ORIGINAL ARTICLE

Diagnostic approach for detection and identification of emerging enteric pathogens revisited: the *(Ali)arcobacter lanthieri* case

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Abstract

An immunocompetent patient without a history of recent travel or animal exposure developed persistent abdominal bloating and cramps without diarrhoea or fever. Negative additional investigations excluded gastritis, infectious colitis, inflammatory bowel disease and neoplasia, but routine stool culture detected a *Campylobacter*-like organism. The isolate was obtained with use of a polycarbonate filter technique, emphasizing the importance of culture to support and validate the occurrence of emerging and new bacterial enteric pathogens. The ensuing extensive laboratory examinations proved challenging in identifying this potential pathogen. Phylogenetic marker analysis based on the 16S ribosomal RNA and *rpoB* gene sequences revealed that the isolate was most closely related to *Arcobacter lanthieri* and *Arcobacter faecis*. Subsequent analysis of a draft whole genome sequence assigned the isolate to *A. lanthieri*. We report the presence of five virulence genes, *cadF*, *ciaB*, *mviN*, *hecA* and *iroE*, indicating a possible pathogenic nature of this organism. This case demonstrated the importance of the use of agnostic methods for the detection of emerging pathogens in cases of enteric disease with a wide array of gastrointestinal symptoms.

Keywords: *Arcobacter*, *Campylobacteraceae*, diagnosis, gastrointestinal disease, public health, zoonosis
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Introduction

The genus *Arcobacter* was proposed in 1991 [1] and allocated together with the genus *Campylobacter* into the family *Campylobacteraceae*. Since then it has come to comprise some 30 validly named species isolated from a wide variety of hosts and the environment. In 2018 Pérez-Cataluña *et al.* [2] suggested the subdivision of the genus *Arcobacter* into seven different genera on the bases of phylogenetic and genomic analyses. The novel genus names were validated, but the proposal was subsequently refuted by On *et al.* [3].

At present, five *Arcobacter* species have been associated with intestinal disease in humans, with clinical symptoms such as diarrhoea, abdominal pain, nausea, vomiting and fever [4]. *Arcobacter butzleri* and *A. cryaerophilus* are the predominant species recorded, but infections with *A. skirrowii*, *A. thereius* and more recently *A. mytili* and *A. lanthieri* have also been reported [5–8]. Contaminated drinking water, contact with pets and manipulation and consumption of foods of animal origin are likely to be the infection sources [9,10].

The present case describes the clinical characteristics of a patient with gastrointestinal symptoms without diarrhoea, leading to the detection of a *Campylobacter*-like organism and difficulties encountered during the extensive laboratory examinations performed in the quest for a correct identification.

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Materials and methods

Patient information and microbiologic examination

All clinical data were obtained prospectively. A single clinical stool specimen was collected according to standard protocols. Microbiologic stool testing to identify bacterial or parasitologic pathogens was performed: standard bacteriologic culture for presence of *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Campylobacter* spp. and *Arcobacter* spp., glutamate dehydrogenase antigen screening and anaerobic culture on chromogenic agar for *Clostridioides difficile*. In the absence of any travel of the patient, only parasitologic antigen detection for endemic the parasites *Cryptosporidium* spp. and *Giardia lamblia* was carried out.

For the isolation of *Campylobacter*-like organisms, 0.5 g stool was inoculated into 5 mL tryptone soy broth (CM0129; Oxoid, Basingstoke, UK) supplemented with 5% lysed horse's blood and the CAT antibiotic supplement (SR0174; Oxoid), then incubated at 35°C in a microaerobic atmosphere (6% O_2, 7% CO_2, 7% H_2 and 80% N_2) for up to 24 hours. Next, the method as descrived by Steele and McDermott was applied with minor modifications [11]. In brief, six drops of enrichment broth were transferred onto the surface of a 0.6 µm Whatman Nuclepore polycarbonate filter and allowed to filter passively at 35°C for 1 hour under 5% CO_2 atmosphere onto blood agar medium (bioMérieux, Marcy l'Etoile, France). After removal of the filter, the plate was incubated further for up to 48 hours under a microaerobic atmosphere. Suspected colonies were subcultivated on blood agar plates for further testing by standard conventional biochemical methods.

Identification of bacterial strain by conventional methods

MALDI-TOF MS analysis. Identification of the isolate with Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) by direct smear and tube extraction was attempted [12]. Mass spectra were generated by a Microflex LT MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) using Flex Control software and compared to the Bruker MSP database (version DB6903) and a validated in-house database for identification of *Arcobacter* spp. of human interest [13] using Bruker Compass software.

Phylogenetic marker analyses. In the clinical laboratory, the 16S ribosomal RNA (rRNA) gene was amplified using primers 27F and 1429R, sequenced using the primer 518F (BaseClear, Leiden, Netherlands) and analysed using BLASTn (http://blast.ncbi.nlm.nih.gov/) [14]. The isolate was then sent to the microbiology laboratory at Ghent University for further identification, where it was labelled as isolate R-75363. The near-complete 16S rRNA gene sequence was determined as described previously [15]. Amplicons were submitted for Sanger sequencing (Eurofins, Luxembourg) and sequence assembly was performed using BioNumerics 7.6 software (Applied Maths, Sint-Martens-Latem, Belgium). A BLASTn analysis and a similarity-based search against a quality-controlled database of 16S rRNA sequences (EzBioCloud; https://www.ezbiocloud.net/) was performed [16]. The *rpoB* gene was amplified using *Campylobacteraceae*-specific primers and PCR conditions [17,18]. The PCR product was sequenced and compared to sequences of the nucleotide collection database using BLASTn. A sequence similarity of 97.7% was used as the cutoff value for species delineation [19].

Genome-based analysis

DNA extraction. High-quality DNA extracts were prepared using a Maxwell 16 tissue DNA purification kit (AS1030; Promega, Madison, WI, USA) and an automated Maxwell 16 DNA preparation instrument (AS2000; Promega). Paired-end 150 bp libraries were sequenced on an Illumina HiSeq 4000 sequencer (Wellcome Centre for Human Genetics, United Kingdom; Illumina, San Diego, CA, USA). Quality control and assembly were performed as described previously [20]. Automatic gene annotation was carried out by the Rapid Annotations using the Subsystems Technology (RAST) server [21].

In silico analysis. Two *in silico* genus- and species-specific multiplex PCR (mPCR) assays [22,23] and a computer-simulated 16S rRNA restriction fragment length polymorphism (RFLP) analysis [24] were carried out using the free software programmes FastPCR and NEBcutter V2.0 respectively [25,26].

Whole-genome analysis. Orthologous average nucleotide identity (OrthoANI) and *in silico* DNA-DNA hybridization (*is*DDH) values were determined using the OrthoANIu tool [27,28] and the Genome-to-Genome Distance Calculator (GGDC 2.1) respectively [29,30]. A phylogenomic tree based on 107 essential single-copy core genes was reconstructed using bcgTree with default parameters [31]. Visualization and annotation of the phylogenetic tree was performed using iTOL [32].

Antibiotic resistance and virulence factors

Antibiotic susceptibility testing was performed using gradient strip (Etest; bioMérieux) technique according to methods validated earlier and for a fixed set of antimicrobial agents [33]. Because breakpoint values for *Arcobacter* species are lacking, European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for *Campylobacter coli* were used for

erythromycin, ciprofloxacin and tetracycline. For ampicillin, EUCAST non–species-related breakpoints were used, and for gentamicin the EUCAST epidemiologic cutoff value of *Enterobacterales* was applied [34,35]. Resistance genes were predicted using ResFinder [36]. The presence of ten putative virulence genes and urease genes reported in *A. butzleri* [37] and the cytolethal distending toxin (*cdt*) genes reported in *A. faecis* LMG 28519T and *A. lanthieri* LMG 28516T [38] in the R-75363 genome was verified by BLAST analyses.

Results

Patient information and microbiologic examination

A 49-year-old immunocompetent man without any relevant medical history presented to his general practitioner with abdominal bloating, heaviness and abdominal cramps of 3 weeks' duration. Stool appeared normal, without diarrhoea, mucus or blood loss. Fever was not observed and body weight was stable. In view of a tentative clinical diagnosis of gastritis, trial therapy with the proton pump inhibitor pantoprazole was initiated. Because symptoms persisted during the next month, the patient was referred to a gastroenterologist for further examination. He was not receiving any medication and had not received antibiotics in the previous 6 months. He lived in a mixed industrial–agricultural environment and reported no recent (<3 months) travel. He had no professional or recreational contact with animals, and there were no pets at home. Physical examination revealed nothing abnormal. A computed tomographic scan of the abdomen as well as gastroscopy and colonoscopy revealed no abnormalities and excluded gastritis, infectious colitis, inflammatory bowel disease and neoplasia.

All microbiologic stool testing remained negative, except bacteriologic culture of a *Campylobacter*-like organism that formed nonswarming, small, beige to off-white, translucent, circular colonies with entire margins on blood agar medium. No further stool samples were collected for culture because the patient spontaneously recovered without any specific treatment. Gram stain revealed slender Gram-negative, comma-shaped curved rods. Limited phenotypic characterization is summarized in Table 1 and revealed a motile oxidase- and catalase-positive organism.

Identification of bacterial strain by conventional methods

MALDI-TOF MS analysis. Although some of the matching hits were with *Arcobacter* species, the analysis of the clinical isolate R-75363 resulted in log scores below 1.7 using both the commercial and the in-house spectral database, indicating unreliable identification.

Phylogenetic marker analyses. The 930 bp 16S rRNA gene fragment revealed 99.6% and 99.0% sequence similarity with *A. lanthieri* AF1440T and *A. faecis* AF1078T respectively, thus placing isolate R-75363 into the genus *Arcobacter* but without a clear-cut identification at the species level [39]. No further accurate taxonomic alienation could be obtained with the analysis of the almost complete 16S rRNA sequence (1414 bp), as sequences of multiple species showed a similarity above the 98.65% species cutoff level (Table 2) [39].

A BLASTn search of an 846 bp fragment of the *rpoB* gene yielded six hits above the species level cutoff (Table 3) [19]. Two of these hits represented *A. lanthieri* strains. The four remaining hits originated from *Arcobacter* sp. isolates FW-4, FW-53, FW-54 and FW-61. Alonso *et al.* [40] reported that these isolates represented a novel taxon closely related to *A. lanthieri* for which the name '*(Ali)arcobacter hispanicus*' was effectively but not validly published [2].

Genome-based analysis

The R-75363 draft genome sequence consisted of 27 contigs of 2 175 890 bp and has a G + C content of 26.47% [2]. The N50 contig size was 126 888 bp, with the largest contig being 553 723 bp.

In silico mPCR and RFLP analysis. A 400 bp amplicon was predicted for the mPCR by Houf *et al.* [22], which would be indistinguishable from the amplicon of *A. butzleri* on agarose gel electrophoresis. By contrast, no PCR amplification was predicted for the mPCR by Douidah *et al.* [23]. The same 16S rRNA-RFLP pattern reported for all six of the isolates yielding hits above the species level cutoff for *rpoB* gene was found [40].

Whole-genome analysis. The OrthoANI analysis and *is*DDH results are shown in Supplementary Table S1. Comparisons with type strain genomes of validly published extant species result in an average nucleotide identity (ANI) value of 96.19% and an *is*DDH value of 84.60% for *A. lanthieri* LMG 28516T, which indicates that isolate R-75363 represents the same species [41–43]. However, isolate R-75363 also has an ANI value of 98.62% and an *is*DDH value of 87.40% for '*(Ali)arcobacter hispanicus*'. The phylogenetic tree (Fig. 1) based on 107 single-copy marker genes confirmed *A. lanthieri* and '*A. hispanicus*' as its closest neighbours. Concerning the *is*DDH value, if there are significant differences between the three formulas, as is the case for strain R-75363, the decision should be based on formula 2. Based exclusively on the *is*DDH value, the isolate would no longer be allocated to the species *A. lanthieri* but identified as '*A. hispanicus*'.

Antibiotic resistance and virulence factors

Cdt genes were not detected. The isolate showed resistance to three of the six antimicrobials tested (Table 4), i.e. ampicillin,

TABLE 1. Phenotypic characteristics of *Arcobacter* R-75363 isolated from patient

Test	Result
Growth on:	
ASA	+
COH	+
MCK	+
Growth in aerobic conditions at:	
28°C	+
37°C	+
42°C	−
Growth in microaerophilic conditions at:	
28°C	+
37°C	+
42°C	−
Characteristic	
Catalase activity	+
Oxidase activity	+
Urease activity	−
Motility	+
Indole	−
H2S (on TSI)	−
Nitrate reduction	+
Esculin hydrolysis	−
Glucose	−
Sucrose	−
Mannitol	−
Maltose	−
Xylose	−
Trehalose	−

+, positive result; −, negative result. ASA, *Arcobacter* selective agar; COH, Colombia agar +5% horse's blood; MCK, MacConkey agar; TSI, triple sugar iron agar.

TABLE 2. BLASTn and EzBioCloud identification results above 98.65% species cutoff level for R-75363 based on 16S rRNA gene sequence (1414 bp)

BLASTn

Organism	Strain	Query cover (%)	Identity (%)	Accession no.
Arcobacter sp.	FW-61	100	100.00	KX925314
	FW-54	100	100.00	KX925313
	FW-4	100	100.00	KX925311
	FW-53	100	99.93	KX925312
	FW54	95	100.00	LT629999
Arcobacter lanthieri	FW-40	100	99.79	KX925316
	FW-34	100	99.79	KX925315
	AF1441	99	99.79	KC551775
	AF1429	99	99.72	KC551771
	AF1431	99	99.72	KC551773
	AF1440T	99	99.65	KC551774
	AF1430	99	99.57	KC551772
	AF1581	99	99.57	KC551776
Arcobacter faecis	775	100	99.08	MN513226
	AF1078T	99	99.01	KC551780
	AF1033	99	98.94	KC551778
	AF1028	99	98.86	KC551777
	AF1058	99	98.79	KC551779
'*Arcobacter vitoriensis*'	F199	100	99.08	KX913922
	FW-59	100	99.01	KX913921

EzBioCloud Organism	Strain	Completeness (%)	Identity (%)	Accession no.
Arcobacter lanthieri	AF1440T	100	99.79	JARU01000021
Arcobacter faecis	AF1078T	100	99.08	JARS01000021

ciprofloxacin and erythromycin. However, the only resistance determinant predicted by ResFinder was the DNA gyrase A (*gyrA*) mutation Thr-85-Ile, which is responsible for resistance to ciprofloxacin [44]. Furthermore, *macA* and *macB* genes encoding for macrolide exporter proteins were identified, which correlates with erythromycin resistance, thereby confirming the results of the phenotypic test.

Five putative virulence genes were present (i.e. *cadF*, *ciaB*, *mviN*, *hecA*, *iroE*) but no urease genes, again confirming phenotypic test results. *Cdt* genes were not detected.

Discussion

Most clinical laboratories use a pyramidal stepwise approach for the detection of gastrointestinal pathogens, aiming at a balance between broad identification of potential pathogens and cost-effectiveness based on local epidemiology. In the present study, the use of the filter method allowed isolation of fastidious bacteria such as strain R-75363, emphasizing the importance of broad-spectrum classic culture techniques to suit not only as second-line tools when rapid molecular tests give positive results but also as screening tests in case of negative rapid testing results.

Identification of bacterial colonies directly from the primary isolation plates by MALDI-TOF MS is validated in clinical laboratories [12]. For *Campylobacteraceae*, identification accuracy at the species level is still restricted [45]. MALDI-TOF MS analysis did not yield a reliable identification of strain R-75363 because neither commercial nor in-house databases held reference spectra for *A. lanthieri* [13]. Constant updating of reference databases is crucial to allow rapid identification of emerging pathogens, especially for heterogeneous bacteria.

Although several DNA-based techniques such as mPCR [22,23] and 16S rRNA-RFLP [24] have been developed to identify *Arcobacter* species, these methods no longer allow the identification of all species, as the present case illustrates. Comparative 16S rRNA gene sequence analysis yielded genus-level identification only. This is often the endpoint in routine diagnosis in the clinical microbiology laboratory, where

TABLE 3. Pairwise similarity scores (%) for *rpoB* gene (846 bp) sequence with sequences of nucleotide collection (nt) database using BLASTn (https://www.ncbi.nlm.nih.gov)

Species	Stain	Query coverage	Identity (%)	Accession no.
Arcobacter sp.	FW-53	73%	100.00	KY002773
Arcobacter sp.	FW-54	73%	99.68	KX962637
Arcobacter sp.	FW-4	73%	99.68	KY002771
Arcobacter sp.	FW-61	73%	99.03	KY002769
Arcobacter lanthieri	FW-40	73%	98.06	KY002772
Arcobacter lanthieri	FW-34	73%	98.23	KY002770

timeliness and the clinical usefulness of the returned information are crucial.

Though the taxonomic resolution of protein encoding genes is superior [10], the *rpoB* gene sequence analysis also failed to provide identification at the species level. Because no unambiguous identification was obtained with the conventional identification tools, a draft whole genome sequence was determined. The ANI and *is*DDH indices provide reliable information and are included in the minimal guidelines to define *Arcobacter* species [30,46]. Using formula 3 in the *is*DDH analysis, as recommended by On *et al.* [30], isolate R-75363 could be identified as *Arcobacter lanthieri*. Others state that this decision should be based on formula 2; based solely on this value, the isolate should be allocated to the yet undescribed species '*(Ali)arcobacter hispanicus*' as isolate FW-54 [28,29]. Nevertheless, '*A. hispanicus*' shows values above the threshold relative to *A. lanthieri*, thereby placing both isolate R-75363 and the alleged '*A. hispanicus*' into the species *A. lanthieri*. This might merely seem to be a taxonomic issue; whether this carries clinical relevance cannot yet be ascertained. Recent phylogenetic analyses have suggested that *Arcobacter* species group into four clusters. Clinical isolate R-75363 would be included in the genus *Aliarcobacter* gen. nov. together with species considered human pathogens [2]. However, we should be aware of the impact such proposals have on clinical diagnostics. Changes in nomenclature and classification present challenges to both clinical and public health microbiologists because identification is used for communication to physicians and between medical centres regarding disease presentation, prognosis, treatment and outbreak investigation.

Next to the determination of species identity, the genotypic antimicrobial resistance screening of the isolate partially supplied a prediction to the phenotypic testing results but was

TABLE 4. Antimicrobial susceptibility of isolate recovered in this study and resistance predicted using ResFinder

Antimicrobial agent	MIC (mg/L)	Susceptibility Category	Susceptibility breakpoint (mg/L)	ResFinder
Ampicillin	8	R	≤2	No
Tetracycline	2	S	≤2	No
Ciprofloxacin	32	R	≤0.5	Yes
Erythromycin	12	R	≤8	No
Azithromycin	6	S	≤8	No
Gentamicin	0.19	S	≤2	No

R, resistant; S, susceptible.

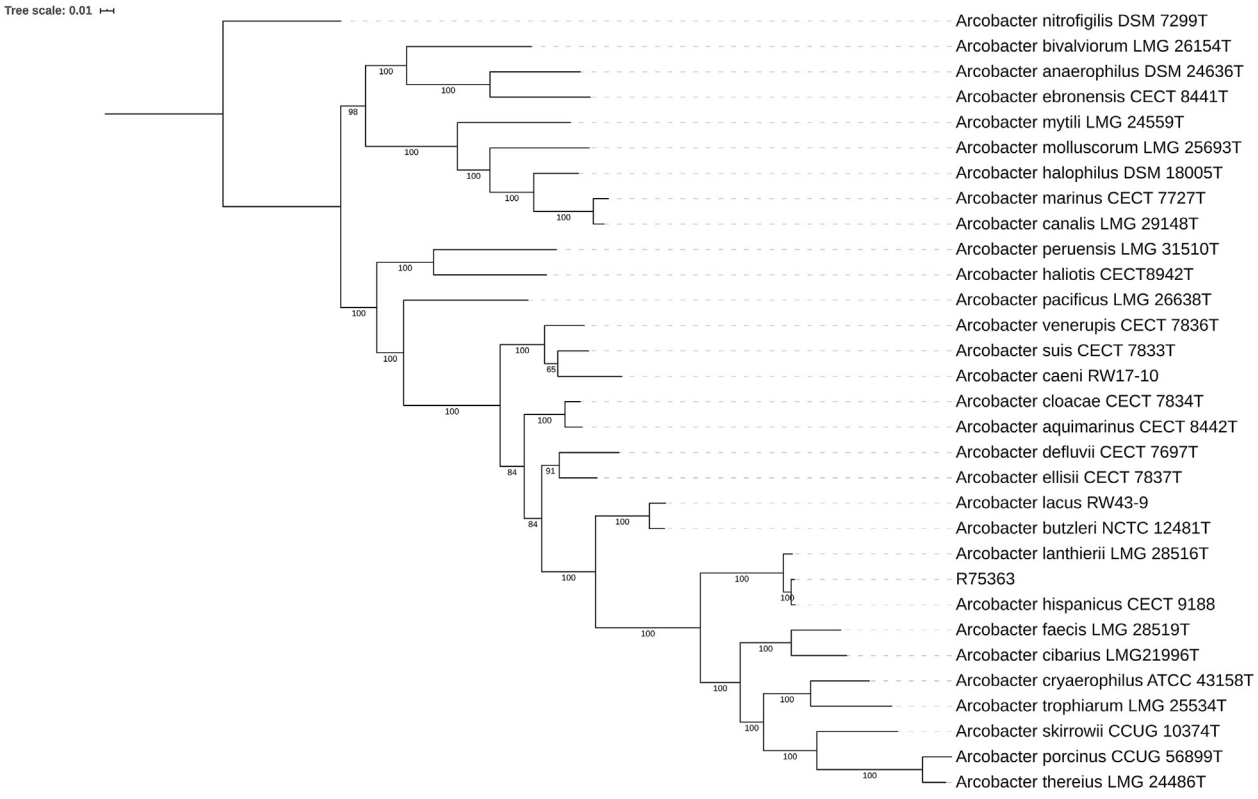

FIG. 1. Phylogenetic tree based on 107 single-copy core genes with bcgTree by partitioned maximum-likelihood analysis. Percentage of replicate trees in which associated taxa clustered together in bootstrap test (1000 replicates) are shown next to branches. *Arcobacter nitrofigilis* DSM 7299 was used as outgroup. Bar indicates 0.01 changes per nucleotide position.

insufficient to guide clinical decision making, and susceptibility testing of the bacterial aetiologic agents remains warranted. Several of the virulence associated genes described in *Arcobacter butzleri* were present in isolate R-75363, indicating a possible pathogenic nature of this organism. Although Zambri *et al.* [38] reported the transcriptional expression of cytolethal distending toxin (*cdtA*, *cdtB*, *cdtC*) genes in *A. faecis* and *A. lanthieri* reference strains with high frequency, these genes were not present in the genome sequence of strain R-75363, which is in agreement with previous studies reporting the absence of these genes in the genus *Arcobacter* [37,47].

Strain R-75363 originated from a stool sample of a single patient, and its pathogenic relevance cannot be established. Because the patient spontaneously recovered, no follow-up sampling or detection for chronic faecal shedding was performed. Hence, the isolate may represent mere, and possibly transient, colonization. Nevertheless, *A. lanthieri* strains have been recovered from human stool specimens before and were shown to exhibit a high degree of cytotoxicity [8]. The true pathogenic role of *A. lanthieri* may become apparent through more case reports or clinical series of infections. Neither close contact to animals or contaminated wastewater could be linked to the gastrointestinal complaints of the patient or to the isolation of this particular *Arcobacter* strain.

In conclusion, this case demonstrated the importance of using agnostic methods for the detection of emerging enteric pathogens, such as *Arcobacter* spp., as well as the need for clear taxonomic nomenclature in which new bacterial species are delignated, preferably based on biologic and clinical features rather than artificial genomic-based criteria. As genome sequencing becomes cheaper and more accessible, it is likely to become the preferred method for the characterization of many groups of microbial isolates. The traditional approaches to the molecular and phenotypic characterization of microorganisms will continue to be relevant but may be better substantiated or validated by the information extracted from genome sequences.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2020.100829.

Conflict of interest

None declared.

References

[1] Vandamme P, Falsen E, Rossau R, Hoste B, Segers P, Tytgat R, et al. Revision of *Campylobacter*, *Helicobacter*, and *Wolinella* taxonomy: emendation of generic descriptions and proposal of *Arcobacter* gen. nov. Int J Syst Bacteriol 1991;41:88–103.

[2] Pérez-Cataluña A, Salas-Masso N, Dieguez AL, Balboa S, Lema A, Romalde JL, et al. Revisiting the taxonomy of the genus *Arcobacter*: getting order from the chaos. Front Microbiol 2018;9.

[3] On SL, Miller WG, Biggs PJ, Cornelius AJ, Vandamme P. A critical rebuttal of the proposed division of the genus *Arcobacter* into six genera using comparative genomic, phylogenetic, and phenotypic criteria. Syst Appl Microbiol 2020.

[4] Vandenberg O, Dediste A, Houf K, Ibekwem S, Souayah H, Cadranel S, et al. *Arcobacter* species in humans. Emerg Infect Dis 2004;10:1863–7.

[5] Van den Abeele AM, Vogelaers D, Van Hende J, Houf K. Prevalence of *Arcobacter* species among humans, Belgium, 2008–2013. Emerg Infect Dis 2014;20:1731–4.

[6] Wybo I, Breynaert J, Lauwers S, Lindenburg F, Houf K. Isolation of *Arcobacter skirrowii* from a patient with chronic diarrhea. J Clin Microbiol 2004;42:1851–2.

[7] Vasiljevic M, Fenwick AJ, Nematollahi S, Gundareddy VP, Romagnoli M, Zenilman J, et al. First case report of human bacteremia with *Malacobacter* (*Arcobacter*) *mytili*. Open Forum Infect Dis 2019;6(7).

[8] Bruckner V, Fiebiger U, Ignatius R, Friesen J, Eisenblatter M, Hock M, et al. Characterization of *Arcobacter* strains isolated from human stool samples: results from the prospective German prevalence study Arcopath. Gut Pathog 2020;12:3.

[9] Houf K, De Smet S, Baré J, Daminet S. Dogs as carrier of the emerging pathogen *Arcobacter*. Vet Microbiol 2008;130:208–13.

[10] Collado L, Figueras MJ. Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. Clin Microbiol Rev 2011;24:174–92.

[11] Steele TW, Mcdermott SN. The use of membrane filters applied directly to the surface of agar plates for the isolation of *Campylobacter jejuni* from feces. Pathology 1984;16(3):263–5. https://doi.org/10.3109/00313028409068535.

[12] Clinical and Laboratory Standards Institute (CLSI). Methods for the identification of cultured microorganisms using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. CLSI guideline M58. Wayne, PA: CLSI; 2017.

[13] Van den Abeele AM, Vogelaers D, Vandamm P, Vanlaere E, Houf K. Filling the gaps in clinical proteomics: a do-it-yourself guide for the identification of the emerging pathogen *Arcobacter* by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Microbiol Meth 2018;152:92–7.

[14] Lane D. 16S/23S rRNA sequencing in nucleic acid techniques in bacterial systematics. Chichester: Wiley; 1991. p. 115–48.

[15] Vandamme P, Holmes B, Bercovier H, Coenye T. Classification of Centers for Disease Control group eugonic fermenter (EF)-4a and EF-4b as *Neisseria animaloris* sp. nov. and *Neisseria zoodegmatis* sp. nov., respectively. Int J Syst Evol Microbiol 2006;56:1801–5.

[16] Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, et al. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 2017;67:1613–7.

[17] Korczak BM, Stieber R, Emler S, Burnens AP, Frey J, Kuhnert P. Genetic relatedness within the genus *Campylobacter* inferred from *rpoB* sequences. Int J Syst Evol Microbiol 2006;56:937–45.

[18] Levican Asenjo A. Sanitary importance of *arcobacter*. Tarragona, Spain: Universitat Rovira i Virgili; 2013.
[19] Adekambi T, Shinnick TM, Raoult D, Drancourt M. Complete rpoB gene sequencing as a suitable supplement to DNA-DNA hybridization for bacterial species and genus delineation. Int J Syst Evol Microbiol 2008;58:1807–14.
[20] Peeters C, De Canck E, Cnockaert M, De Brandt E, Snauwaert C, Verheyde B, et al. Comparative genomics of pandoraea, a genus enriched in xenobiotic biodegradation and metabolism. Front Microbiol 2019;10.
[21] Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 2008;9.
[22] Houf K, Tutenel A, De Zutter L, Van Hoof J, Vandamme P. Development of a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri, Arcobacter cryaerophilus* and *Arcobacter skirrowii*. FEMS Microbiol Lett 2000;193:89–94.
[23] Douidah L, De ZL, Vandamme P, Houf K. Identification of five human and mammal associated *Arcobacter* species by a novel multiplex-PCR assay. J Microbiol Methods 2010;80:281–6.
[24] Figueras MJ, Levican A, Collado L. Updated 16S rRNA-RFLP method for the identification of all currently characterised *Arcobacter* spp. BMC Microbiol 2012;12:292.
[25] Kalendar R, Khassenov B, Ramankulov Y, Samuilova O, Ivanov KI. FastPCR: an in silico tool for fast primer and probe design and advanced sequence analysis. Genomics 2017;109:312–9.
[26] Vincze T, Posfai J, Roberts RJ. NEBcutter: a program to cleave DNA with restriction enzymes. Nucleic Acids Res 2003;31:3688–91.
[27] Lee I, Kim YO, Park SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3.
[28] Yoon SH, Ha SM, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 2017;110:1281–6.
[29] Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M. Genome sequence–based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 2013;14.
[30] On SLW, Miller WG, Houf K, Fox JG, Vandamme P. Minimal standards for describing new species belonging to the families *Campylobacteraceae* and *Helicobacteraceae: Campylobacter, Arcobacter, Helicobacter* and *Wolinella* spp. Int J Syst Evol Microbiol 2017;67:5296–311.
[31] Ankenbrand MJ, Keller A. bcgTree: automatized phylogenetic tree building from bacterial core genomes. Genome 2016;59:783–91.
[32] Letunic I, Bork P. Interactive Tree of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Res 2019;47(W1):W256–9.
[33] Van den Abeele AM, Vogelaers D, Vanlaere E, Houf K. Antimicrobial susceptibility testing of *Arcobacter butzleri* and *Arcobacter cryaerophilus* strains isolated from Belgian patients. J Antimicrob Chemother 2016;71:1241–4.
[34] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0. Basel: EUCAST; 2019.
[35] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 18th informational supplement. CLSI document M100-S18. Wayne, PA: CLSI; 2008.
[36] Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67:2640–4.
[37] Miller WG, Parker CT, Rubenfield M, Mendz GL, Wösten MMSM, Ussery DW, et al. The complete genome sequence and analysis of the Epsilonproteobacterium *Arcobacter butzleri*. PLoS One 2007;2:1–21.
[38] Zambri M, Cloutier M, Adam Z, Lapen DR, Wilkes G, Sunohara M, et al. Novel virulence, antibiotic resistance and toxin gene-specific PCR-based assays for rapid pathogenicity assessment of *Arcobacter faecis* and *Arcobacter lanthieri*. BMC Microbiol 2019;19:11.
[39] Kim M, Oh HS, Park SC, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 2014;64:346–51.
[40] Alonso R, Martínez-Malaxetxebarria I, Girbau C, Carmona S, Velasco H, Fernández-Astorga A. Genomic diversity of *Arcobacter* spp. isolated from surface waters. J Microbiol Biotechnol 2018;7(2).
[41] Richter M, Rossello-Mora R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A 2009;106:19126–31.
[42] Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 2007;57:81–91.
[43] Konstantinidis KT, Tiedje JM. Genomic insights that advance the species definition for prokaryotes. Proc Natl Acad Sci U S A 2005;102:2567–72.
[44] Wieczorek K, Osek J. Antimicrobial resistance mechanisms among *Campylobacter*. Biomed Res Int 2013;2013:340605.
[45] Alispahic M, Hummel K, Jandreski-Cvetkovic D, Nobauer K, Razzazi-Fazeli E, Hess M, et al. Species-specific identification and differentiation of *Arcobacter, Helicobacter* and *Campylobacter* by full-spectral matrix-associated laser desorption/ionization time of flight mass spectrometry analysis. J Med Microbiol 2010;59(Pt 3):295–301.
[46] Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, et al. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 2018;68:461–6.
[47] Johnson LG, Murano EA. Lack of a cytolethal distending toxin among *Arcobacter* isolates from various sources. J Food Protect 2002;65:1789–95.