View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by IUPUIScholarWorks

ORIGINAL ARTICLE

Characterization of *Pontibacter altruii*, sp. nov., isolated from a human blood culture

M. Roiko[1], **M. May**[2] **and R. F. Relich**[3]

1) *Altru Health System, Department of Pathology and Laboratory Services, Grand Forks, ND, USA,* 2) *University of New England College of Osteopathic Medicine, Department of Biomedical Sciences, Biddeford, ME, USA and* 3) *Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA*

Abstract

The genus *Pontibacter* is a recent addition to the family *Cytophagaceae*, phylum *Bacteroidetes*. Previous reports of its cultivation and molecular detection are from a variety of environmental sources, including marine and desert habitats. We report the first description of a *Pontibacter* sp., which was initially identified as *Elizabethkingia meningoseptica*, isolated from a human clinical specimen. On the basis of 16S rRNA gene sequence, unique mass spectral profile and phenotypic characterization, this isolate represents a novel species within the genus *Pontibacter* that has been named *Pontibacter altruii*, sp. nov., strain Grand Forks.

© 2017 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

Keywords: Antibiotics, *Bacteroidetes*, Blood culture, Mass spectrometry, Novel species, *Pontibacter*

Original Submission: 14 April 2017; **Accepted:** 23 May 2017

Article published online: 3 June 2017

Corresponding author. R.F. Relich, Division of Clinical Microbiology, Suite 6027, Indiana University Health Pathology Laboratory, 350 West 11th Street, Indianapolis, IN 46202, USA

E-mail: rrelich@iupui.edu

Background

The phylum *Bacteroidetes* is an extremely large and diverse taxon composed of Gram-negative, non-sporulating, aerobic and anaerobic, rod-shaped bacteria. Among the many members of this phylum is the genus *Pontibacter* [1,2]. Members of this genus, like most of their relatives, occupy a vast array of habitats that include soil (mud and desert), marine and freshwater environments; however, until now, human clinical isolates of *Pontibacter* have not been reported [2]. Here we report the isolation and characterization of a novel *Pontibacter* sp., the first isolate in the genus to originate from a human. This bacterium was the sole isolate from one of four blood culture specimens collected from a woman with apparent sepsis, who later recovered upon treatment with broad-spectrum antimicrobial therapy; however, the role of this organism in the patient's illness is unknown. Detailed below is the clinical case description, details of strain isolation, and characterization of *Pontibacter altruii* sp. nov., strain Grand Forks.

Case description

A 72-year-old woman presented to the emergency department of a local hospital in Grand Forks, ND, USA with complaints of increasing shortness of breath and was noted to be acutely confused and in respiratory and renal failure. The patient's past medical history was significant for hypertension, hyperlipidaemia, type 2 diabetes mellitus and chronic hypoxic respiratory failure. A computed tomography scan of the patient's torso revealed mediastinal lymphadenopathy and pulmonary nodule enlargement, mitral valve calcification, cholelithiasis and diffuse pulmonary interstitial thickening with ground-glass opacities consistent with possible interstitial pulmonary oedema secondary to congestive heart failure. A transthoracic echocardiogram showed possible vegetations on the mitral and tricuspid valves, but vegetations were not apparent by trans-oesophageal echocardiogram. The patient

© 2017 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

http://dx.doi.org/10.1016/j.nmni.2017.05.016

was admitted to the intensive care unit and was immediately prescribed empiric antimicrobial therapy for treatment of suspected pneumonia. On admission, blood cultures were collected by venipuncture and submitted in two paediatric blood culture bottles, documented as low-volume collections; a sputum culture was ordered, but was cancelled because a sputum specimen could not be procured. Results of tests for autoimmune diseases, influenza and disseminated fungal infections were all negative.

Following 72 h of incubation in a continuous monitoring blood culture system (BACTEC™ FX; BD Diagnostics, Sparks, MD), the aerobic culture vial (BD BACTEC™ Plus Aerobic/F) from one of two sets of blood cultures flagged positive. Microscopic examination of a Gram-stained smear of the blood culture broth revealed pleomorphic Gram-negative rods. Aliquots of the blood culture broth were subcultured to solid media, including sheep blood agar (tryptic soy agar containing 5% defibrinated sheep blood), chocolate agar and MacConkey agar (Remel, Lenexa, KS, USA). Following 24 h of incubation at 35 °C in 5% CO_2, a pure culture of small, white, smooth colonies grew on blood and chocolate agars, but no growth was noted on the MacConkey or other plates (Columbia CNA agar and anaerobe cultivation media). Gram-negative rods identical to those seen in the blood culture broth were observed in a Gram-stained smear of the colonial growth (Fig. 1). The isolate tested positive for cytochrome oxidase and catalase, but tested negative for indole production using a spot indole testing procedure. Identification of the isolate was attempted by automated (VITEK® 2 Gram-Negative Identification card; bioMérieux, Durham, NC, USA) and manual (API 20NE; bioMérieux) phenotypic methods, and automated (VITEK® 2) testing was performed for preliminary antimicrobial susceptibility testing.

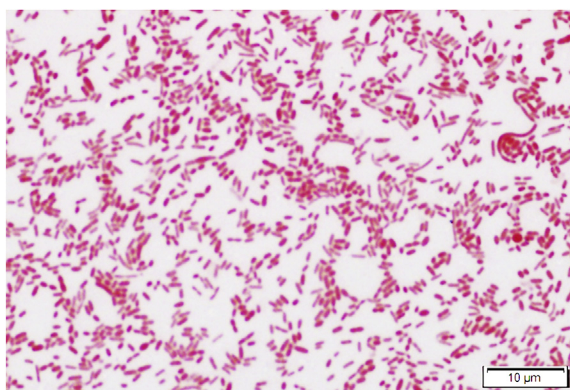

FIG. 1. Gram stain of *Pontibacter altruii*, sp. nov. strain Grand Forks reveals that cells are pleomorphic Gram-negative rods that vary in both length and width. On average, cells measured 0.6 × 1.3 μm in greatest dimension (average of 100 cells). Original magnification, 1000×.

On admission, the patient started intravenous vancomycin (2000 mg on day 1, 1000 mg on day 2) and meropenem (500 mg/8 h). The patient stabilized overnight in the intensive care unit and was transferred to the general ward 17 h after admission. After 2 days of antibiotic treatment, vancomycin was discontinued due to elevated creatinine. The same day, a rash was observed and meropenem was switched to intravenous aztreonam (1 g/8 h) as the patient had a history of penicillin allergy. Following blood culture detection, but before susceptibility testing of the isolate, the patient also received intravenous levofloxacin (750 mg/48 h). During her inpatient stay, the patient also received warfarin, intravenous furosemide and bi-level positive airway pressure therapy for prevention of thromboembolism associated with atrial fibrillation, treatment of atrial fibrillation, hypertension and respiratory failure, respectively. On hospital day 17, she was switched to oral levofloxacin (750 mg/48 h for 10 days) and discharged to home in a stable condition. Two additional sets of blood cultures collected 1 day after the initial positive culture remained negative. At home, the patient was ordered to continue positive airway pressure therapy. To date, the patient is not known to have experienced a recrudescence of this infectious process.

Materials and methods

Morphological and physicochemical analyses

Morphological attributes of this isolate, including cell shape and size, were rendered from 1000× photomicrographs of Gram-stained smears of colonial growth using an Olympus BX51 bright-field microscope equipped with a DP70 12-bit digital camera and Olympus cellSens software (Olympus, Waltham, MA, USA). Size measurements of 100 individual cells were made using ImageJ 1.X software [3] calibrated to the scale bar attached to the photomicrographs.

Following primary isolation, the isolate was subjected to automated phenotyping using the VITEK® 2 Gram-Negative Identification card following the manufacturer's instructions for use. Briefly, colonies of the isolate were suspended in sterile 0.45% (w/v) non-bacteriostatic saline to create a cell suspension in the range of 0.5–0.63 McFarland units. Next, the suspension was applied, under vacuum, to a Gram-negative identification panel containing immobilized chromogenic substrates. Following incubation, the biochemical profile and presumptive identification were derived by the VITEK® 2 software. In addition, the isolate was preliminarily identified using the API 20NE system. Characterization of the isolate using standard tubed biochemical media was later pursued, the results of which are listed in Table 1.

© 2017 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases, *NMNI*, **19**, 71–77
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

TABLE 1. Comparison of phenotypic characteristics of the genus *Pontibacter* and the proposed new species, *Pontibacter altruii*, sp. nov.

Characteristics	*Pontibacter* genus	*Pontibacter altruii* sp. nov.
Cell shape and size	Rods: 0.3–0.7 × 1.2–1.9 μm	Rods: 0.6 × 1.3 μm
Motility	Gliding	Gliding
Pigment production	Pink, non-diffusible	No pigment
Growth environment	Strict aerobe	Strict aerobe
Oxidase	Positive	Positive
Catalase	Positive	Positive
Alkaline phosphatase	Positive	Positive
Esculin hydrolysis	Positive	Positive
Gelatin hydrolysis	Positive	Positive
DNA hydrolysis	Positive	Positive
Nitrate reduction	Negative	Negative
Indole production	Negative	Negative
H_2S production	Negative	Weak-positive
0% NaCl tolerance	Positive	Positive
Casein hydrolysis	Negative	NT
Tween-80 hydrolysis	Negative	NT
Chitin hydrolysis	Negative	NT
Cellulose hydrolysis	Negative	NT
Carbohydrate utilization	Positive	Dextrose: Negative Lactose: Weak-positive Maltose: Negative Mannitol: Negative Sucrose: Negative Xylose: Negative

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using Etest (bioMérieux) and disc diffusion; testing was performed according to standard methods. Briefly, a suspension of bacterial colonies from a culture <24 h old were suspended in sterile 0.45% (w/v) non-bacteriostatic saline and the turbidity of the suspension was adjusted to match a 0.5 McFarland standard. Cation-adjusted Mueller–Hinton agar plates (150 mm; Remel, Lenexa, KS, USA) were subsequently inoculated with the suspension. When dry, either Etest strips or antibiotic-impregnated discs were applied with forceps, and inoculated plates were incubated at 35 °C in an ambient atmosphere for 24 h.

Nucleic acid sequencing and mass spectrometry

Total DNA was extracted from the isolate using a generic lysis and silica adsorption-based separation protocol (NucliSENS® EasyMAG®, bioMérieux; [4,5]). A portion of the 16S ribosomal RNA gene was amplified with primers 27-F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492-R (5′-ACGGCTACCTTGTTACGACTT-3′) [6] using Roche Life Science Expand High Fidelity^{plus} reagents according to the manufacturer's recommendations (Roche, Basel, Switzerland). The amplicon sequence was derived by standard dideoxy labelling methods at the Interdisciplinary Center for Biotechnology Research (University of Florida). Identification via ribosomal RNA gene sequence was attempted by BLAST query of the National Center for Biotechnology Information database GenBank [7,8].

Identification of the isolate was attempted by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry using a MALDI Biotyper instrument (Bruker-Daltonics, Billerica, MA, USA); half of the spots were overlaid with 70% formic acid for on-target protein extraction [9]. Briefly, small portions of isolated colonies were smeared onto six separate spots on a 96-spot stainless steel MALDI-TOF target plate. Three of the spots were overlaid with 1 μl of 70% formic acid and allowed to completely dry. Next, all 6 spots were overlaid with 1 μl of matrix solution (α-cyano-4-hydroxycinnamic acid), dried and analysed. Each spot was lased 240 times to generate mass spectral profiles. Although mass peaks were recorded for all spots, no identification could be rendered from the MALDI Biotyper research use only library (BDAL, MBT Compass, 2016).

Phylogenetic analysis

The rRNA gene sequence derived from the isolate was used to construct a weighted neighbour-joining ('weighbour') tree employing Jukes–Cantor correction (100 bootstrapped replicates) [10]. The 16S rRNA gene sequence from the within-phylum organism *Bacteroides fragilis* (strain Bfr920) was used as the outgroup. The phylogenetic analysis was carried out using the Ribosomal Database Project (RDP) platform (rdp.cme.msu.edu) [11].

Results

Clinical microbiology

After 3 days of incubation, one aerobic, paediatric vial from the two collected grew an unknown isolate on sheep blood and chocolate agars upon subculture. Primary phenotypic testing provisionally identified the organism as *Elizabethkingia meningoseptica*; however, low confidence scores for both VITEK® 2-based and API 20NE-based identifications were obtained, prompting referral of the isolate to a commercial reference laboratory for definitive identification. The reference laboratory could not provide a species-level identification, but instead reported the isolate's identification as an unidentified Gram-negative rod, most common relative *Pontibacter*, based on partial 16S rRNA gene sequencing.

Colonial characteristics

The isolate grows moderately slowly on sheep blood and chocolate agars, but does not grow on Gram-negative selective media, including MacConkey agar, and fails to grow in anaerobic conditions. Pigment production, a prominent feature of other *Pontibacter* spp., was not demonstrable on sheep blood and tryptic soy agars following 7 days of incubation. Following 24 h of incubation, colonies are small (~1 mm), smooth and glistening (Fig. 2A). After 72 h at 35 °C, colonies reach maximum size

© 2017 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases, *NMNI*, **19**, 71–77
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

(4–5 mm) and are umbonate (Fig. 2B). Following 96 h of incubation, a noticeable haze of growth emanating from the colonies is observable (Fig. 2C), and is an indication of gliding motility, a form of motility described for other pontibacters [2]. Turbidity is seen in broth cultures after several days of incubation.

Phenotypic characterization

The isolate exhibits all defining phenotypes common to members of the genus *Pontibacter*, including morphology, Gram-stain reaction, gliding motility, strictly aerobic growth, cytochrome oxidase and catalase production. Biochemical parameters assessed and comparison to other *Pontibacter* spp. are listed in Table 1.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing demonstrated low MICs or large zones of inhibition surrounding most agents tested, except for the aminoglycosides amikacin, gentamicin and tobramycin (Table 2).

Molecular characterization

The ribosomal RNA gene sequence showed 96% identity to *Pontibacter rhizosphera* and 95% identity to *Pontibacter korlensis*. Mass spectrum analysis of the isolate could not furnish an identification. The failure of both mass spectrometry and 16S rRNA gene sequencing to definitively identify the isolate suggested that it had not been previously characterized.

Phylogenetic analysis

Using weighted neighbour-joining methods, the isolate formed its own branch within the genus *Pontibacter*. The deepest branching species from the root was *Pontibacter ruber*, followed by a small group containing *Pontibacter deserti* and *Pontibacter populi*, which was followed by the novel isolate (Fig. 3).

TABLE 2. Antimicrobial susceptibility testing results.

Antibiotic/test[a]	MIC or zone of inhibition diameter
β-lactamase[b]	Positive
Ampicillin	0.38 μg/mL
Azithromycin	0.38 μg/mL
Aztreonam	48 mm
Cefepime	1.5 μg/mL
Ceftazidime	6 μg/mL
Ceftriaxone	8 μg/mL
Ciprofloxacin	0.94 μg/mL
Colistin	12 μg/mL
Doripenem	0.125 μg/mL
Ertapenem	0.064 μg/mL
Erythromycin	0.19 μg/mL
Gentamicin	128 μg/mL
Levofloxacin	0.094 μg/mL
Meropenem	0.094 μg/mL
Moxifloxacin	0.064 μg/mL
Penicillin G	0.50 μg/mL
Piperacillin-tazobactam	0.023 μg/mL
Rifampin	45 mm
Ticarcillin-clavulanate	0.19 μg/mL
Tigecycline	0.064 μg/mL
Tobramycin	>256 μg/mL
Trimethoprim-sulfamethoxazole	0.94 μg/mL

[a]For drugs other than piperacillin-tazobactam and rifampin, MICs were determined by Etest using cation-adjusted Mueller–Hinton agar incubated in an ambient atmosphere at 35 °C for 24 h. Haemophilus Test Medium agar was used for piperacillin-tazobactam Etest and rifampin disc diffusion testing with incubation for 48 h at 35 °C.
[b]β-lactamase testing was performed by Nitrocefin disc testing.

Discussion

Isolation of a novel member of the genus *Pontibacter* is described. *Pontibacter* spp. are known to occupy diverse habitats, but this is the first description of a *Pontibacter* sp. isolated from a human clinical specimen. Because all previous descriptions in the literature are from environmental sources (Table 3), coupled with the fact that this isolate was only isolated from one of four blood culture vials, a criterion commonly used by clinical microbiology laboratories for defining blood culture contamination with host microbiota, the organism's pathogenic potential

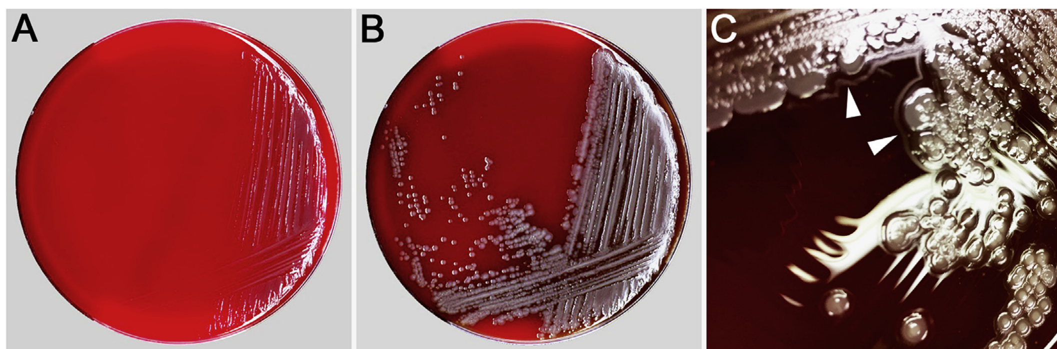

FIG. 2. Colonial morphology and growth characteristics of *Pontibacter altruii*, sp. nov. strain Grand Forks grown on standard sheep blood agar. (A) After 24 h of incubation at 35 °C in an ambient atmosphere, colonies are small, smooth and white. (B) Following 72 h of incubation, colonies measure up to 5 mm and are smooth, white and umbonate. (C) Following 96 h of incubation, a light haze of growth is noticeable at the margin of colonies (darts) indicating gliding motility.

© 2017 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases, *NMNI*, **19**, 71–77
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

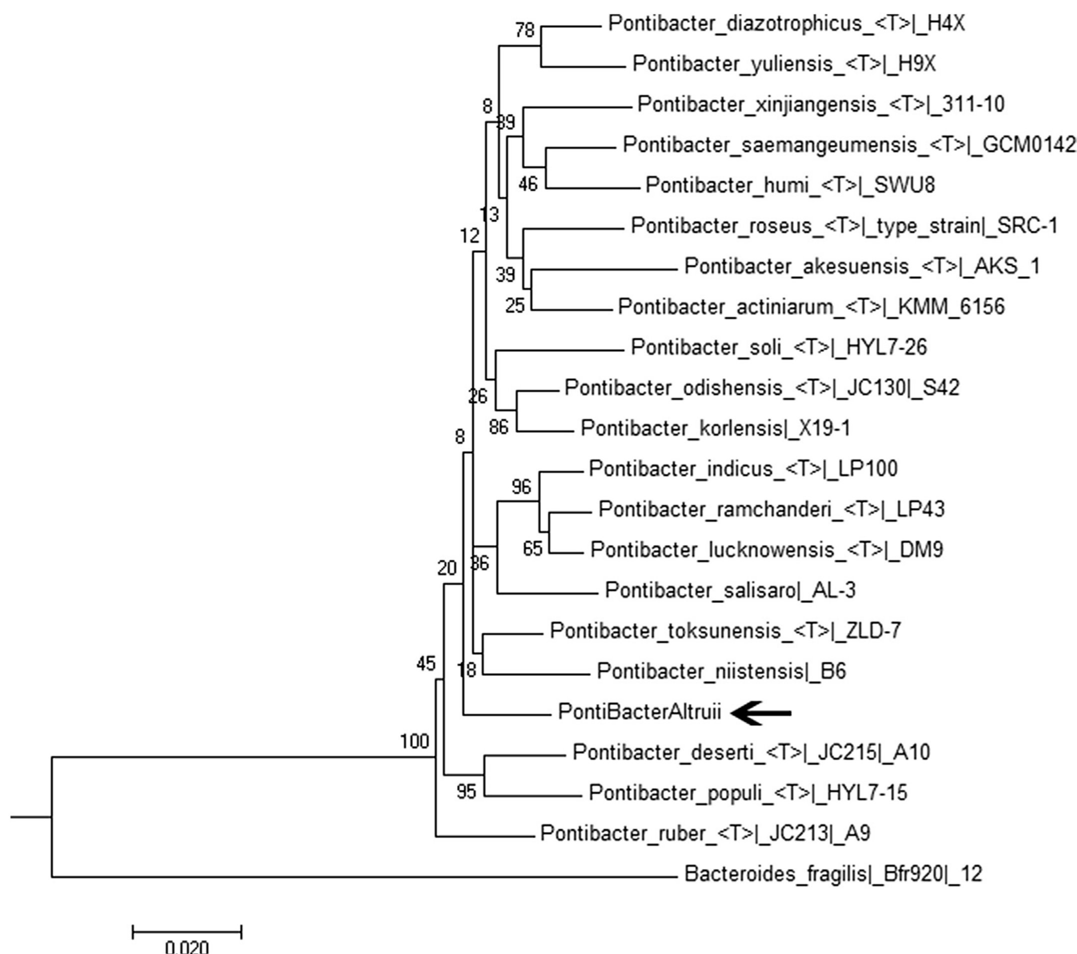

FIG. 3. Phylogenetic analysis of the genus *Pontibacter*, including *Pontibacter altruii* sp. nov. A weighted neighbour-joining tree employing Jukes–Cantor correction (100 bootstrapped replicates) was generated using 16S ribosomal RNA gene sequence from 20 previously described *Pontibacter* species, *P. altruii*, sp. nov. and *Bacteroides fragilis* (outgroup). The position of *P. altruii*, sp. nov. (arrow) was somewhat ambiguous; however, it unambiguously clustered within the genus rather than creating a unique lineage.

is unknown. In addition, direct testing of the patient's blood for this bacterium by methods such as PCR and next generation sequencing could not be performed, as the original blood specimens had already been discarded by the time such analyses were considered. The potential for this organism's misidentification through automated and manual, kit-based phenotypic testing platforms was exemplified in this report, as our isolate was identified as *E. meningoseptica*. In addition, the relatively recent designation of this genus suggests that *Pontibacter* spp. could have been isolated from other human infections in the past, but was given one or more misnomers. Many biochemical reactions are consistent with those of *E. meningoseptica*, but our isolate is indole negative, a characteristic that can be used to distinguish our isolate from *E. meningoseptica*. The data also highlight the need for confirming unusual identifications with additional methods, such as nucleic acid sequencing. This is, predictably, a relatively common scenario when commercially available microbial identification systems are challenged with rare and obscure taxa.

To the best of our knowledge, this is the first report of broad antimicrobial susceptibility profiling of a *Pontibacter* isolate. Although no categorical antimicrobial susceptibility interpretive guidelines exist for pontibacters, the MICs and zones of inhibition gleaned from in-depth testing shed some light on the susceptibility of this organism to a variety of antibiotic classes. For example, these data suggest either acquired or intrinsic resistance to aminoglycosides. This profile is consistent with that of other members of this genus, which also exhibit resistance to at least one member of the aminoglycosides [1,2,12]. However, MICs for most other antibiotics are low, suggesting that this isolate is susceptible to most routinely used antibiotics.

Ribosomal and phylogenetic analyses indicate that our isolate represents a novel species, and is appropriately assigned to the genus *Pontibacter*. The ribosomal RNA gene sequence and mass

© 2017 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases, *NMNI*, **19**, 71–77
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

TABLE 3. ***Pontibacter*** **species described in the literature are from a wide variety of environmental sources.**

Pontibacter species	Source	Reference
P. amylolyticus	Deep-sea hydrothermal vent	Wu et al., 2016 [13]
P. actiniarum	Marine	Nedashkovskaya et al., 2005 [1]
P. akesuensis	Desert soil	Zhou et al., 2007 [14]
P. chinhatensis	Pond sediment containing hexachlorocyclohexane isomer waste	Singh et al., 2015 [15]
P. deserti	Desert soil	Subhash et al., 2014 [16]
P. diazotrophicus	Takalamakan desert	Xu et al., 2014 [17]
P. humi	Mountain soil	Srinivasan et al., 2014 [18]
P. indicus	Hexachlorocyclohexane-contaminated soil	Singh et al., 2014 [19]
P. jeungdoensis	Solar saltern	Joung et al., 2013 [20]
P. korlensis	Desert sand	Zhang et al., 2008 [21]
P. locisalis Sy30T	Soil from abandoned saltern	Zhou et al., 2016 [22]
P. lucknowensis	Hexachlorocyclohexane dump site	Dwivedi et al., 2013 [23]
P. mucosus	Hexachlorocyclohexane-contaminated pond sediment	Nayyar et al., 2016 [24]
P. niistensis	Forest soil	Dastager et al., 2010 [25]
P. odishensis	Dry soil of solar saltern	Subhash et al., 2013 [26]
P. populi	Soil of *Euphrates poplar* forest	Xu et al., 2012 [27]
P. ramchanderi	Hexachlorocyclohexane-contaminated pond sediment	Singh et al., 2013 [28]
P. rhizosphera	Rhizosphere soil of *Nerium indicum*	Raichand et al., 2011 [12]
P. roseus	Muddy waters of drainage system	Mukherjee et al., 2015 [29]
P. ruber	Desert soil	Subhash et al., 2014 [16]
P. saemangeumensis	Seawater	Kang et al., 2013 [30]
P. salisaro	Clay tablet solar saltern	Joung et al., 2011 [31]
P. toksuensis	Arid soil	Zhang et al., 2013 [32]
P. ummariensis	Hexachlorocyclohexane-contaminated soil	Mahato et al., 2015 [33]
P. xinjiangensis	Soil	Wang et al., 2010 [34]
P. yuliensis	Soil of a *Populus euphratica* forest in the Taklamakan desert	Cao et al., 2014 [35]
Pontibacter sp. nov. BAB1700	Drilling well sediments	Joshi et al., 2012 [36]

spectrum are unique to this organism. Its phylogenetic position based on 16S ribosomal RNA gene sequence indicates that it is a relatively divergent member of the genus, though not the most divergent. Based on these data, we propose the formal name *P. altruii* sp. nov., strain Grand Forks after the laboratory and geographic location of its isolation, respectively.

Conclusion and isolate naming

Description of *P. altruii* sp. nov., strain grand forks

P. altruii sp. nov. (al tru' i i) N.L. neut. Of Altru Health System laboratory, the clinical laboratory responsible for recognizing the novelty of the species. Cells are Gram-negative, rod shaped, pleomorphic, and move by gliding motility. Aerobic. Non-sporulating. Chemoorganotroph. Acid is produced from lactose. Esculin, DNA and gelatin are hydrolysed. Catalase, oxidase and hydrogen sulphide are produced. Unique 16S rRNA gene sequence (GenBank Accession no. KX982528) distinct from the most closely related species in the genus *Pontibacter*. First isolated from the blood culture of a woman with sepsis in Grand Forks, North Dakota, USA. The type strain is Grand Forks.

Transparency declaration

The authors have no competing interests to declare. Ethics approval and consent to participate. Specimens were de-identified and so exempted from consent requirements.

Availability of data

Ribosomal RNA sequence has been deposited in GenBank under the accession number KX982528. *P. altruii* strain Grand Forks can be readily obtained by the authors.

Funding

This work was supported by internal funds from Altru Health System, the University of New England College of Osteopathic Medicine and the Indiana University School of Medicine.

Author contributions

MR made the original isolation and performed the phenotypic characterization and 16S sequencing. MM performed the phylogenetic and taxonomic analysis. RFR performed mass spectrometry, antimicrobial susceptibility testing, biochemical analysis, and imaging of the isolate. This manuscript was drafted by MR, MM and RFR.

Acknowledgements

The authors wish to thank Brian Emery of the Special Bacteriology Reference Laboratory, Bacterial Special Pathogens

© 2017 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases, *NMNI*, **19**, 71–77
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Branch, US CDC for his help with initial phenotypic characterization, and Courtney Pearson of the University of New England for her assistance with manuscript preparation.

References

[1] Nedashkovskaya OI, Kim SB, Suzuki M, Shevchenko LS, Lee MS, Lee KH, et al. *Pontibacter actiniarum* gen. nov., sp. nov., a novel member of the phylum "Bacteroidetes," and proposal of Reichenbachiella gen. nov. as a replacement for the illegitimate prokaryotic generic name Reichenbachia Nedashkovskaya, et al. 2003. Int J Syst Evol Microbiol 2005;55:2583–8.

[2] Nedashkovskaya OI, Kim SB. Genus XIV. Pontibacter. In: Krieg NR, Ludwig W, Whitman W, et al., editors. Bergey's manual of systematic Bacteriology. 2nd ed. New York: Springer Science & Business Media; 2011. p. 410–2.

[3] Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 2012;9:671–5.

[4] Tang Y-W, Sefers SE, Li H, Kohn DJ, Procop GW. Comparative evaluation of three commercial systems for nucleic acid extraction from urine specimens. J Clin Microbiol 2005;43:4830–3.

[5] Béssède E, Renaudin H, Clerc M, de Barbeyrac D, Bebear C, Pereyre S. Evaluation of the combination of the NucliSENS easyMAG and the EasyQ applications for the detection of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in respiratory tract specimens. Eur J Clin Microbiol Infect Dis 2010;29:187–90.

[6] Decker C, Olu K, Arnaud-Haond S, Duperron S. Physical proximity may promote lateral acquisition of bacterial symbionts in vesicomyid clams. PLoS One 2013;8(7):e64830.

[7] Altschul SF, Madden TL, Schäffer 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.

[8] Benson DA, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Res 2015;43:D30–5.

[9] Theel ES, Schmitt BH, Hall L, Cunningham SA, Walchak RC, Patel R, et al. Formic acid-based direct, on-plate testing of yeast and *Corynebacterium* species by Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2012;50:3093–5.

[10] Bruno WJ, Socci ND, Halpern AL. Weighted neighbor joining: a likelihood-based approach to distance-based phylogeny reconstruction. Mol Biol Evol 2000;17:189–97.

[11] Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res 2014;42:D633–42.

[12] Raichand R, Kaur I, Singh NK, Mayilraj S. *Pontibacter rhizosphera* sp. nov., isolated from rhizosphere soil of an Indian medicinal plant *Nerium indicum*. Antonie Van Leeuwenhoek 2011;100:129–35.

[13] Wu Y-H, Zhou P, Jian SL, Liu ZS, Wang CS, Oren A, et al. *Pontibacter amylolyticus* sp. nov., isolated from a deep-sea sediment hydrothermal vent field on the Southwest Indian Ridge. Int J Syst Evol Microbiol 2016. http://dx.doi.org/10.1099/ijsem.0.000944.

[14] Zhou Y, Wang X, Liu H, Zhang KY, Zhang YQ, Lai R, et al. *Pontibacter akesuensis* sp. nov., isolated from a desert soil in China. Int J Syst Evol Microbiol 2007;57:321–5.

[15] Singh AK, Garg N, Lal R. *Pontibacter chinhatensis* sp. nov., isolated from pond sediment containing discarded hexachlorocyclohexane isomer waste. Int J Syst Evol Microbiol 2015;65:2248–54.

[16] Subhash Y, Sasikala C, Ramana CV. *Pontibacter ruber* sp. nov. and *Pontibacter deserti* sp. nov., isolated from the desert. Int J Syst Evol Microbiol 2014;64:1006–11.

[17] Xu L, Zeng X-C, Nie Y, Luo X, Zhou E, Zhou L, et al. *Pontibacter diazotrophicus* sp. nov., a novel nitrogen-fixing bacterium of the family Cytophagaceae. PLoS One 2014;9(3):e92294.

[18] Srinivasan S, Lee J-J, Kim MK. *Pontibacter humi* sp. nov., isolated from mountain soil. Curr Microbiol 2014;69:263–9.

[19] Singh AK, Garg N, Lata P, Kumar R, Negi V, Vikram S, et al. *Pontibacter indicus* sp. nov., isolated from hexachlorocyclohexane-contaminated soil. Int J Syst Evol Microbiol 2014;64:254–9.

[20] Joung Y, Kim H, Lee BI, Kang H, Jang TY, Kwon OS, et al. *Pontibacter jeungdoensis* sp. nov., isolated from a solar saltern in Korea. J Microbiol 2013;51:531–5.

[21] Zhang L, Zhang Q, Luo X, Tang Y, Dai J, Li Y, et al. *Pontibacter korlensis* sp. nov., isolated from the desert of Xinjiang, China. Int J Syst Evol Microbiol 2008;58:1210–4.

[22] Zhou Y-X, Xie Z-H, Zhao JX, Du ZJ, Chen GJ. *Pontibacter locisalis* Sy30T sp. nov. isolated from soil collected from an abandoned saltern. Antonie Van Leeuwenhoek 2016;109:415–20.

[23] Dwivedi V, Niharika N, Lal R. *Pontibacter lucknowensis* sp. nov., isolated from a hexachlorocyclohexane dump site. Int J Syst Evol Microbiol 2013;63:309–13.

[24] Nayyar N, Kohli P, Mahato NK, Lal R. *Pontibacter mucosus* sp. nov., isolated from hexachlorocyclohexane contaminated pond sediment. Int J Syst Evol Microbiol 2016. http://dx.doi.org/10.1099/ijsem.0.001013.

[25] Dastager SG, Raziuddin QS, Deepa CK, Li WJ, Pandey A. *Pontibacter niistensis* sp. nov., isolated from forest soil. Int J Syst Evol Microbiol 2010;60:2867–70.

[26] Subhash Y, Tushar L, Sasikala C, Ramana CV. *Erythrobacter odishensis* sp. nov. and *Pontibacter odishensis* sp. nov. isolated from dry soil of a solar saltern. Int J Syst Evol Microbiol 2013;63:4524–32.

[27] Xu M, Wang Y, Dai J, Jiang F, Rahman E, Peng F, et al. *Pontibacter populi* sp. nov., isolated from the soil of a Euphrates poplar (*Populus euphratica*) forest. Int J Syst Evol Microbiol 2012;62:665–70.

[28] Singh AK, Garg N, Sangwan N, Negi V, Kumar R, Vikram S, et al. *Pontibacter ramchanderi* sp. nov., isolated from hexachlorocyclohexane-contaminated pond sediment. Int J Syst Evol Microbiol 2013;63:2829–34.

[29] Mukherjee S, Lapidus A, Shapiro N, Cheng J-F, Han J, Reddy T, et al. High quality draft genome sequence and analysis of *Pontibacter roseus* type strain SRC-1(T) (DSM 17521(T)) isolated from muddy waters of a drainage system in Chandigarh, India. Stand Genomic Sci 2015;10:8.

[30] Kang JY, Joung Y, Chun J, Kim H, Joh K, Jhang KY. *Pontibacter saemangeumensis* sp. nov., isolated from seawater. Int J Syst Evol Microbiol 2013;63:565–9.

[31] Joung Y, Kim H, Ahn TS, Jon K. *Pontibacter salisaro* sp. nov., isolated from a clay tablet solar saltern in Korea. J Microbiol 2011;49:290–3.

[32] Zhang L, Zhu L, Wei L, Li C, Wang Y, Shen X. *Pontibacter toksunensis* sp. nov., isolated from soil, and emended descriptions of *Pontibacter roseus* and *Pontibacter akesuensis*. Int J Syst Evol Microbiol 2013;63:4462–8.

[33] Mahato NK, Tripathi C, Nayyar N, Singh AK, Lai R. *Pontibacter ummariensis* sp. nov., isolated from a hexachlorocyclohexane contaminated soil. Int J Syst Evol Microbiol 2015;66:1080–7.

[34] Wang Y, Zhang K, Cai F, Zhang L, Tang Y, Dai J, et al. *Pontibacter xinjiangensis* sp. nov., in the phylum "Bacteroidetes," and reclassification of [*Effluviibacter*] *roseus* as *Pontibacter roseus* comb. nov. Int J Syst Evol Microbiol 2010;60:99–103.

[35] Cao H, Nie Y, Zeng XC, Xu L, He Z, Luo X, et al. *Pontibacter yuliensis* sp. nov., isolated from soil. Int J Syst Evol Microbiol 2014;64:968–72.

[36] Joshi MN, Sharma AC, Pandya RV, et al. Draft genome sequence of *Pontibacter* sp. nov. BAB1700, a halotolerant, industrially important bacterium. J Bacteriol 2012;194:6329–30.

© 2017 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases, *NMNI*, **19**, 71–77
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).