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ARCOBACTER BUTZLERI: FIRST ISOLATION REPORT FROM CHICKEN CARCASSES IN COSTA RICA

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ABSTRACT

Arcobacter butzleri isolation from chicken carcasses in Costa Rica is reported for the first time. The isolated strains (P and R) were presumptively identified by their phenotypic characteristics. Definitive identification was made using a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*.

These first isolations indicate the necessity of further investigation about the prevalence, distribution, ecology and interactions with human beings of this and other *Arcobacter* species.

Key words: *Arcobacter butzleri*, chicken carcasses, isolation, epidemiology

The genus *Arcobacter*, belongs to the family *Campylobacteraceae*, class Proteobacteria, subclass Gracillicutes and comprises polar flagellates, spirally, curved, Gram negative rods formerly known as aerotolerant *Campylobacter*-like organisms (4).

The first isolates were obtained by Ellis *et al*. (5) from aborted bovine fetuses. Further studies related these microorganisms with mastitis and abortion in the bovine, ovine, equine and porcine species (4, 15).

Currently, the genus *Arcobacter* comprises the following species: *A. cryaerophilus*, *A. butzleri*, *A. skirrowii, A. nitrofrigilis*, *A. cibarius*, *A. halophilus, A. mytili*, *A. thereius, A. marinus* and the "*Candidatus A. sulfidicus"* (1, 9, 13). At present, *A. butzleri* is considered the most common species of the genus being considered as a zoonotic and emerging foodborne pathogen that could be associated with bacteremia and human diarrheic illness. It has been also isolated from food of animal origin, especially from poultry, carcasses and offal, milk, mussels, as well as from water bodies, sewage and fecal samples of different animal species (6, 7, 12, 17, 19).

In Latin America little information about *Arcobacter* species is available, being isolated in Chile (6, 7), Argentina (8), Brazil (3) and Mexico (18). In order to provide additional information about the occurrence of *A. butzleri* in different parts of the world, we report here the first isolation of this zoonotic and emerging foodborne pathogen from chicken carcasses in Costa Rica.

During the search of *Campylobacter* spp. in chicken carcasses, from two pre-enriched samples seeded on cefoperazone charcoal deoxycholate agar (CCDA), a commercial blood free medium for the isolation of *C. jejuni, C. coli, C. upsaliensis* and *C. lari* at 37°C, little pinpoint, translucent colonies were isolated. These colonies differed from the characteristics gray, moist, flat-spreading colonies

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of *Campylobacter.* However, Gram stain and wet mount observations under phase contrast microscopy revealed Gram negative curved bacilli with rapid darting and corkscrew-like motility, respectively, being also catalase and oxidase positive. Growth tests at 25°C and 37°C in aerobic atmosphere and at 42°C in microaerobic atmosphere were performed. Positive results were obtained only at the two first conditions. These results allowed presumptive indetification of isolates as *Arcobacter* sp. Strains were identified phenotypically as *A. butzleri* using the standard tests described in Table 1 (1). Definitive identification was made using the multiplex polymerase chain reaction (*m*-PCR) proposed by Houf *et al.* (Fig.1) (11), confirming that both strains (strain P and strain R) corresponded to the species *A. butzleri*.

Table 1. Differential characteristics of three zoonotic *Arcobacter* species and *Campylobacter jejuni*

Characteristic	***A. cryaerophilus***	***A. butzleri***	***A. skirrowii***	***C. jejuni***
Oxidase	+	+	+	+
Catalase	+	+	V	+
Nitrate reduction	+	+	+	+
Hyppurate hydrolisis	-	-	-	+
Indoxyl acetate hydrolisis	+	+	+	+
Growth in:				
Air at 25°C	+	+	+	-
4% (W/V) NaCl	-	-	+	-
Mac Conkey agar	V	+	-	-
Resistance to: cephoperazone (64 mgl^{-1})	+	+	+	+

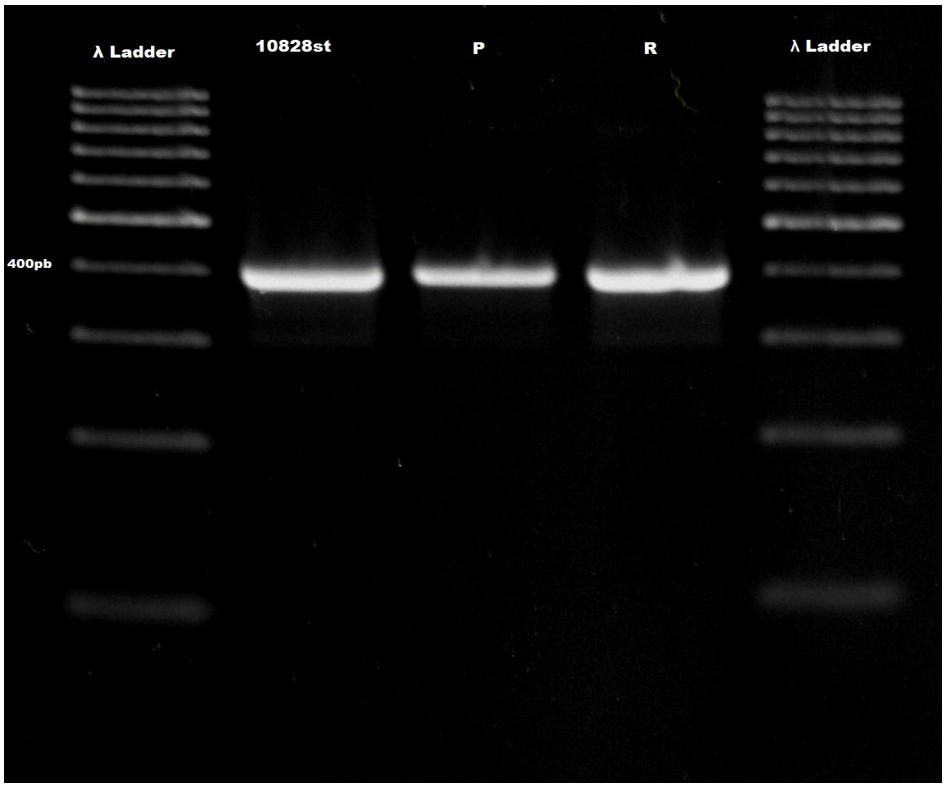

Figure 1. First lane: 100-bp ladder; second lane: A. butzleri 10828st reference strain; third lane: strain P; fourth lane: strain R; fifth lane: 100-bp ladder.

A. butzleri is an emerging pathogen that has been associated with abortion and enteritis in animals and with diarrhea and bacteremia in adults and children. This A*rcobacter* species seems to be the most frequent in human beings. *A. butzleri* recognizes a wide reservoir range, being isolated from domestic and free living mammals and birds, from retail chicken meat, from shellfish and environmental water bodies as well. It has been suggested that water and chicken meat may play an important role in the transmission of these organism (6, 7, 12, 14, 16).

At present no standard isolation method for *Arcobacter* has been proposed, however there is consensus that an enrichment step and the use of antimicrobials are necessary for this purpose (2,9,14).

The first strains of *A. butzleri* isolated from chicken meat in Costa Rica grew in CCDA after a previous enrichment period of 24h in Bolton broth, both incubated in microaerobic conditions at 37°C. This is not surprising because *A. butzleri* is resistant to the antimicrobials present in both media (2,14). Furthermore, arcobacters are able to grow under aerobic and anaerobic conditions over a wide temperature range (15–37°C) but optimal growth occurs under microaerobic conditions (3–10%O_2) (4).

Correct identification of arcobacters is not easy, and too often, arcobacters are misidentified as campylobacters, especially when phenotypical methods are applied. Due to their relative metabolic inertness biochemical identification of arcobacters is not recommended (9) being necessary the use of some molecular techniques such as the *m*-PCR described by Houf et al. (11). As shown in Fig 1, both strains amplified a 401-bp fragment, specific for *A. butzleri*. No PCR product was generated for other *Arcobacter* species.

Like for *Campylobacter*, a high prevalence of *A. butzleri* is observed on chicken carcasses (9, 14, 16). The first isolation of *A. butzleri* from chicken carcasses in Costa Rica not only reveals the presence of this bacterium in our country but, due their potential importance for public health, also creates the necessity of further investigation about the prevalence, distribution, ecology and interactions with human beings of this and other *Arcobacter* species.

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