Folia Microbiol (2015) 60:155–158
DOI 10.1007/s12223-014-0355-x

First report of *Wohlfahrtiimonas chitiniclastica* from soft tissue and bone infection at an unusually high northern latitude

Siiri Kõljalg · Kaidi Telling · Kristi Huik · Marko Murruste · Virve Saarevet · Merit Pauskar · Irja Lutsar

Received: 25 March 2014 / Accepted: 25 September 2014 / Published online: 11 October 2014

Abstract *Wohlfahrtiimonas chitiniclastica* is a rare human pathogen mostly associated with parasitic flies. This is the report on *W. chitiniclastica* infection in the soft tissue and bone at a 58°N latitude in the northern temperate climate zone. The importance of correct identification of clinically relevant bacteria is highlighted.

Keywords *Wohlfahrtiimonas* · Infection · MALDI-TOF · Vitek · Climate change · Parasites

Introduction

The genus *Wohlfahrtiimonas* belongs to Gammaproteobacteria and consists of two species *W. chitiniclastica* and *W. larvae*. *W. chitiniclastica* was first described in 2008 from larva of the parasitic fly *Wohlfahrtia magnifica* (Tóth et al. 2008). According to GenBank records, *W. chitiniclastica* has been isolated from environmental and human sources in only numbered cases, all from areas with relatively warm climate. Human infections with *W. chitiniclastica* are very rare, and only two cases of bloodstream infections, both in southern countries, have been reported (Rebaudet et al. 2009; Almuzara et al. 2011).

W. chitiniclastica is phylogenetically most closely related to *Ignatzschineria*, genus in which all species are associated with parasitic flies able to cause myiasis in humans and animals (Tóth et al. 2001; Gupta et al. 2011). Two cases of *Ignatzschineria* sp. bacteremia in patients with wound myiasis have been published (Maurin et al. 2007; Roudiere et al. 2007).

Here, the first case of *W. chitiniclastica* isolated from the soft tissue and bone is reported. It is also the report of *W. chitiniclastica* isolation at the most northern latitude.

S. Kõljalg · K. Telling · K. Huik · M. Pauskar · I. Lutsar
Department of Microbiology, University of Tartu, Tartu, Estonia

S. Kõljalg (✉)
United Laboratories, Tartu University Hospital, Tartu, Estonia
e-mail: Siiri.Koljalg@kliinikum.ee

M. Murruste · V. Saarevet
Department of General and Plastic Surgery, Tartu University Hospital, Tartu, Estonia

Case report

A 64-year-old male with a 4-year history of gangrene in the distal part of the legs and amputations of the toes on both feet was hospitalized in November 2013 in Tartu University Hospital, Estonia, after a bicycle accident with hypothermia and signs of alcohol abuse.

Upon admission, his central body temperature was 32.5 °C, but other vital signs were normal. The stumps were inflammatory and covered with malodorous plaques. The dorsal skin of the left foot was necrotic. The posterior tibial and popliteal pulses were palpable. No parasites were found at sanitary care. His white blood cell count was 10,470 cells/μL (86 % neutrophils), C-reactive protein 75 mg/L, arterial blood pH 7.250, lactate 4.7 mmol/L, and creatinine 45 μmol/L. Blood ethanol concentration was 1.99 %.

Immediately after hospitalization, the transmetatarsal amputation of digits I–V of the left foot and of digit III of the right foot was performed. Intravenous amoxicillin clavulanate was started postoperatively and given for 8 days. After surgery, the blood inflammatory markers started to normalize and the wounds healed. The patient was discharged on the ninth day of hospitalization in good condition.

The samples of the bone from the resection line were collected during surgery and sent to the laboratory of microbiology for aerobic culture and microscopy. Unfortunately, no

histopathological investigations were conducted. The samples were cultured onto blood and chocolate agar and incubated at 37 °C in aerobic conditions. Microscopic investigation of acridine orange-stained slides were negative for bacteria. After 18 h of incubation, two different types of bacterial colonies present in equal amounts on semi-quantitative swab culture were revealed in the sample of the left foot. Similar amount of swarming bacterial colonies was present in the sample of the right foot. Microbial identification using Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS; Bruker Daltonics, Germany) established *W. chitiniclastica* and *Myroides odoratimimus* in the sample of the left foot and *Proteus mirabilis* in the sample of the right foot (identification score values 2.350, 2.389, and 2.259, respectively). The confirmation of the identification results was attempted using Vitek 2 System (bioMérieux, Marcy l'Etoile, France), but *W. chitiniclastica* strain 7602 was identified as *Comamonas testosteroni* with 99 % probability (bionumber 0000000100500003). The isolated *W. chitiniclastica* strain 7602 was characterized with the following positive test results in Vitek GN card: L-lactate and succinate alkalization, L-lactate assimilation, tyrosine arylamidase activity, and positive reaction in Ellmann's test.

DNA of the isolated *W. chitiniclastica* strain 7602 was amplified and sequenced in 16S ribosomal RNA (rRNA) with primers (8F and 1510R) adapted from Zoetendal et al. (Zoetendal et al. 1998). The sequence was analyzed using Basic Local Alignment Search Tool (blast.ncbi.nlm.nih.gov), and it had 99 % similarity with *W. chitiniclastica*-type strain DSM 18708 16S rRNA and 82 % similarity with *Comamonas testosteroni*-type strain DSM 50244 16S rRNA. According to MALDI-TOF MS and sequence similarity results, the strain was finally identified as *W. chitiniclastica* and the partial sequence of the 16S rRNA gene was deposited in the GenBank database under accession no. KJ169569. The isolated *W. chitiniclastica* strain has been deposited at the WFCC Estonian Human Microbiota Biobank under accession number HUMB7602.

Antibiotic susceptibility of *W. chitiniclastica* strain 7602 was measured using agar-gradient method with antibiotic-immersed strips (Liofilchem s.r.l., Roseto degli Abruzzi, Teramo, Italy) on Mueller-Hinton agar (Oxoid Limited, Basingstoke, United Kingdom) including the following antibiotics (MIC μg/mL) proposed by Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute 2013) for other bacteria that do not belong to the Enterobacteriaceae family: piperacillin-tazobactam (0.75), ceftazidime (4), cefepime (1.5), imipenem (0.125), meropenem (0.094), gentamicin (0.25), amikacin (1.5), trimethoprim-sulfamethoxazole (2.0), colistin (1.5), levofloxacin (0.5), and ciprofloxacin (1.0). *W. chitiniclastica* strain 7602 was susceptible to all tested antibiotics according to CLSI breakpoints (Clinical and Laboratory Standards Institute 2013).

Discussion

W. chitiniclastica is a gram-negative strictly aerobic oxidase positive rod belonging to the class Gammaproteobacteria which was first isolated from the third stage larva of the fly *Wohlfahrtia magnifica* (Diptera: Sarcophagidae) (Tóth et al. 2008). Only two cases of *W. chitiniclastica* infection in humans—bacteremia and fulminant sepsis in Southeastern France and Argentina (Rebaudet et al. 2009; Almuzara et al.

Table 1 The origins of *Wohlfahrtiimonas chitiniclastica* strains deposited in The National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov) 16S ribosomal RNA gene database

Wohlfahrtiimonas chitiniclastica strains			
GenBank accession no.	Source	Country	Reference
AM397063	Fly *Wohlfahrtia magnifica*	Hungary	Tóth et al. 2008
AM401582	Aquatic plant	Egypt	Unpublished
AM420063	HUMAN subgingival plaque	Niger	Bolivar et al. 2012
DQ868763	HUMAN gangrene	Germany	Unpublished
EU008090	Unknown	India	Unpublished
EU484335	HUMAN bacteremia	France	Rebaudet et al. 2009
HM035991	Soil	China	Unpublished
HQ407260	Fly *Musca domestica*	India	Gupta et al. 2012
HQ407275	Fly *Musca domestica*	India	Gupta et al. 2012
JF692205	HUMAN sepsis	Argentina	Almuzara et al. 2011
JQ796717	Fly *Chrysomya megacephala*	China	Unpublished
KC422630	Fish	India	Unpublished
KJ169569	HUMAN soft tissue and bone infection	Estonia	Current publication

2011)—have been reported so far. Both patients were homeless with alcohol abuse and poor hygienic habits. Similarly, our patient was an alcohol abuser but not homeless. *W. chitiniclastica* has been isolated from different parasitic flies (Tóth et al. 2008; Cao et al. 2013) ubiquitous in areas of warmer climate but not in Northern Europe. The source of the two described human infections was attributed to flies. Recently, the case of septicemia secondary to wound myiasis in a deer was reported in USA (Thaiwong et al. 2014). There are few reports of *W. chitiniclastica* from houseflies (Gupta et al. 2012) and other environmental sources (Table 1), but not at high northern latitudes.

In our case, *W. chitiniclastica* was part of a polymicrobial infection represented in equal amounts with another gram-negative opportunistic pathogen, *Myroides odoratimimus,* in aerobic blood agar culture. Previously, the bacterial interactions in polymicrobial infections have shown to be strain specific (Sibley et al. 2008). Although *Myroides odoratimimus* infections are mainly associated with patients with an impaired immune defense, a case of soft tissue infection, septic shock, and pneumonia in an immunocompetent patient was described by Benedetti et al. (2011). It has been suggested that aggregative properties and biofilm formation are putative virulence factors for *Myroides* sp. The impact of simultaneous presence of *W. chitiniclastica* and *Myroides odoratimimus* in relation to tissue damage processes remains unclear.

Amoxicillin clavulanate was used for empirical treatment and continued for 8 days as improvement of clinical signs occurred. The clinical efficacy of amoxicillin clavulanate against *Wohlfahrtiimonas* and *Myroides* is unknown and successful treatment may be related to wound debridement.

Estonia is situated in the northern part of the temperate climate at 58°N, but the summer in 2013 was much warmer than the average in Northern Europe (National Oceanic and Atmospheric Administration. National Climatic Data Center 2013). Climate changes have been associated with altered range of several infectious diseases or their vectors (Bezirtzoglou et al. 2011). It is possible that flies carrying *W. chitiniclastica* have reached more northern latitudes than the previous as our patient has no international travel history. Although the natural habitat of *W. chitiniclastica* is not well investigated, the possibility of unrecorded colonization of the flies or other vectors in Estonia cannot be ruled out.

Microbial identification has reached a new level with the introduction of MALDI-TOF MS microbial cell protein-based identification system improving our knowledge about epidemiology of infectious diseases. In this case, *W. chitiniclastica* would have remained undetected with the traditional identification methods. As DNA sequencing is not routinely used in many laboratories, these bacteria may be more common but misidentified in clinical samples.

Our report provides new insights into clinical aspects and geographical spread of *W. chitiniclastica*. We emphasize the importance of correct identification of clinically relevant bacteria in order to observe global distribution of infectious diseases and their possible geographical changes.

Acknowledgments This study was supported by the Estonian Ministry of Education and Research (financing of scientific collections No. KOGU-HUMB) and the University of Tartu (grant No. SARMBARENG).

Conflict of interest On behalf of all the authors, the corresponding author states that there is no conflict of interest.

References

Almuzara MN, Palombarani S, Tuduri A, Figueroa S, Gianecini A, Sabater L, Ramirez MS, Vay CA (2011) First case of fulminant sepsis due to *Wohlfahrtiimonas chitiniclastica*. J Clin Microbiol 49: 2333–2335

Benedetti P, Rassu M, Pavan G, Sefton A, Pellizzer G (2011) Septic shock, pneumonia, and soft tissue infection due to *Myroides odoratimimus*: report of a case and review of *Myroides* infections. Infection 39:161–165

Bezirtzoglou C, Dekas K, Charvalos E (2011) Climate changes, environment and infection: facts, scenarios and growing awareness from the public health community within Europe. Anaerobe 17:337–340

Cao X-M, Chen T, Xu L-Z, Yao L-S, Qi J, Zhang X-L, Yan Q-L, Deng Y-H, Guo T-Y, Wang J, Hu K-X, Xu B-L (2013) Complete genome sequence of *Wohlfahrtiimonas chitiniclastica* strain SH04, isolated from *Chrysomya megacephala* collected from Pudong International Airport in China. Genome Announc 1(2):e0011913

Clinical and Laboratory Standards Institute (2013) Performance standards for antimicrobial susceptibility testing; M100-S23, 23rd informational supplement. Clinical and Laboratory Standards Institute, Wayne

Geneva Study Group on Noma (GESNOMA), Bolivar I, Whiteson K, Stadelmann B, Baratti-Mayer D, Gizard Y, Mombelli A, Pittet D, Schrenzel J (2012) Bacterial diversity in oral samples of children in Niger with acute noma, acute necrotizing gingivitis, and healthy controls. PLoS Negl Trop Dis 6:e1556. doi:10.1371/journal.pntd.0001556

Gupta AK, Dharne MS, Rangrez AY, Verma P, Ghate HV, Rohde M, Patole MS, Shouche YS (2011) *Ignatzschineria indica* sp. nov. and *Ignatzschineria ureiclastica* sp. nov., isolated from adult flesh flies (Diptera: Sarcophagidae). Int J Syst Evol Microbiol 61:1360–1369

Gupta AK, Nayduch D, Verma P, Shah B, Ghate HV, Patole MS, Shouche YS (2012) Phylogenetic characterization of bacteria in the gut of house flies (*Musca domestica* L.). FEMS Microbiol Ecol 79:581–593

Maurin M, Delbano JN, Mackaya L, Colomb H, Guier C, Mandjee A, Recule C, Croize J (2007) Human infection with *Schineria larvae*. Emerg Infect Dis 13:657–659

National Oceanic and Atmospheric Administration. National Climatic Data Center. (2013) Global Analysis - August 2013. http:// www.ncdc.noaa.gov/sotc/global/2013/8. Accessed 03 Dec 2013

Rebaudet S, Genot S, Renvoise A, Fournier PE, Stein A (2009) *Wohlfahrtiimonas chitiniclastica* bacteremia in homeless woman. Emerg Infect Dis 15:985–987

Roudiere L, Jean-Pierre H, Comte C, Zorgniotti I, Marchandin H, Jumas-Bilak E (2007) Isolation of *Schineria* sp. from a man. Emerg Infect Dis 13:659–661

Sibley CD, Duan K, Fischer C, Parkins MD, Storey DG, Rabin HR, Surette MG (2008) Discerning the complexity of community interactions using a Drosophila model of polymicrobial infections. PLoS Pathog 4(10):e1000184

Thaiwong T, Kettler NM, Lim A, Dirkse H, Kiupel M (2014) *Wohlfahrtiimonas chitiniclastica*—a first report of emerging zoonotic pathogen in the US. J Clin Microbiol 2014 Mar 26 [Epub ahead of print]

Tóth E, Kovács G, Schumann P, Kovács AL, Steiner U, Halbritter A, Márialigeti K (2001) *Schineria larvae* gen. nov., sp. nov., isolated from the 1st and 2nd larval stage of *Wohlfahrtia magnifica* (Diptera: Sarcophagidae). Int J Syst Evol Microbiol 51:401–407

Tóth EM, Schumann P, Borsodi AK, Kéki Z, Kovács AL, Márialigeti K (2008) *Wohlfahrtiimonas chitiniclastica* gen. nov., sp. nov., a new gammaproteobacterium isolated from *Wohlfahrtia magnifica* (Diptera: Sarcophagidae). Int J Syst Evol Microbiol 58:976–981

Zoetendal EG, Akkermans AD, De Vos WM (1998) Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. Appl Environ Microbiol 64:3854–3859