

ARCOBACTER BUTZLERI AN EMERGING ENTEROPATHOGEN: COMMUNICATION OF TWO CASES WITH CHRONIC DIARRHEA

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SHORT COMMUNICATION

ABSTRACT

The first two cases of chronic diarrhea due to *Arcobacter butzleri* in Chile are reported. The clinical findings, the absence of other enteropathogens, virus or parasites, the epidemiological association between both patients, the treatment outcome and the fact that *A. butzleri* was the only bacteria isolated, support the assumption that it was the etiological agent of these chronic diarrhea cases.

Key words: *Arcobacter butzleri*, chronic diarrhea, emerging pathogen

The genus *Arcobacter*, belonging to the family *Campylobacteraceae*, includes polar flagellated, curved or spiral Gram negative bacteria described first in 1977 as *Vibriol Spirillum* organisms and later as aerotolerant *Campylobacter* species (1). Currently, the genus *Arcobacter* is composed of four species (11): i. *A. nitrofrigidis* is a nitrogen fixing microorganism associated to the roots of *Spartina alterniflora*, a salt marsh plant; ii. *A. skirrowii* has been isolated from lambs with diarrhea and from aborted fetuses of pigs, bovines and ovine and more recently from chronic diarrhea in a human being (14); iii. *A. cryaerophilus* and iii. *A. butzleri* have been associated with abortion and enteritis in animals and with diarrhea and bacteremia in adults and children. The latter is also considered an emerging food pathogen and it seems to be most frequently isolated from human beings than the other species (6,12,13). We report here two cases of chronic diarrhea affecting two brothers from whom *A. butzleri* was isolated.

Case 1. A 2 years 6 months old boy was admitted at the Pediatric Gastroenterology Unit of the Valdivia County Hospital (Valdivia City – Chile, 73° 11' Western, 39° 46' Southern latitude) with an episode of acute gastroenteritis associated with vomiting, mucous diarrheic stools with no blood or pus and

moderate dehydration, requiring hospitalization. Three stool samples obtained every other day were examined for ova and parasites. Additional two fecal samples were obtained. One was tested for rotavirus and the other was cultured only for enteropathogenic *Enterobacteriaceae*. All the tests were negative.

Parenteral fluid therapy, dietetic antidiarrheic regimen and no antimicrobial therapy were prescribed. The patient improved quickly being discharged after 48 h hospitalization.

During the further three months he suffered from several short intermittent diarrheic episodes until he was admitted at the Pediatric Gastroenterology Unit again, with a two days history of mucous diarrhea with no signs of dehydration. At that time the child weighed 14.3 Kg and his height was 96 cm. Stool samples were taken for parasites, rotavirus, enteropathogenic *Enterobacteriaceae*, and *Campylobacter* and *Arcobacter*. Stool culture for the classical thermophilic enteropathogenic *Campylobacter* species was done by direct inoculation onto a modified Skirrow medium plate (3). For the emerging *Campylobacter* and *Arcobacter* species the membrane filter method (2) was used. In brief, a 47 mm 0.45µm membrane filter (Millipore) was placed on each of two sheep

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blood agar plates and various drops (10-12) of 1/10 fecal suspension in saline solution were placed onto the membrane filters. The membranes were removed 30 min later following filtration of both the fluid and bacterial cells. The modified Skirrow medium plate was incubated at 42°C for 48 h and one of the sheep blood agar plates (for emerging *Campylobacter* species) at 37°C for up to five days, under microaerophilic atmosphere, and the other (for *Arcobacter* species) at 26°C under aerobic conditions.

No parasites, rotavirus, classical enteropathogenic bacteria nor *Campylobacter* spp. were found. However in blood agar plates incubated at 37°C under microaerophilic atmosphere and at 26°C under aerobic conditions, little, round, concave pinpoint, non hemolytic colonies were isolated. Gram stain from colonies of both plates showed Gram negative curved and s-shaped rods that were actively motile in wet preparations under phase contrast microscopy. Oxidase and catalase tests gave positive reactions. Subcultures incubated aerobically at 42°C failed to grow but the microorganisms were able to grow at 37, 26 and 15°C. Their growth and morphological characteristics as well as their biochemical properties were compatible with those of *A. butzleri* (Table 1).

After the identification of the Gram negative curved rods, erythromycin (50 mg/kg/day divided into four doses for 10 days) and dietetic regimen were prescribed. The favorable outcome without further diarrhea relapses was rapidly followed

and the two control stool cultures for *Arcobacter*, one at the 5th day of treatment and the other at the 3^d day after ending treatment, were negative.

Case 2. This is a 1 year old girl, with a previous history of two acute diarrheic episodes, one at 8 and the other at 10 months of age. In both opportunities the diarrheic syndrome coursed with no dehydration and no blood and leucocytes in stools were observed being the patient successfully treated only with dietetic antidiarrheic regimen.

The patient, the sister of Case 1, during the last three months suffered intermittently of abdominal cramps and pain, sometimes with not well formed stools. Having in mind this clinical picture and the isolation of *A. butzleri* from her brother, a stool sample was taken and the same laboratory studies described above were performed and only *A. butzleri* was isolated.

She was treated with erythromycin (50mg/kg/day divided into four doses for 10 days) and dietetic regimen. Abdominal pain and cramps disappeared after treatment and *A. butzleri* could no longer be detected in the two control stool cultures performed.

Since the first clinical case of *Arcobacter* enteritis was reported in 1987 (9), several communications indicated that *A. butzleri* could be considered as an emerging pathogen for humans. It has been associated with acute (4) and severe diarrheal illness (5), recurrent abdominal cramps (10), persistent or chronic diarrhea (7), bacteremia (15) and neonatal sepsis (8).

One of our cases coursed as a chronic diarrhea and the other showed abdominal pain and cramps as main clinical features. Both clinical presentations have been described previously. Chronic diarrhea cases were reported by Marinescu *et al.* in two children (7), whereas an outbreak of abdominal cramps was described among school students in Italy by Vandamme *et al.* (10). More recently, Vandenberg *et al.* (12) showed that *A. butzleri* is more frequently associated with a persistent and watery diarrhea and less associated with bloody diarrhea.

Consumption of contaminated poultry and contaminated water (6,13) have been described as risk factors for human infection. In both cases consumption of contaminated poultry and water was ruled out as infection risk factors because they drink only potable water and well cooked chicken meat. However, the mother referred that the children used to play in an area visited by sparrows and where bird feces could be found. We took fecal samples from 60 sparrows captured

Table 1. Phenotypic properties of zoonotic *Arcobacter* species and the patients' strains.

Test	<i>A. butzleri</i>	<i>A. cryaerophilus</i>	<i>A. skirrowii</i>	Patients' strains
Oxidase	+	+	+	+
Catalase	V	V	+	+
Nitrate reduction	+	+	+	+
Urease	-	-	-	-
H ₂ S (TSI)	-	-	-	-
Hydrolysis of: hippurate	-	-	-	-
indoxylacetate	+	+	+	+
Growth at: 15°C	+	+	+	+
25°C	+	+	+	+
42°C	V	-	-	-
Growth: in 1% glycine	-	V	-/V	-
in 3.5% NaCl	V	-	V	W
on McConkey agar	V	V	-	+
on Minimal Medium	+	-	-	+
Susceptibility to: NA	V	V	S	S
cephalothin	R	R	R/S	R

+, positive reaction; -, negative reaction; TSI, triple sugar iron agar; W, weak reaction; NA, nalidixic acid; V, variable reaction; S, susceptible; R, resistant.

in that area, isolating *A. butzleri* in 7% of them (unpublished data). However we were not able to establish epidemiological relationships between the strains isolated from the patients and those isolated from the sparrows. Nevertheless, they showed the same susceptibility levels to erythromycin (0.5 µg), ampicillin (≥256 µg), gentamicin (0.75 µg), ciprofloxacin (0.125 µg), cloramphenicol (64 µg) and tetracycline (8 µg), using the E-test method. Therefore, we believe that the children could have been infected by direct contact with sparrow feces. Being *A. butzleri* a zoonotic bacteria, contact with animals or animal dejections could also be considered as a risk for human infection.

Since the strains were susceptible *in vitro*, both children were successfully treated with erythromycin and after the symptoms subsiding, *A. butzleri* could no longer be detected in two consecutive stool cultures.

A. butzleri is considered an emerging human pathogen (6,12,13). However, little is known about its clinical significance. Thus, case-control and virulence studies need to be conducted in order to establish pathogenicity factors and clinical features of *A. butzleri* infections.

Although *Arcobacter* intestinal infections in humans have been reported since 1987 (8), there is no standardized method for the isolation of these bacteria. Therefore, there is a need of optimized primary isolation procedures. This represents an important step for the definition of a laboratory identification protocol and their clinical and epidemiological significance.

The isolation of *A. butzleri* in association with clinical symptoms and in the absence of other enteric pathogens as well as the successful treatment with erythromycin suggest that this microorganism was the responsible of both cases. We believe this to be the first cases of chronic diarrhea in humans due to *A. butzleri* in Chile and probably, in South America.

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RESUMO

***Arcobacter butzleri* um enteropatógeno emergente: comunicação de dois casos de diarreia crônica**

Os primeiros dois casos de diarreia crônica por *Arcobacter butzleri* no sul do Chile são apresentados. As características clínicas, a ausência de outros enteropatógenos, vírus ou parasitas, o resultado do tratamento, bem como a associação epidemiológica entre ambos pacientes e o fato de *A. butzleri* ter sido a única bactéria isolada permitem assumir que este

microrganismo seria o agente etiológico destes dois casos de diarreia crônica.

Palavras chaves: *Arcobacter butzleri*, diarreia crônica, patógeno emergente

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