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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.

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, Weeksella* 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 multisensitive 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

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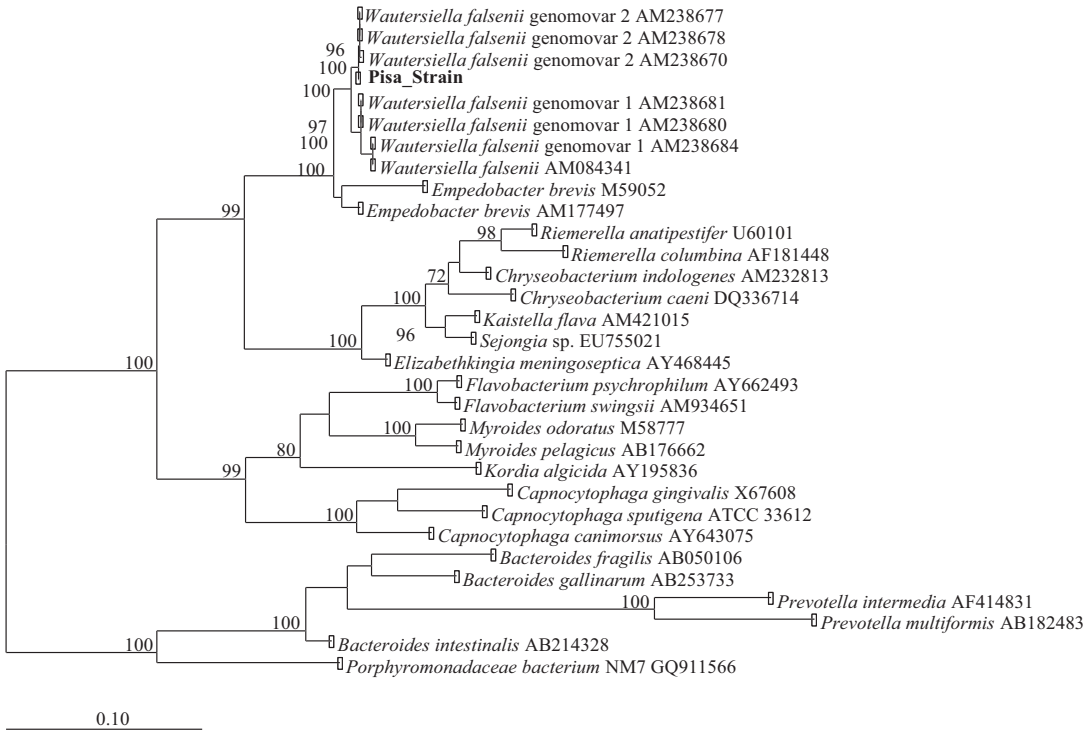

Fig. 1. Phylogenetic tree based on the 16S rRNA gene sequences of the isolate described in this study (Pisa Strain) and some representative members of related genera in the family *Flavobacteriaceae*, with a particular focus on the genus *Wautersiella*. Bootstrap percentages greater than 70%, based on maximum likelihood analyses of 1000 replications, are shown at nodes. Bar represents 10 nucleotide substitutions in 100 nucleotides.

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), ceftazidime (>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 tigecycline (≤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′-GAGTTTGAT(CT)(AC)TGGCTCAG-3′, modified from Lane, 1991) [4] and BAC R1492 (5′-GG(CGAT)(AT)ACCTTGTTACGACTT-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: 16SF343ND 5′-TACGGGAGGCAGCAG-3′; 16SF785ND 5′-GGATTAGATACCCTGGTA-3′; 16SR515ND 5′-ACCGCGGCTGCTGGCAC-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* genomovar 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* genomovar 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. genomovar 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 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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References

[1] Kämpfer P, Avesani V, Janssens M, Charlier J, De Baere T, Vaneechoutte M. Description of *Wautersiella falsenii* gen. nov., sp. nov., to accommodate clinical isolates phenotypically resembling members of the genera *Chryseobacterium* and *Empedobacter*. Int J Syst Evol Microbiol 2006;56:2323–9.

[2] Schreckenberger PC, Daneshvar MI, Weyant SR, Hollis DG. *Acinetobacter*, *Achromobacter*, *Chryseobacterium*, *Moraxella*, and other non-fermentative gram-negative rods. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, editors. Manual of clinical microbiology. 8th ed., Washington, DC: American Society for Microbiology; 2003. p. 749–79.

[3] Zhang RG, Tan X, Liang Y, Meng TY, Liang HZ, Lv J. Description of *Chishuiella changwenlii* gen. nov., sp. nov., isolated from freshwater, and transfer of *Wautersiella falsenii* to the genus *Empedobacter* as *Empedobacter falsenii* comb. nov.. Int J Syst Evol Microbiol 2014;64(8):2723–8.

[4] Lane DJ, Harrison AP, Stahl DA, Pace B, Giovannoni SJ, Olsen GJ, et al. Evolutionary relationship among sulfur and iron oxidizing eubacteria. J Bacteriol 1991;174:269–78.

[5] Vannini C, Rosati G, Verni F, Petroni G. Identification of the bacterial endosymbionts of the marine ciliate *Euplotes magnicirratus* (*Ciliophora*, Hypotrichia) and proposal of '*Candidatus Devosia euplotis*'. Int J Syst Evol Microbiol 2004;54(Pt 4):1151–6.

[6] Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389–402.

[7] Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar. et al. ARB: a software environment for sequence data. Nucleic Acids Res 2004;32(4):1363–71.

[8] Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 2013;41:D590–6.

[9] Van der Velden LBJ, de Jong AS, de Jong H, de Gier RPE, Rentenaar RJ. First report of a *Wautersiella falsenii* isolated from the urine of an infant with pyelonephritis. Diagn Microbiol Infect Dis 2012;74(4):404–5.

[10] Marchandin H, Michon AL, Jumas-Bilak E. Atypical bacteria in the CF airways: diversity, clinical consequences, emergence and adaptation. In: Sriramulu D, editor. Cystic fibrosis-renewed hopes through research. 2012.

[11] Traglia GM, Dixon C, Chiem K, Almuzara M, Barberis C, Montaña S, et al. Draft genome sequence of *Empedobacter* (Formerly *Wautersiella*) *falsenii* comb. nov. Wf282, a strain isolated from a cervical neck abscess. Genome Announc 2015;3(2):e00235–315.

[12] Harris D, Pacheco A, Lindner AS. Detecting potential pathogens on hospital surfaces: an assessment of carpet tile flooring in the hospital patient environment. Indoor Built Environ 2009;19(2):239–49.

[13] Maleki-Ravasan N, Oshaghi MA, Afshar D, Arandian MH, Hajikhani S, Akhavan AA, et al. Aerobic bacterial flora of biotic and abiotic compartments of a hyperendemic Zoonotic Cutaneous Leishmaniasis (ZCL) focus. Parasites Vectors 2015;8:63.

[14] Perkins SD, Angenent LT. Potential pathogenic bacteria in metalworking fluids and aerosols from a machining facility. FEMS Microbiol Ecol 2010;74:643–54.

[15] Murat JB, Grenouillet F, Reboux G, Penven E, Batchili A, Dalphin JC, et al. Factors influencing the microbial composition of metalworking fluids and potential implications for machine operator's lung. Appl Environ Microbiol 2012;78:34–41.

[16] Woskie SR, Virji MA, Hallock M, Smith TJ, Hammond SK. Summary of the findings from the exposure assessments for metalworking fluid mortality and morbidity studies. Appl Occup Environ Hyg 2003;18:855–64.

[17] Kennedy SM, Chan-Yeung M, Teschke K, Karlen B. Change in airway responsiveness among apprentices exposed to metalworking fluids. Am J Respir Crit Care 1999;159:87–93.

[18] Barhad B, Pilat L, Teculescu D. Recent progress in the study of occupational lung diseases in Romania. Br J Ind Med 1975;32:164–8.

[19] Greaves IA, Eisen EA, Smith TJ, Pothier LJ, Kriebel D, Woskie SR, et al. Respiratory health of automobile workers exposed to metal-working fluid aerosols: respiratory symptoms. Am J Ind Med 1997;32:450–9.

[20] Park DU, Jin KW, Koh DH, Kim BK, Kim KS, Park DY. Association between use of synthetic metalworking fluid and risk of developing rhinitis-related symptoms in an automotive ring manufacturing plant. J Occup Health 2008;50:212–20.