease and for indole production, which grows aerobically on common isolation media such as blood agar [1] or MacConkey agar plates at 37 °C. Wautersiella was proposed as monospecific genus within the family Flavobacteriaceae in 2006 [1]. W. falsenii is the only acknowledged species among the genus Wautersiella [1]. Two genomovars, namely 1 and 2, were included to accommodate two groups of closely related isolates from clinical origins and with phenotypic resemblance to isolates of the genera Chryseobacterium, Weekiella and Empedobacter and of CDC groups II-e and II-h [2]. Recently, Zhang et al. [3] evidenced that the type strain of the type species of the genera Empedobacter and Wautersiella shared biochemical and phenotypical characteristics, suggesting that they might belong to the same genus [3].

Case and discussion

The patient

The patient, a 32-year-old man, metalworker, affected by lymphoblastic leukemia, was admitted in Hematology Unit-Pisa (Italy) in good clinical condition for the bone marrow transplantation from his HLA-matched relative. Laboratory findings in the first day of hospitalization included a white blood cell count of 3500/μL, hemoglobin 11.9 g/dL, platelet 114,000/μL. Two days later, a central venous catheter was positioned. Due to disease relapsing in day 3, he underwent high-dose chemotherapy with cytarabine and mitoxantrone. On day 12, the patient developed fever (>39°C) and a swelling near the right axilla in day 13; he received a broad range therapy with piperacillin/tazobactam, teicoplanin and gentamicin. Blood was drawn for cultures and a multisensitve strain of Klebsiella pneumoniae was isolated. Due to the persistence of fever, in day 24 a new therapy scheme was adopted: liposomal amphotericin B, meropenem, tigecycline, daptomycin and colistin. A wound swab, performed on the swelling, was negative for bacteria and fungi. On day 31, Candida antigen was detected in the serum patient. Due to the onset of cough and cold symptoms, a microbiological monitoring of the respiratory samples was performed on day 35 and a strain of W. falsenii was isolated. The patient was discharged on the 42nd hospital day, after blood transfusion, with the following home therapy: fluconazole (200 mg) twice per day, acyclovir (400 mg) twice per day, clarithromycin (500 mg) once per day, erythropoietin (40,000 UI) once per week and filgrastim. Laboratory findings on the last hospital day included a white blood cell count of 2790/μL, hemoglobin 8.1 g/dL, platelet 14,000/μL.

The respiratory samples were cultured on common isolation media, both nutrients and selective media and were incubated at...
37 °C. Suspected colonies were identified using a matrix-assisted laser desorption ionization-time of flight mass spectrometer (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany), with a score of 1.94 (probable genus-level identification). Antimicrobial susceptibility testing was performed using the Sensititre system (Thermo Fisher Scientific). The Minimal Inhibitory Concentrations (MICs) results were interpreted for nineteen antimicrobials, using the EUCAST breakpoints (European Committee on Antimicrobial Susceptibility Testing, 2014). The W. falsenii isolate was resistant to amikacin (>16 μg/ml), amikacin-clavulanic acid (>8 μg/ml), ampicillin-sulbactam (32 μg/ml), cefotaxime (>4 μg/ml), ceftazi-dime (>8 μg/ml), doripenem (>8 μg/ml), gentamicin (>4 μg/ml), imipenem (8 μg/ml) and piperacillin-tazobactam (16 μg/ml). MICs values were not interpreted for ciprofloxacin (1 μg/ml), colistin (>8 μg/ml), ertapenem (>1 μg/ml), fosfomycin (>64 μg/ml), levofloxacin (≤4 μg/ml), meropenem (32 μg/ml), nitrofurantoin (>64 μg/ml) and ticarcycline (≤12 μg/ml). The isolate was susceptible only to cefepime (≤1 μg/ml) and trimethoprim-sulfamethoxazole (≤0.5 μg/ml). Accordingly, the isolate showed phenotypic resistance to the majority of available antimicrobials. This resistance pattern, typical of an environmental bacterium, explains well both the predominance of this organism and lack of response to the previously administered antimicrobials.

The identification of colonies was confirmed by 16S polymerase chain reaction. Genomic DNA was extracted and amplified with the primers F7Bac_2deg (5′-GAGTGTGAT(C/T)ACCTGCTACG-3′, modified from Lane, 1991) [4] and BAC R1492 (5′-GGCATGATACTTGCATTCT-3′, modified from Lane, 1991) [4]. Amplified and purified fragments were further sequenced in both directions with proper internal primers for the bacterial 16S rRNA gene sequence: 16SF3443 (5′-TACGGGAGGCAGCAGCAGCAG-3′; 16SF785N 5′-GGATTAGATACCCTGGTA-3′; 16SF515ND 5′-ACCGGCGCTGCTCAGCAC-3′ [5]. Affiliation of sequences was first determined by NCBI BLAST analysis [6], then sequences were inserted in the ARB 5.2 software [7], Silva 104 database [8] and alignment was refined manually for phylogenetic studies. The 16S rRNA gene showed 99% identity (1440/1441 bp) with W. falsenii genovar 2 (GeneBank accession no. AM238678). A phylogenetic analysis of almost-complete 16S rRNA gene sequences revealed that the isolate studied formed a distinct cluster with W. falsenii genovar 2, well supported by bootstrap analysis. The phylogenetic tree showing the results of the performed phylogenetic analysis is shown in Fig. 1.

**Accession number**

Nucleotide sequence data was deposited to the European Nucleotide Archive (ENA) under accession number LN886517.

**Conclusions**

Little is known regarding the epidemiology and clinical significance of this organism. To date, few case-reports detailing the clinical disease associated with W. falsenii were reported, all describing localized infection. Twenty-six strains of W. falsenii isolated from the first publication were from clinical origin [1]. Of these, 5 were isolated from blood cultures, 1 from ear discharge, 1 from oral cavity, 1 from pleural fluid, 2 from pus, 2 from respiratory tract (subsp. genovar 1), 1 from vaginal swab, 5 from wound cultures, and 8 were from an unknown origin [1]. In the year 2012, the first isolation of W. falsenii from a urine sample of an infant with a complicated urinary tract infection was described [9]. W. falsenii was found in a respiratory sample from a cystic fibrosis patient, without an individual interpretation of its clinical significance [10]. In 2015, was also isolated from a cervical neck abscess sample from a female with acute otitis media [11]. W. falsenii was implied as a potential agent of hospital-acquired infections via hospital carpet [12]. The organism was also isolated from soil, polluted sediment, rodent skin [13] and appears in two articles on potential pathogenic agents in metal working aerosols.
and fluid [14,15]. As reported by Perkins and Angenent [14], the metalworking industry utilizes recirculating metalworking fluids containing, in addition to chemical substances, bacteria of potential epidemiologic significance [16,17]. In the past years, epidemiological assessments with machine operators in the metalworking industry identified remarkable respiratory effects (e.g., Refs. [16–20]). This supports our hypothesis: the patient may have contracted W. falsenii in the workplace and afterwards W. falsenii may have found in the patient a pleasant host in which to replicate. Indeed, the patient was recovered in a protective isolation; he was in a single room with HEPA filtration and a controlled ventilation system; accordingly, no clear source of infection was identified. Microbiological sampling of room surfaces and water filters did not give results for W. falsenii. The infection described in this study was not severe; however, respiratory infections should be considered important, since they may be the source of more severe infections, particularly in immunocompromised patients.

To our knowledge, this study reports the first isolation of W. falsenii genomovar 2 from a respiratory sample. We speculate that our patient was harboring W. falsenii genomovar 2 and both the immunosuppression and the antimicrobial treatments provided a chance for this organism to emerge. The significance of this result in terms of clinical microbiology is yet to be evaluated. Although infection with W. falsenii remains rare, this bacterium should not be underestimated, mainly because of its natural resistance to many available antibiotics. Little is known regarding the epidemiology of this genus and nothing about the clinical differences between W. falsenii genomovar 1 and W. falsenii genomovar 2 infections. Further studies are necessary to establish the clinical significance, resistance patterns, and carrier rate of this emerging opportunistic pathogen.

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