

Human Acute Gastroenteritis Associated with *Arcobacter butzleri*

Tuba Kayman, M.D.,¹ Halil Ibrahim Atabay, Ph.D.,² Seçil Abay, Ph.D.,³ Harun Hızlısoy, M.Sc.,³ Çelenk Molva, M.Sc.,⁴ and Fuat Aydın, Ph.D.,³ ¹Department of Clinical Microbiology, Kayseri Education and Research Hospital, Kayseri, Turkey, ²Department of Medical Microbiology, Faculty of Medicine, Sifa University, Izmir, Turkey, ³Department of Microbiology, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey, and ⁴Department of Food Engineering, Faculty of Engineering, Izmir Institute of Technology, Izmir, Turkey

Introduction

Arcobacter spp. are considered emerging food-borne pathogens (1). Contaminated water and meat play an important role in the transmission of these bacteria to humans (2,3). Currently, the genus *Arcobacter* has 13 recognized species: *A. butzleri*, *A. cryaerophilus*, *A. skirrowii*, *A. nitrofigilis*, *A. cibarius*, *A. halophilus*, *A. mytili*, *A. thereius*, *A. marinus*, *A. trophiarum*, *A. defluvii*, *A. molluscorum*, and *A. ellisii* (4,5). Only three species, namely, *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*, have been associated with human and animal diseases (1). These microorganisms cause a number of infections, such as abortion, mastitis, and septicemia, in animals. In humans, they have been reported to cause gastrointestinal tract infections and septicemia (6,7). *Arcobacter* septicemia secondary to underlying diseases, such as cirrhosis (8) and acute gangrenous appendicitis (9), has also been documented in humans.

The role of other *Arcobacter* species in human and animal diseases remains unclear (1). It has been reported that *Arcobacter* species are found more commonly on poultry rather than red meat (10), suggesting that poultry may be a major reservoir for the pathogen. We report here a patient with abdominal pain, diarrhea, nausea, and sweating from whom *A. butzleri* was isolated. This is the first documented human case of *A. butzleri* gastroenteritis in Turkey.

Case Report

A 30-year-old male patient with acute abdominal pain, diarrhea, nausea,

and sweating was admitted to the Kayseri Education and Research Hospital, Kayseri, Turkey. The patient had experienced four episodes of diarrhea but no vomiting. He had no other signs and symptoms of disease. The patient's physical examination was unremarkable. A stool sample was collected from the patient and submitted to the microbiology laboratory for routine enteric bacterial culture and an ova and parasite examination.

Macroscopically, the stool specimen was watery, with no gross blood or mucus observed. On microscopic examination, the stool specimen was negative for the presence of erythrocytes, leukocytes, protozoa, and parasite eggs. The stool specimen was plated onto eosin methylene blue agar (Oxoid, UK) and Hektoen enteric agar (Merck, Germany) for isolation of conventional enteric pathogens as well as modified charcoal cefoperazone deoxycholate agar (mCCDA) (Oxoid) for the cultivation of *Campylobacter* species. The eosin methylene blue and Hektoen enteric agars were incubated at 37°C under aerobic conditions for 18 to 24 h. The mCCDA plate was incubated for 48 to 72 h at 42°C in a microaerophilic atmosphere, which was achieved by using a microaerophilic gas-generating kit (Oxoid). The media used for the culture of routine enteric pathogens (*Salmonella* and *Shigella* spp.) were negative for these organisms. However, on the mCCDA plate, grey-white colonies with a distinct metallic sheen were observed. All colonies growing on the mCCDA appeared similar, with no other colonial morphotypes observed.

Three colonies from mCCDA were subcultured to blood agar plates containing 5% defibrinated sheep blood to ensure purity of the isolate. After overnight incubation, the isolate was confirmed as a gram-negative rod by Gram-stained smear, and a number

of phenotypic tests were performed, including the oxidase test, the catalase test, ability to grow at temperatures ranging from 25 to 42°C, growth on MacConkey agar at 15 and 25°C, a wet-preparation motility test under phase-contrast microscopy, urease production, nitrate reduction, H₂S production, hydrolysis of hippurate, and susceptibility to 30 µg of nalidixic acid and 30 µg of cephalothin by the disk susceptibility test. The antibiotic susceptibility pattern of the isolate against several other antibiotics was also determined. The isolate was presumptively identified as *Arcobacter* sp. on the basis of colonial morphology, growth characteristics, and biochemical reactions, as described by Aydın et al. (10).

The putative isolate was then subjected to a genus-specific PCR and multiplex PCR (mPCR) for identification to the genus and species level, respectively. For the genus-specific PCR, the primer combination ARCOI-ARCOII, targeting a section of the 16S rRNA genes, was used (11). Each amplification process was performed in duplicate, using a 25-µl reaction mixture containing 2 ml template DNA, 2.5 ml of 10× PCR buffer [750 mM Tris-HCl, pH 8.8, 200 mM (NH₄)₂SO₄, 0.1% (vol/vol) Tween 20, and 1.5 mM MgCl₂], 10 µM of each primer, 0.2 mM each of the four dNTPs (Fermentas, Germany), and 1.5 U *Taq* DNA polymerase (Fermentas, Germany). The samples were subjected to an initial denaturation step (94°C for 5 min), followed by 35 amplification cycles. Each amplification cycle consisted of 1 min at 94°C (denaturation), 1 min at 56°C (primer annealing), and 1 min at 72°C (primer extension). A primer extension step (72°C for 7 min) followed the final amplification cycle. The amplified products were resolved in 0.6% (wt/vol) Tris-acetate-EDTA TAE agarose gel, and the band patterns were analyzed in the gel documentation

Corresponding Author: Fuat Aydın, Ph.D., Department of Microbiology, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey. Tel.: +90 352 338 22 72-144. Fax: +90 352 337 27 40. E-mail: faydin23@hotmail.com

system (Vilber Lourmat, France). Sterile distilled water served as the negative control. The 1223-bp region of 16S rRNA was amplified, and the genetic sequence was identified as *Arcobacter* sp. (Fig. 1).

The isolate identified as *Arcobacter* sp. was examined by mPCR, using the primers developed by Houf et al. (12) to determine the species. PCRs were performed in a 25- μ l reaction mixture containing 2 ml of template DNA, 2.5 ml of 10 \times PCR buffer, 10 μ M of each of the primers (ARCO, BUTZ, CRY1, CRY2, and SKIR), 0.2 mM each of the four dNTPs (Fermentas, Germany), and 1.0 U *Taq* DNA polymerase (Fermentas). PCR involved initial denaturation at 94°C for 3 min, 35 cycles of denaturation (94°C, 45 s), primer annealing (60°C, 45 s) and chain extension (72°C, 1 min), and final extension (72°C, 7 min). The amplified products were resolved in 1% (wt/vol) TAE agarose gel, and the band patterns were analyzed in the gel documentation system (Vilber Lourmat, France). Sterile distilled water served as the negative control. The mPCR showed that the strain was *A. butzleri* (Fig. 2).

For the purpose of species identification, DNA sequence analysis of the 1,223-bp genus PCR product was performed. BLAST analysis showed the sequence to have the closest homology (100%) with the 16S rRNA of *A. butzleri* (accession numbers DQ464342.1, DQ464341.1, AY621116.1, AF314538.1, U34386.1, and U34388.1).

The antibiotic susceptibility test was performed using a disk diffusion method (13). The *A. butzleri* isolate was susceptible to amikacin (30 μ g), erythromycin (15 μ g), doxycycline (30 μ g), piperacillin-tazobactam (100/10 μ g), trimethoprim-sulfamethoxazole (1.25/23.75 μ g), ciprofloxacin (5 μ g), levofloxacin (5 μ g), and nalidixic acid (30 μ g). The isolate was resistant to ampicillin (10 μ g), cefuroxime (30 μ g), and clindamycin (2 μ g) (antibiotic disks from Oxoid). The patient was treated with 1 g of ciprofloxacin/day for 7 days. His symptoms subsided within 2 days of the initiation of antibiotic therapy.

Discussion

Although several reports describe the isolation and identification of arcobacters from different sources, especially from animals and the environment (10,14-16), to our knowledge, no

publications describe the recovery of arcobacters from human infections in Turkey. Arcobacters were first described in the 1970s by Ellis and colleagues, who isolated these organisms from aborted fetal tissues of pigs and cattle. Because of their similarity to campylobacters and their ability to grow aerobically at 30°C, they were originally considered to be aerotolerant campylobacters (1). After detailed taxonomic studies, these microorganisms were later included in the genus *Arcobacter* (17). Our isolate was catalase, oxidase, and nitrate reduction positive but urease and H₂S negative. The isolate grew on MacConkey agar at 15 and 25°C and was susceptible to nalidixic acid but resistant to cephalothin.

In our case, the patient had eaten barbecued chicken 1 day before the onset of his diarrheal symptoms. Considering that *Arcobacter* species are commonly isolated from various animal sources, especially poultry (14,15), it seems possible that the source of our patient's infection was improperly cooked chicken. Because arcobacters have been isolated from the stool specimens of healthy people (18), another stool specimen was collected from our patient 1 month after the onset of the illness and cultured for the presence of arcobacter by using direct-plating and enrichment techniques. However, this time *A. butzleri* was not isolated from the patient's fecal specimen.

In several countries, *A. butzleri* has been reported to cause acute gastroenteritis and chronic diarrhea in humans. An outbreak caused by *A. butzleri* was reported in an Italian school (19). This outbreak involved 10 children who complained of abdominal pain without diarrhea. Lerner et al. (20) recovered *A. butzleri* from two hospitalized patients with diabetes mellitus type I, hyperuricemia, and alcohol abuse, who complained of diarrhea and abdominal cramps. In Chile, Fernandez et al. (21) isolated *A. butzleri* from two patients with chronic diarrhea, and in another study (22), *A. butzleri* was found at a prevalence of 8% as an etiological agent of travellers' diarrhea in Mexico, Guatemala, and India.

To our knowledge, this is the first reported human case of acute gastrointestinal infection caused by *A. butzleri* in Turkey. In light of this case, it is

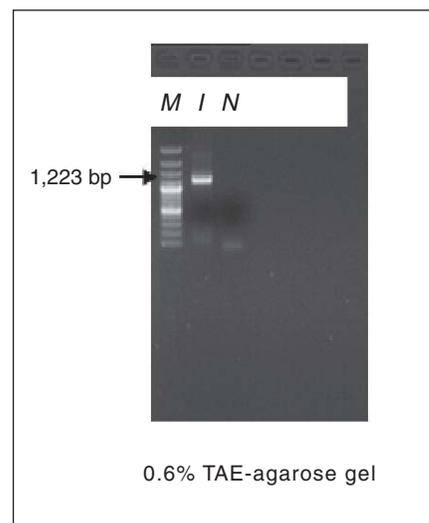


Figure 1. Identification of representative *Arcobacter* isolate to the genus level by PCR. M, marker (100 bp plus DNA ladder); I, the isolate; N, negative control.

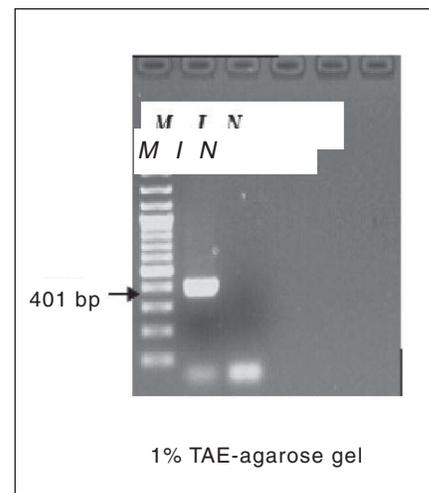


Figure 2. Identification of representative *Arcobacter* isolate by mPCR. M, Marker (100 bp plus DNA ladder); I, the isolate; N, negative control.

recommended that *A. butzleri* be considered a possible cause of gastroenteritis in humans

References

1. Vandenberg, O., M.B. Skirrow, and J.P. Butzler. 2005. *Campylobacter* and *Arcobacter*, p. 1541-1562. In S. Borriello, P. Murray, and P.R. Funke (ed.), Topley & Wilson's microbiology and microbial infections, 10th ed. Edward Arnold Ltd., Italy.
2. Lehner, A., T. Tasara, and R. Stephan. 2005. Relevant aspects of *Arcobacter* spp. as potential foodborne pathogen. *Int. J. Food Microbiol.* 102:127-135.

3. Snelling, W.J. et. al. 2006. Under the microscope: *Arcobacter*. Lett. Appl. Microbiol. 42:7-14.
4. Collado, L. and M.J. Figueras. 2011. Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. Clin. Microbiol. Rev. 24:174-192.
5. Figueras, M.J. et. al. 2011. *Arcobacter ellisii* sp. nov., isolated from mussels. Syst. Appl. Microbiol. 34:414-418.
6. Burnens, A.P., U.B. Schaad, and J. Nicolet. 1992. Isolation of *Arcobacter butzleri* from a girl with gastroenteritis on *Yersinia* selective agar. Med. Microbiol. Lett. 1:251-256.
7. On, S.L., A. Stacey, and J. Smyth. 1995. Isolation of *Arcobacter butzleri* from a neonate with bacteraemia. J. Infect. 31:225-227.
8. Yan, J.J. et. al. 2000. *Arcobacter butzleri* bacteremia in a patient with liver cirrhosis. J. Formosa Med. Assoc. 99:166-169.
9. Lau, S.K. et. al. 2002. Identification by 16S ribosomal RNA gene sequencing of *Arcobacter butzleri* in a patient with acute gangrenous appendicitis. J. Clin. Pathol. Mol. Pathol. 55:182-185.
10. Aydin, F. et. al. 2007. Prevalence and distribution of *Arcobacter* species in various sources in Turkey and molecular analysis of isolated strains by ERIC-PCR. J. Appl. Microbiol. 103:27-35.
11. Harmon, K.M. and I.V. Wesley. 1996. Identification of *Arcobacter* isolates by PCR. Lett. Appl. Microbiol. 23:241-244.
12. Houf, K. et. al. 2000. Development of a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. FEMS Microbiol. Lett. 193:89-94.
13. CLSI. 2010. Performance standards for antimicrobial susceptibility testing 20th informational supplement. M100-S20. CLSI, Wayne, PA.
14. Atabay, H.I. et. al. 2002. The prevalence of arcobacters on chicken carcasses sold in retail markets in Turkey and identification of the isolates using SDS-PAGE. Int. J. Food Microbiol. 81:21-28.
15. Ertas, N. et. al. 2010. Investigation of the existence of *Arcobacter* species in environmental water resources and raw milk by multiplex PCR. J. Food Protect. 73:2099-2102.
16. Atabay, H.I. et. al. 2008. Isolation of various *Arcobacter* species from domestic geese (*Anser anser*). Vet. Microbiol. 128:400-405.
17. Vandamme, P. et. al. 1992. Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. Int. J. Syst. Bacteriol. 42:344-356.
18. Miller, G.W. et. al. 2009. First multi-locus sequence typing scheme for *Arcobacter* spp. BMC Microbiol. 9:196.
19. Vandamme, P. et. al. 1992. Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. J. Clin. Microbiol. 30:2335-2337.
20. Lerner, J., V. Brumberger, and V. Preac-Mursic. 1994. Severe diarrhea associated with *Arcobacter butzleri*. Eur. J. Clin. Microbiol. Infect. Dis. 13:660-662.
21. Fernandez, H., S. Krause, and M.P. Villanueva. 2004. *Arcobacter butzleri* an emerging enteropathogen: communication of two cases with chronic diarrhea. Brazil J. Microbiol. 35:216-218.
22. Jiang, Z.D. et. al. 2010. Microbial etiology of travelers' diarrhea in Mexico, Guatemala, and India: importance of enterotoxigenic *Bacterioides fragilis* and *Arcobacter* species. J. Clin. Microbiol. 48:1417-1419.