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Prevalence of *Arcobacter* species isolated from human and various animals in east of Turkey

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ABSTRACT

This study was conducted to determine presence and distribution of *Arcobacter* spp. by conventional culture and a multiplex PCR (m-PCR) in the intestinal content samples that are collected from the cattle, sheep and goats and in the faecal samples of people who reffered to the hospitals in the east of Turkey, because of complains about gastroenteritis and diarrhea. In the examination of the total 800 samples, containing 200 swab samples from each of animal species (cattle, sheep and goat) and 200 human faecal samples, *Arcobacter* spp. were isolated from 2.25 % (18/800) of the samples. The isolated *Arcobacter* strains were identified by genus and species specific PCR assays. The isolation percentages were calculated as 2.12% (17/800) for *A. butzleri* and 0.12% (1/800) for *A. cryaerophilus.* None of the human and animal samples were found to be positive for *A. skirowii.* In conclusion, this report confirmed that the presence of *Arcobacter* species in human and various animals feacal samples in the east of Turkey. In addition, the present study is the first study to demonstrate the existence of *A. butzleri* and *A. cryaerophilus* in goats intestinal content samples in Turkey.

1. Introduction

Arcobacter is a genus previously known as aerotolerant *Campylobacter*, have recently been considered emergent enteropathogens and potential zoonotic agents (Ho et al., 2006). Currently, the genus *Arcobacter* contains 21 species of which three (*Arcobacter butzleri*, *A. cryaerophilus* and *A. skirrowii*) have emerged in recent years as potential human pathogens and associated with gastrointestinal infections (Collado and Figueras, 2011). In particular, *A. butzleri* has been linked with several cases of gastrointestinal diseases, with diarrhea being the main symptom in humans (Kiehlbauch et al, 1991; On et al.1995). Among *Campylobacter* spp. and related organisms, *A. butzleri* has been described as the fourth most prevalent pathogen found in diarrhoeic samples (Figueras et al., 2014; Fernandez et al., 2015). In addition, it has been reported that *Arcobacter* spp. were isolated from faecal samples of clinically healthy animals including poultry, cattle, swine etc. as well as from variety of foods and water sources (Rice et al., 1999; Ongor et al., 2004; Grove-White et al., 2014; Girbau et al., 2015).

Despite of the range of the isolation methods used previously, no single standard method for the isolation of *Arcobacter* spp. from faecal samples has been established. Numerous selective media for the isolation of arcobacters have been described, almost all containing several antibiotics as inhibitory agents. A different approach, involving the passage of motile *Arcobacter* through a membrane filter onto a nonselective growth agar medium, is recognized as allowing the isolation of *Arcobacter* sensitive to antibiotics incorporated

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into the selective media(Ongor et al., 2004; Hamill et al., 2008). The major drawback of this technique is the labor-intensive character of the method, the lower sensitivity of the medium compared to conventional selective media, and overgrowth of plates by competing fecal contaminants such as bacteria in Enterobactericeae family (Aspinall et al., 1993; Lopez et al., 1998). As a consequence, the use of a selective agar and the filter method in combination is recommended to optimize recovery of arcobacters from faecal samples (Ongor et al., 2004). Due to the difficult phenotypic characterization of *Arcobacter* spp., DNA-based methods as PCR currently have been reported for identify of arcobacters at genus and species level (Harmon and Wesley, 1997; Houf et al., 2000). m-PCR (targeting the 16S and 23S rRNA genes), was developed for the simultaneous detection and identification of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* by Houf *et al.* (2000).

Information on the presence of arcobacters in various sources in Turkey is limited, so the present study was conducted to determine the prevalence of *Arcobacter* spp. isolated from different animals and human in east of Turkey. According to our knowledge, the present study is the first report of the isolation of *Arcobacter* spp. from goat intestinal content samples.

2. Materials and Methods

2.1. Samples collection

A total number 800 samples were collected from various sources including of intestinal contents samples of animals (cattle, sheep, goats) (200 each) slaughtered at a local abattoir in the east of Turkey; faecal samples of people (200) who reffered to the local hospitals with the complaints of gastroenteritis and diarrhea. Faecal samples were obtained from human using sterile cotton swabs. The intestinal contents were also collected from the lumen of the intestine. The swab samples were transferred to the laboratories within tubes containing 0.9% NaCl under cool conditions (at 4 °C).

2.2. Isolation of Arcobacter spp.

Faecal samples weere examined according to the method described by Ongor et al. (2004). Briefly, samples were homogenized by vortexing and aseptically inoculated into 10 ml Brucella broth (Difco, Detroit, MI, USA) containing Cefoperazone – Amphotericin - Teicoplanin (CAT) supplement (SR174E, Oxoid, UK) described by Atabay and Corry (1998). All enrichment samples were incubated for 48 h at 30°C under aerobic conditions. The enriched samples were dispensed using a micropipette onto 0.45µ pore size cellulose acetate filter liad on the surface of a Muller-Hinton Agar (CM337, Oxoid) with %5 lysed horse blood. The plates were incubated aerobically at 37°C for 1 h before filters was removed. Then the inoculated plates were incubated under aerobic conditions at 30°C for 48-72 h. The isolates were presumptively identified as *Arcobacter* spp. by colony appearance, microscopic morphology and motility. Then, Two or three pure colonies from each positive sample were selected for following experiments and stored at 20 °C in Nutrient Broth (CM067B; Oxoid) with 15% glycerol.

2.3. Multiplex-PCR (m-PCR) to detect Arcobacter at species level

Single colony from each isolate was used to extract bacterial DNA by the chloroform extraction method as described previoulsy by Acik and Cetinkaya (2005) in Turkey. The PCR was performed in a gradient thermalcycler (TC-512, Techne, UK) in a total reaction volume of 50 µl containing 5 µl of 10 X PCR buffer (750 mM Tris–HCl, pH 8.8, 200 mM (NH4)2SO4, 0.1% Tween 20), 5 µl 25 mM MgCl2, 250 µM of each deoxynucleotide triphosphate, 1.25 U of Taq DNA polymerase (MBI Fermentas, Hanover, MD, USA), 1 µM of each primer (Iontek, Bursa, Turkey) and 5 µl of template DNA. A pair of primers derived from 16S rRNA (Harmon and Wesley 1997) was first used to identify arcobacters at genus level. Then, positive DNA samples were examined further using three pairs of specific primers for *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*, which were desribed by Houf *et al.* (2000) for differentiation at species level (Table 2). Amplification procedures used for both genus and species- specific PCR (m-PCR) were described previously (Harmon and Wesley, 1997; Houf et al., 2000). In the genus-specific PCR, products with the molecular size of 1223 bp and in the m-PCR, the sizes of 401, 257, 641

bp were considered indicative for identification as *Arcobacter* spp., *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*, respectively. PCR amplicons were detected by electrophoresis in 2% (w/v) agarose gel and stained with ethidium bromide and then visualized using an ultraviolet transilluminator.

Reference *A. butzleri* (ATCC 49616), *A. cryaerophilus* (ATCC 43157) and *A. skirrowii* (ATCC 51132) strains were included as positive controls and distilled water was used as negative control in all assays.

2.4. Statistical analysis

A chi-squared (χ^2) test was used to estimate differences between the isolation rates of Arcobacter spp. in animals and human, whereby a probability of less than 0.05 was considered as statistically significant.

3. Results

Of the 800 faecal samples tested by conventional culture and PCR, 18 (2.25%) were detected to be positive for *Arcobacter* spp. the isolation rate of *Arcobacter* spp. determined to be the highest in the intestinal content samples of goat with 4% (8/200) and the lowest in the intestinal content samples of sheep with 1% (2/200). The distribution of isolation rates by sample and animal species is present in Table I.

In m-PCR analysis of DNA samples extracted from the colonies of 18 samples, *A. butzleri* was identified from 17 (94.4%). This proportion was calculated to be 2.1 % when the total number of 800 samples was considered. The presence of *A. butzleri* was found to be highest in intestinal content samples of human, sheep, cattle, followed by intestinal content samples of goat (87.5%). Only one goat sample yielded *A. cryaerophilus* wheras none of the sheep, cattle and human samples were found positive for *A. cryaerophilus*. In addition, no *A. skirrowi* were isolated and identified from samples of human and animals. No significant differences were found between the isolation rates of *Arcobacter* spp. obtained from animals and human ($P<0.001$).

4. Discussion

Arcobacters have been isolated from many different sources such as animals and their products, human and foods. In many studies, arcobacters have been associated with animal diseases including abortion, mastitis and diarrhoea (Vandamme De Ley, 1991; Lopez et al., 1998; Van Driessche et al., 2003; Hamill et al., 2008) and reproduction disorders (De Oliveira et al., 1997). *Arcobacter* species have been also isolated from clinically healthy animals and human (Houf et al., 2000; On et al., 2002, Rice et al., 1999). There is limited research on *Arcobacter* presence in goat samples. Shah et al. (2013) reported that none of the goat faecal samples was found to carry *Arcobacter* spp. On the contrary, 10.7% of faecal samples from goats were reported to be positive for *Arcobacter* by De Semet et al. (2007). In thi study, the prevalence of *Arcobacter* spp. in the goat samples was relatively low, which is compareble to those by De Semet et al. (2007). Differences in *Arcobacter* prevalence in goat samples were also observed between other animals. The prevalence of *Arcobacter* in cattle samples in this study was lower to be prevalence found by Ongor et al. (2004) in Turkey and to prevalance previously reported by Fernandez et al. (2015) in Chile. Other studies have also reported the occurence of arcobacters in clinically healthy cattle, with a prevalence varying from 3.6 to 40.1% (Harmon and Wesley, 1997; Golla et al., 2002; Kabeya et al., 2003; Van Driessche et al., 2003; Grove-White et al., 2014). In addition, previous studies reported high prevalance of *Arcobacter* spp. in sheep (Golla et al., 2002; Rivas et al., 2004). The prevelance of *Arcobacter* spp. in the sheep samples in this study 1%, which is lower than found in a previously study (Golla et al., 2002). A variety of factors, such as age, season, number of animals examined, type of samples and isolation methods, could be proposed to be responsible for the differences in the isolation rates of the agents. Shah *et al.* (2013) reported that prevalence of *Arcobacter* was showed in significant difference in detection rates between adult and young cattle. In addition, the prevalence of *Arcobacter* spp. isolated from water samples in winter was significantly higher than summer (Tazegul, 2010).

In Turkey, there are not records related the prevalence of *Arcopacter* spp. in human, due to not recognize the important of *Arcobacter* for public healthy and not apply isolation and identification of agents in rutine laboratory in

hospitals. In a study conducted at the local region in Turkey by Kayman et al. (2012), 9 out of 3287 human faecal samples (0,27%) were detected to be positive for *Arcobacter* spp. In the present study, the prevalence of *Arcobacter* species in human faecal samples (1.5%) was significantly higher to the prevalence found by Kayman *et al.* (2012). However, the prevalence of *Arcobacter* in this study was similar to the prevalence observed by Abeele et al. (2014) in Belgium (1.3%). Abeele et al. (2014) reported that prevalence of other intestinal pathogens *Campylobacter*, *Salmonella* and *C. difficile* was 5.6%, 2% and 1.6%, respectively. These results suggests that prevalance of *Arcobacter* in human was low compared to those of other foodborne pathogens and *Arcobacter* spp. were the fourth most common pathogen group.

Arcobacter butzleri has been described as the predominant species isolated from animals and human samples, and it was confirmed as the most prevalent agent in this study. 94.4% and 5.6% of the tested *Arcobacter* species were found to be positive for *A. butzleri* and *A. cryaerophilus*, respectively, whereas none of the examined samples were found positive for *A. skirrowii*. *Arcobacter cryaerophilus* was detected from only one goat samples in this study. To our knowledge, this is the first report in which in *A. cryaerophilus* was isolated from goat intestinal contents samples. The findings in this study were similar to results previously reported in the animals and human samples. Ongor et al. (2004) reported that prevalence of *A butzleri, A. cryaerophilus* and *A. skirrowii* were found to be positive 7% , 2% and 0.7% of cattle faecal samples, respectively, in east of Turkey. In other studies, the high prevalence of the *A. butzleri* in various animals and human was also reported by other authors (Rahmi, 2014; Yesilmen et al., 2014; Fernandez et al., 2015; Zacharow et al., 2015) Fernandez et al. (2015) reported that only *A. butzleri* was isolated (3.6%) from childeren with diarrhea and unable to recovery A. *cryaerophilus* and *A. skirrowii*. A number of factors such as the type and concentrations of antimicrobial compounds in media might influence the growth and isolation rate *Arcobacter* spp. in particularly, *A. cryaerophilus* and *A. skirrowii*. In addition, some changes in the isolation methods, such as cultivation under microaerobic conditions or the use of blood agar plates without any antibiotic, may improve the isolation rates of *A. skirrowii* and A. *cryaerophilus* due to these species are more difficult to culture than *A. butzleri*.

In conclusion, this report confirmed the presence of *Arcobacter* species in human and various animals feacal samples in the east region of Turkey. Therefore, they can play vital role in contamination of environment and food chain and pose a thtreat for human healthy. According to our knowledge, the present study is the first report of the isolation of *Arcobacter* spp. from goat faecal samples. In additon this is the first report in which in *A. cryaerophilus* was isolated from goat intestinal contents samples.

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Table 1. Results of culture and m-PCR

	PCR and Culture results		m-PCR results		
Type of samples	No. of samples	*Arcobacter* spp.	*A. butzleri*	*A. cryaerophilus*	*A. skirrowii*
Cattle	200	5 (2.5%)	5 (100%)	0	0
Sheep	200	2 (1%)	2(100%)	0	0
Goat	200	8 (4%)	7 (87.5%)	1(12.5%)	0
Human	200	3 (1.5%)	3 (100%)	0	0
Total	**800**	**18 (2.25%)**	**17 (94.4%)**	**1 (5.6%)**	**0**

P<0.001

Table 2. Primers used in this study.

Species	Primers	Sequences (5'-3')	PCR Products (bp)	References
Arcobacter spp.	Arco I Arco II	AGAGATTAGCCTGTATTGTATC TAGCATCCCCGCTTCGAATGA	1223	Harmon and Wesley, 1997
A.butzleri	BUTZ ARCO	CCTGGACTTGACATAGTAAGAATGA CGTATTCACCGTAGCATAGC	401	Houf et al., 2000
A.skirrowii	SKIR ARCO	GGCGATTTACTGGAACACA CGTATTCACCGTAGCATAGC	641	Houf et al., 2000
A.cryaerophilus	CRY1 CRY2	TGCTGGAGCGGATAGAAGTA AACAACCTACGTCCTTCGAC	257	Houf et al., 2000

Refereces

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