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**Genotyping of various *Arcobacter* species isolated from domestic geese by randomly amplified polymorphic DNA (RAPD) analysis**

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The present study was undertaken to genotype *Arcobacter* (A.) *butzleri*, *A. cryaerophilus* and *A. skirrowii* isolates from domestic geese from three different flocks in Turkey. Fifteen *Arcobacter* isolates were analysed to determine the RAPD profiles based on the amplified DNA fragment patterns using a universal primer for genotyping. 7 *A. cryaerophilus*, 2 *A. butzleri* and 6 *A. skirrowii* isolates produced 6, 2 and 3 distinct profiles, respectively. The isolates of the same patterns originated from the same flocks. The findings of the present study may support previous reports of the existence of a large degree of heterogeneity among *Arcobacter* isolates. Observation of such levels of genetic diversity may suggest that there are multiple contamination sources in the environment and/or the determined genotypes may have undergone genetic rearrangements. This first report of genotyping of various *Arcobacter* species isolated from healthy geese is expected to improve the understanding of the ecology and epidemiology of this emerging pathogen.

**Keywords:** *Arcobacter*, genotyping, RAPD-PCR, geese

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**Summary**

The present study was undertaken to genotype *Arcobacter* (A.) *butzleri*, *A. cryaerophilus* and *A. skirrowii* isolates from domestic geese from three different flocks in Turkey. Fifteen *Arcobacter* isolates were analysed to determine the RAPD profiles based on the amplified DNA fragment patterns using a universal primer for genotyping. 7 *A. cryaerophilus*, 2 *A. butzleri* and 6 *A. skirrowii* isolates produced 6, 2 and 3 distinct profiles, respectively. The isolates of the same patterns originated from the same flocks. The findings of the present study may support previous reports of the existence of a large degree of heterogeneity among *Arcobacter* isolates. Observation of such levels of genetic diversity may suggest that there are multiple contamination sources in the environment and/or the determined genotypes may have undergone genetic rearrangements. This first report of genotyping of various *Arcobacter* species isolated from healthy geese is expected to improve the understanding of the ecology and epidemiology of this emerging pathogen.

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**Zusammenfassung**


**Schlüsselwörter:** *Arcobacter*, Genotypisierung, RAPD-PCR, Gänse
Introduction

The genus *Arcobacter* (A.) was formed to include a number of microorganisms initially referred to as ‘aerotolerant campylobacters’ (Neill et al., 1982; Vandamme et al., 1991). These bacteria are distinguished from *Campylobacter* by their ability to grow at aerobic conditions and at lower temperatures (Vandamme et al., 1992). *Arcobacter* currently contains six validly described species: *A. butzleri*, *A. cryaerophilus* (with two subgroups), *A. skirrowii*, *A. nitrofigilis*, *A. cibarius* and *A. halophilus* (Vandamme et al., 1992; Houf et al., 2005; Stuart et al., 2005).

*A. butzleri*, *A. cryaerophilus* and *A. skirrowii* have been associated with various animal diseases including abortion, diarrhoea and mastitis (Logan et al., 1982; Kiehlbauch et al., 1991; On et al., 2002). These three species have also been isolated in cases of human diseases such as enteritis and bacteremia with *A. butzleri* being the most frequently reported species (Mansfield and Forsythe, 2000; Vandenberg et al., 2004; Wybo et al., 2004; Snelling et al., 2006). *Arcobacters* were initially isolated from bovine foetuses (Ellis et al., 1977). They have also been isolated from different foods such as poultry carcasses, pork, beef and various water samples (Atabay et al., 1998a, 2002a, 2006; Rice et al., 1999; Kabeya et al., 2004; Gude et al., 2005), which suggests that *arcobacters* are transmitted via food and water. The studies conducted to determine the prevalence of *Arcobacter* spp. in foods of animal origin reported that arcobacters are more prevalent in poultry than in red meat (Corry and Atabay, 2001).

Four species of *Arcobacter*, *A. butzleri*, *A. cryaerophilus*, *A. skirrowii* and *A. cibarius*, have so far been isolated in poultry and poultry products (Houf et al., 2005). *Arcobacter* spp. were also isolated in healthy livestock (Onor et al., 2004; Van Driessche et al., 2005).

Due to relative biochemical inertness and fastidious growth requirements, routine identification and differentiation of *Arcobacter* spp. are problematic (Atabay et al., 1998b). In addition, phenotypic similarities between *Campylobacter* and *Arcobacter* could lead to misidentification of *Arcobacter* as *Campylobacter* (Houf et al., 2000). Moreover, selective agent(s) used in *Arcobacter* isolation media can be detrimental to some species strains of *Arcobacter* (Houf et al., 2001). Therefore, the true incidence and/or prevalence of *Arcobacter* species may be underestimated (Atabay et al., 2006).

In order to determine the epidemiology of *Arcobacter* spp. in detail, a variety of molecular genotyping techniques, which will help to elucidate epidemiological relationships among the various *Arcobacter* isolates, have previously been applied to arcobacters by a number of researchers (Houf et al., 2003; Morita et al., 2004). These methods include ribotyping (Kiehlbauch et al., 1991), amplification of the repetitive elements or random sequences using PCR primer and PCR conditions

A universal RAPD typing primer (Primer 6: 5'-AA-CAGCActCTGTCAGC-3' (Integrated DNA Tech., Coralville, USA)) was used in the present study as previously described (MacGowan et al., 1993; Unver et al., 2006). The amplification was carried out in a 50 µl reaction mixture including PCR buffer, 1.5 mM MgCl₂, 25 pmol primer 6, 0.2 mM each of the dNTP mixture, 2 U Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania) and 450 ng DNA with 5 min of denaturation at 94 °C followed by 4 cycles each consisting of 1 min denatur-
Results and Discussion

Fifteen Arcobacter isolates from 90 cloacal samples were analysed to determine the RAPD profiles based on the distinct amplified DNA fragment patterns and they produced a total of 11 different RAPD profiles. The RAPD analysis was able to discriminate all the Arcobacter isolates examined. Distinct RAPD patterns obtained are shown in Figure 1. A. cryaerophilus, A. butzleri and A. skirrowii isolates produced 6 (C1-C6), 2 (B1, B2) and 3 (S1-S3) different profiles, respectively. Table 1 summarizes the RAPD patterns of the Arcobacter isolates and the locations where the organisms were isolated. The isolates producing the same patterns originated from the same flock. The numbers of the distinct RAPD patterns were distributed as follows: 3 patterns (S3), 2 patterns (C1), 2 patterns (S2) and the rest of the distinct patterns produced were from a different isolate. No amplification was observed in the “no template control” (data not shown). RAPD results were found to be reproducible when analyzed in independent experiments.

High genetic diversity of Arcobacter strains isolated from numerous sources has been previously shown in independent studies from different regions. Manke et al. (1998) reported 86 different DNA patterns in 121 A. butzleri isolates from 223 turkey carcasses. Atabay et al. (2002b) reported that 11 subtypes were found in 35 A. butzleri isolates recovered from 35 chicken carcasses in Turkey. In another study, 91 A. butzleri and 40 A. cryaerophilus genotypes were detected in 182 A. butzleri and 46 A. cryaerophilus isolates from broiler carcasses in Belgium (Houf et al., 2002). Houf et al. (2003) also reported that 159 A. butzleri and 139 A. cryaerophilus subtypes were determined within 1079 Arcobacter isolates recovered from a poultry abattoir environment. Thirty five A. skirrowii, 121 A. cryaerophilus and 322 A. butzleri isolates recovered from the faeces of healthy pigs in Belgium generated 30, 70 and 123 distinct DNA patterns (Van Driessche et al., 2004). Genetic diversity was also observed in Arcobacter strains isolated from cattle (Van Driessche et al., 2005). The RAPD-PCR genotyping technique, as in the present study, was utilised in all the studies mentioned above in order to subtype Arcobacter isolates from various sources. The current study revealed 10 different RAPD profiles in 15 Arcobacter isolates obtained from three locations in the Northeastern part of Turkey. A high degree of genetic diversity among the Arcobacter isolates detected in the current study is in line with the results of previous studies. These findings may suggest that there exist multiple contamination sources in the environment and/or the determined genotypes may have undergone genetic rearrangements. The mechanism(s) how arcobacters generate this genetic heterogeneity should be further studied.

In the present study, similar genotypes were detected only among Arcobacter isolates recovered from the same flock. This may show that there is a limited genetic diversity, the existence of a relatively small number of parent genotypes and/or shared contamination sources within the flocks or locations examined. However, larger epidemiological studies involving more locations and flocks with more isolates are required in order to confirm this hypothesis.

Since arcobacters show relative inertness to biochemical tests and they require fastidious growth conditions (Atabay et al., 1998b; On, 2001), a variety of molecular genotyping techniques such as ribotyping, ERIC-PCR,
PFGE, AFLP and sequencing of certain genes have been practically used to discriminate the isolates among and, or within species (Atabay et al., 2002b; Houth et al., 2002; Van Driessche et al., 2004, 2005). In the current study, the RAPD-PCR technique utilizing a universal typing primer was successfully used for genotyping the isolates suggesting that the primer used in this study has a considerable discriminatory power to differentiate genotypes of arcobacters. The limited reproducibility of RAPD-PCR is of general concern for using this method. However, this technique utilizing a universal primer was found to be highly repeatable in this study. Therefore, RAPD-PCR can be practically applied in most laboratories since it requires no special and, or complex equipment and takes less time and is less labourous as compared with some other genotyping methods such as PFGE and AFLP.

In conclusion, the detection of genetically diverse Arcobacter species in the faeces of geese in the current study may indicate the presence of multiple sources for contamination in the environment and, or the determined genotypes may have undergone genetic rearrangements. This is the first report of genotyping of various Arcobacter species isolated from the faeces of healthy geese. Detailed molecular epidemiological studies involving high numbers of isolates from different sources are needed in order to understand the epidemiology of arcobacters and to clarify their role(s) as foodborne human pathogens.


References


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