A severe case of persistent diarrhoea associated with Arcobacter cryaerophilus but attributed to Campylobacter sp. and a review of the clinical incidence of Arcobacter spp.

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Abstract

Although rarely, Arcobacter spp. have been associated with diarrhoea and bacteraemia. We report a persistent case in a healthy 26-year-old Spanish male of bloody diarrhoea, which was attributed to Campylobacter but in fact was caused by Arcobacter cryaerophilus, as determined by sequencing of the rpoB gene. The isolate was re-identified by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) and genotyped for five putative virulence genes and for seven genes included in the Arcobacter multilocus sequence typing database. The low score obtained by MALDI-TOF indicates the need to complement the database with more isolates. Only the ciaB gene, which encodes for an invasin, was detected. Despite the isolate belonging to a new sequence type, three of the alleles (glnA, pgm and tkt) had been found previously in isolates from faeces of patients with diarrhoea. This study, together with the reviewed literature, indicates that Arcobacter can produce bacteraemia and that the isolation from patients with diarrhoea range from 0.11% to 1.25%. This study also demonstrates that Arcobacter species are confused with Campylobacter spp., as previously suggested. This is one of the factors that leads to underestimation of their incidence together with the use of inappropriate detection and identification methods.

Keywords: Arcobacter, emerging or re-emerging diseases, gastrointestinal disease, persistent diarrhoea

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Introduction

Bacteria of the genus Arcobacter, which was created with aerotolerant species previously included in the genus Campylobacter, are considered emergent enteropathogens and potential zoonotic agents [1]. The genus currently includes 17 species and some of these species produce abortions, mastitis and gastrointestinal disorders in animals [2–5] and bacteremia, endocarditis, peritonitis and diarrhoea in humans [6–12]. So far there have been very few human diarrhoea cases reported despite it having been found that Arcobacter butzleri was the fourth most common Campylobacter-like organism isolated from human stools [1,13,14]. Persistent watery diarrhoea was the main symptom associated with Arcobacter species, in contrast to the bloody diarrhoea produced by Campylobacter jejuni [1,13]. It has been suggested that Campylobacter isolates cover Arcobacter spp. [13], which are not routinely studied with the ad hoc methods in clinical laboratories. However, the true impact of this confusion is unknown [1].

This study describes in detail the clinical characteristics of an acute case of diarrhoea produced by A. cryaerophilus, which was recognized after sequencing of the rpoB gene from an isolate biochemically identified as Campylobacter sp. The isolate was re-identified with matrix-assisted laser desorption ionization time of flight (MALDI-TOF) and genotyped by multilocus...
sequence typing; its putative virulence genotype was screened by PCR. The study intends to alert clinicians to the possible role that this poorly known bacteria genus plays in the development of human disease by showing all known clinical cases.

Case Report

A 26-year-old male with no previous history of disease visited the doctor complaining of bloody watery diarrhoea of 3 weeks duration (with about three liquid deposits a day), with abdominal pain but without fever, nausea or vomiting. The patient had been seen with similar symptoms 4 months before and was diagnosed with acute gastroenteritis. A stringent diet was the recommended treatment but no laboratory testing was made at that time. Considering this previous history, a blood and stool analysis was carried out. The stool sample was examined for parasites and cultured for Escherichia coli, Salmonella spp., Shigella spp., Yersinia enterocolitica, Aeromonas spp., Plesiomonas spp., Vibrio spp. and Campylobacter spp. Complete blood count was performed and searches were made for hepatitis B antigen, anti-hepatitis C virus antibody and other antibodies against human immunodeficiency virus, cytomegalovirus, adenoviruses and parvovirus B19. The patient was diagnosed with acute gastroenteritis and an empirical antibiotic treatment was initiated with amoxicillin/clavulanic acid.

The laboratory evaluation showed an almost normal complete blood count, except for a slight relative reduction in neutrophils (37%) and an increase in lymphocytes (51.2%). The blood culture was negative but the stool sample showed a positive culture in Campylosel medium (BioMérieux, Marcy l’Etoile, France) after 3 days of incubation at 42°C, under microaerobic conditions. The colonies were identified as Campylobacter sp. based on phenotypic tests (Gram stain, hippurate hydrolysis and resistance to cephalothin). By disc diffusion, the isolate was susceptible to amoxicillin/clavulanic acid and to gentamicin, but was resistant to ciprofloxacin and erythromycin. Considering these results and the good evolution of the patient, the empirical treatment was maintained for 8 days, after which time he had recovered completely with no more diarrhoea episodes. The isolate was sent to the Unit of Microbiology at the University Rovira i Virgili for re-identification using the sequences of the rpoB, as was done routinely for all isolates identified as Campylobacter at the hospital. The DNA extraction, amplification and sequencing were performed using primers and conditions previously described [15]. A BlastN analysis with the obtained rpoB sequence revealed a 99% similarity with the strain of A. cryaerophilus (Strain of subgroup 1B, LMG 10229, accession number EU669900), followed by a 95% similarity with the sequence of the type strain of A. cryaerophilus (Strain of subgroup 1A, LMG 9904T, accession number EU669899), and only 90% similarity with a strain of the next most similar species, A. butzleri (Strain ED-1, accession number AP012047). Therefore, the isolate was identified as being A. cryaerophilus.

Considering the rarity of the recovered bacterium, the patient was contacted again to obtain additional information. He indicated that he regularly eats raw meat and fish, and also had a dog at home and a group of laying hens fenced in the garden. Despite the patient indicating that he had not had any other episodes of diarrhoea, a new stool sample was taken to evaluate his possible carrier state. Rectal samples from the dog and cloacal swab samples from three of the six laying hens he had at that time, as well as two samples of their faeces collected from the ground, were taken for microbiological examination using molecular detection and culture, as described in a previous study [16]. However, all of these samples were negative for Arcobacter.

To our knowledge, only three cases of A. cryaerophilus infection have so far been reported [6,17,18]. Those cases, together with the few available for the other species of the genus, are summarized in Tables 1 and 2. The first and only case of diarrhoea due to A. cryaerophilus dates back to 1988 when it was still included in the genus Campylobacter with the name Campylobacter cryaerophila. The 35-year-old homosexual man with this infection showed intermittent diarrhoea of 4–6 months duration with abdominal pain [17]. The other two are cases of bacteraemia, one in Taiwan that involved an immunocompromised 72-year-old woman with uraemia who showed a haematogenous pneumonia [18], and the other in a 7-year-old boy from China who had fallen into a mud pool while driving a mini motorcycle and suffocated [6]. As seen in Tables 1 and 2, a few other cases of diarrhoea and bacteraemia have been linked to A. butzleri [4,7,19–23] and, more rarely, to Arcobacter skirrowii [24]. For instance, a case of acute diarrhoea caused by A. butzleri in a 30-year-old healthy man was reported from Turkey; it was cured with treatment with ciprofloxacin [23]. A recent case of peritonitis due to Arcobacter sp. has been reported in a peritoneal dialysis patient whose catheter was repositioned [25]. Despite intravenous cefazolin and oral levofloxacin being given as a prophylaxis, the patient only responded after intravenous ticarcillin–clavulanic treatment for 2 weeks, with no need for the catheter to be removed.

The majority of these case studies underline the difficulty in recognizing or identifying these bacteria because they grew slowly and their identification required sequencing of the 16S rRNA gene [6,7,21] or the use of specific multiplex PCR methods [22–24,26]. Considering that several hospitals
now use the MALDI-TOF identification technique for such fastidious, slow-growing microbes, we have re-identified our A. cryaerophilus isolate using that method [27]. The isolate was studied with the Ultraflex TOF/TOF MALDI-TOF instrument, that uses the MALDI BIOTYPER 2.0 software (Bruker Daltonics, Bremen, Germany) after spotting a fresh colony directly onto the target plate and the addition of 1 μL of the matrix, cinamic acid, as described by the manufacturer. The type strain of A. cryaerophilus (LMG 9904T) was used in parallel as a control. The MALDI BIOTYPER output for our clinical strain scored 1.493 with the strain A. cryaerophilus T277 CPB. A score of <1.7 normally indicates an unconfident identification, between 1.7 and 1.99 indicates a genus-level identification, and a score ≥2 indicates a species-level identification. The second higher score was only 1.42 with a strain of the species Pseudomonas proteolytica. Despite the unconfident identification the first match was with an A. cryaerophilus and among the following bacteria listed there was no Campylobacter spp. The type strain of A. cryaerophilus (LMG 9904T) used as control was correctly identified despite it showing a low score (1.885). Clinicians should be aware that in the case of a strain showing this behaviour with MALDI-TOF it is worth confirming its identity by sequencing the rpoB gene, so that the true incidence of these bacteria can be established. The inconclusive results obtained with MALDI-TOF could be explained by the fact that a correct identification with this method depends on the number of bacteria strains included in the database [27]. The BIOTYPER database has only 13 Arcobacter strains and only four of them belong to the species A cryaerophilus. However, the capacity of this method to separate strains belonging to all

**TABLE 1.** Cases of intestinal infections associated with Arcobacter spp.

| Patients’ sex/age | Country | Presentation | Species         | Outcome                                    | Underlying conditions                  | Reference |
|-------------------|---------|--------------|-----------------|--------------------------------------------|----------------------------------------|-----------|
| M/35 years        | Australia | Chronic diarrhoea (6 months) | A. cryaerophilus | NS                                        | Homosexual with history of anxiety and repeated sexual exposure | [17]      |
| 3−7 years         | Italy    | No diarrhoea, abdominal pain, occasional vomiting or fever and abdominal cramps | A. butzleri | Recovered 7-10 days after treatment with ofloxacin treatment | None | [4]       |
| 2. F/52 years     | Germany | 1. Acute watery diarrhoea (15 days) and abdominal cramps | A. butzleri  | 1. Recovered 3 days after treatment with doxycycline | 1. Type 1 diabetes mellitus and alcohol abuse | [19]      |
| 1. M/2 years      | Chile    | 1. Acute mucous diarrhoea and vomiting; 2. Chronic diarrhoea (4 months) with abdominal cramps and pain | A. butzleri | 1. Recovered in 2 days with parenteral fluid therapy, restricted diet but without antimicrobial treatment; 2. Recovered 10 days after treatment with erythromycin | None | [22]      |
| M/73 years        | Belgium  | Chronic diarrhoea (2 months) | A. skinawi | Recovered 10 days after no specific treatment | Prosthetic aortic heart valve | [24]      |
| M/30 years        | Turkey   | Acute watery diarrhoea, abdominal pain, nausea and sweating | A. butzleri | Recovered 2 days after treatment with ciprofloxacin | None | [23]      |
| M/26 years        | Spain    | Persistent bloody and watery diarrhoea (3 weeks) | A. cryaerophilus | Recovered 8 days after treatment with amoxicillin/clavulanic acid | Acute gastroenteritis 4 months earlier | Current study |

NS, not specified.

1. Originally described as Campyllobacter cryaerophila.
2. Four males and six females between 3 and 7 years old.

**TABLE 2.** Extra-intestinal infections associated with Arcobacter spp.

| Patients’ sex/age | Country  | Presentation                                           | Species | Outcome                                    | Underlying disease                  | Reference |
|-------------------|----------|-------------------------------------------------------|---------|--------------------------------------------|-------------------------------------|-----------|
| Neonate           | UK       | Bacteraemia with hypotension, hypothermia and hypoglycaemia | A. butzleri | Recovered 6 days after penicillin and cefotaxime treatment | Mother had prenatal bleeding due to placenta praevia. Delivery at 26 weeks of gestation | [20]      |
| M/72 years        | Taiwan   | Bacteraemia and haematogenus pneumonia               | A. cryaerophilus | Recovered 2 weeks after cefotaxime and tobramycin treatment | Chronic renal failure, haemodialysis with arteriovenous fistula. Two months of fever and progressive cough with purulent sputum. She also had a 1-month history of anorexia and frequent loose stool 2 months before admission | [18]      |
| M/60 years        | Taiwan   | Bacteraemia with fever and haematopenis               | A. butzleri | Recovered 4 days after cefoxaxime treatment | Chronic hepatitis B carrier, liver cirrhosis | [21]      |
| F/69 years        | Hong Kong | Bacteraemia with fever and lower quadrant pain       | A. butzleri | Recovered 3 days after cefuroxime and metronidazole treatment | Gangrenous appendicitis | [7]       |
| F/63 years        | China    | Peritonitis after repositioning of catheter with fever and abdominal pain | Arcobacter sp. | Recovered 2 weeks after treatment with ticarcillin-clavulanate | End-stage renal failure of unknown cause | [25]      |

NS, not specified.
Arcobacter spp. has been recently demonstrated [2,28], therefore, it is possible that the inclusion of more strains in the BIOTYPER database will allow their correct identification, and this will contribute to clarify the clinical importance of this genus.

The seven housekeeping genes (aspA, atpA, glnA, gltA, glyA, pgm and tkt) included in the Arcobacter multilocus sequence typing database created by Miller et al. [29] were sequenced from our isolate of A. cryaerophilus using the primers described by these authors. New alleles were obtained for four genes (i.e. aspA-215, atpA-152, gltA-149 and glyA-473), while the sequences of the glnA (codifying for glutamine synthetase), pgm (phosphoglucomutase) and tkt (transketolase) genes corresponded to the already known alleles 59, 133 and 115, respectively. Therefore, this new clinical isolate (strain 609) belonged to a new sequence type (ST) named ST-392. Interestingly, the allele glnA-59 and the pgm-133 had been obtained from strain 276, which was isolated from the faeces of a patient with gastroenteritis in France in 2004, while the allele tkt-115 had been obtained from strain 305, which was also obtained from faeces of a patient with gastroenteritis in the USA in 2009. Apart from strains 276 and 305, the database includes only two other isolates of A. cryaerophilus recovered from human samples, i.e. strains 281 (from gastroenteritis) and 285 (from human blood), and both isolates share the ST-201. The few available human pathogenic strains in the database do not allow a relationship to be established between the presence of certain alleles, or STs, and virulence. Therefore, it is important that more strains isolated from human infections are included in the database.

**FIG. 1.** Neighbour joining tree based on the concatenated sequences of aspA, atpA, glnA, gltA, glyA, pgm and tkt (3339 bp) showing the position of strain 609 (ST-392) among the 75 strains of Arcobacter cryaerophilus included in the Arcobacter multilocus sequence typing database. Bootstrap values (≥70%) based on 1000 replications are shown at the nodes.
database as we did. In the tree constructed with the concatenated sequences of the seven genes from each of the 75 A. cryaerophilus strains that are currently included in the database (Fig. 1), our strain grouped with two others obtained in Europe from poultry (strain 346, ST-290 and strain 325, ST-268), being therefore the most related strains. However, whether the A. cryaerophilus strain was acquired in our patient through consumption of poultry meat could not be demonstrated.

Five putative Arcobacter virulence genes (ciaB, cadF, cj1349, hecA and irgA) were also searched for in our strain using the primers designed by Douidah et al. [30]. However, only the presence of the ciaB gene was detected, which encodes for an invasion protein in C. jejuni. The same result was obtained for five strains of A. cryaerophilus recovered from shelfish and from animal faeces in our laboratory [31]. In the study of Douidah et al. [30], which included 99 A. cryaerophilus strains isolated from human, chicken, pig, cattle, sheep, horse and dog, the ciaB gene was present in the majority (92.9%) of the strains, followed by cj1349 (51.5%), cadF (34.3%), hecA (4%) and irgA (3%).

Among the faecal samples at the Hospital Universitari Sant Joan de Reus, where the isolate of A. cryaerophilus was obtained, Campylobacter was the most commonly isolated enteropathogen, representing 41.4% (65/157) of the positive stools in the last year, followed by Salmonella spp. (36.3%), Aeromonas spp. (14.6%), Shigella spp. (4.4%), Hafnia alvei (3.2%) and Yersinia enterocolitica (1.3%). Among the Campylobacter and Campylobacter-like organisms, C. jejuni was the most prevalent species (82.7%), followed by Campylobacter coli (16.4%), while A. cryaerophilus was in third place (0.9%), which agrees with previous studies [13,14].

A summarized revision of studies on Arcobacter, including those comparing patients with and without diarrhoea, is provided in Table 3. The prevalence of Arcobacter species in human stools ranged from 0.1% to 1.25% in studies that derived the information from culturing, whereas the detection from faeces by PCR ranged from 0.4% to 13% [9,10,12–37]. In one study, performed in Belgium and France where the prevalence was determined by culture [13], A. butzleri occupied the fourth place (3.5%) among Campylobacter-like organisms, while A. cryaerophilus occupied the seventh place (0.5%). In another study performed in South Africa using multiplex PCR detection, A. butzleri showed a higher prevalence (6.2%) after C. jejuni (10.2%) [10]. In the same study, A. cryaerophilus and A. skirrowii showed lower incidences (2.8% and 1.9%, respectively). In two other studies that detected Arcobacter using the same multiplex PCR method a higher incidence was also observed [12,38]. One was a case–control study of faeces from diabetic patients in Italy [38]. In that study there was 78.9%
on-diarrhoeic faeces of patients with type 2 diabetes, versus the 26.2% found for the controls (non-diabetic non-diarrhoea subjects). In the second study, an 8.0% incidence was reported among US/European travellers who suffered acute diarrhoea while visiting Mexico, Guatemala and India [12]. Other recent studies compared the ability to detect Arcobacter using in parallel molecular and culture methods [36,37]. Collado et al. [36] detected the species A. butzleri in 1.4% of stool samples of patients with diarrhoea, using a genus-specific PCR and a species-specific multiplex PCR method, whereas it was isolated from only 0.7% of samples by culture. In the other study, de Boer et al. [37] developed a multiplex real-time PCR able to detect A. butzleri and campylobacters from the faeces of patients with diarrhoea; testing this method in parallel with culture. Using this method, A. butzleri was detected in 0.4% of samples but was not recovered by culture. The higher prevalence obtained using molecular methods supports the statement that Arcobacter spp. could be underestimated as enteropathogens because of limitations in the current culturing methods, and demonstrates the importance of routinely screening stool samples for the species of this genus using molecular methods in parallel.

The isolation in our patient of A. cryaerophilus in the absence of other enteropathogens and the remission of the diarrhoea symptoms after treatment with amoxicillin/clavulanic acid, to which the bacteria was sensitive, seems to indicate that this bacterium could be considered the aetiologic agent of the diarrhoea process. Despite not being able to find the contagious source of Arcobacter in the environment of our patient, we were able to speculate that, in this case, it could have been acquired through the consumption of poorly cooked poultry meat or fish. Interestingly, the patient showed recurrent episodes with abdominal pain, which seems to be a typical clinical presentation for this genus.

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Conflict of Interest

None declared.

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