Isolation and Identification of *Arcobacter* Species Recovered from Rabbits in Zagazig, Egypt

Iman I.A. Suelam

Diagnostic Laboratory, Veterinary Hospital, Zagazig University, Egypt

**Abstract:** Although *Arcobacter* species is an emerging foodborne pathogen, the role of rabbit as a source of *Arcobacter* infections is unknown. The present study was designed to study the isolation rate of *Arcobacter* species in rabbit and its drinking water. The molecular fingerprinting of 10 *Arcobacter* species isolates was carried out by ERIC-PCR technique. From 50 samples including 20 rabbit meat, 20 rabbit stool and 10 drinking water samples, 35 (70%) *Arcobacter* species strains were isolated. Recovered isolates from the examined meat, stool and water samples were 15, 20 and 0 with the percentages of 75, 100 and 0, respectively. Concerning, *A. butzleri*, it was isolated from 2(10%) of meat samples and 3(15%) of stool samples. *A. skirrowii* was isolated from meat and stool with the frequency of 6(30%) and 8(40%), respectively. *A. cryaerophilus* was isolated from 7(35%) and 9(45%), respectively. ERIC-PCR grouped the total examined 10 isolates of *Arcobacter* species based on the presence or absence of the major amplified bands (A-M) ranged from >2072 bp to 200 bp. The conservative common bands in all isolates were 300 and 400 bp bands. It was found that there were both intra-species and inter-species molecular diversity among the examined *Arcobacter* species clones. The circulating *Arcobacter* species clones in the tested rabbit farm had multiple genotypes (9/10). This may be attributed to the variant sources of infections.

**Key words:** Arcobacter • Isolation • Rabbit • ERIC-PCR • Water • Aerotolerant.

**INTRODUCTION**

Arcobacters are considered potential emerging food and waterborne pathogens. *Arcobacter* species are members of family Campylobacteriaceae and cause a variety of diseases in human and animal. They have the ability to grow aerobically at 30°C which is a distinctive feature that differentiates *Arcobacter* species from *Campylobacter* species [1]. The genus Arcobacter is relatively new, proposed by Vandamme and De Ley [2] and encompasses a group of organisms known initially as aerotolerant campylobacters [3]. The genus Arcobacter currently contains 10 species, of which seven may be considered emerging human food-borne pathogens. *A. butzleri*, *A. skirrowii*, *A. cryaerophilus*, *A. cibarius*, *A. mytili*, *A. thereius* and *A. trophiarum* have all been isolated from foodstuffs, including meat, shellfish and water, or from the feces of livestock [4]. *A. butzleri*, *A. skirrowii* and *A. cryaerophilus* have been isolated from human [5], poultry products [6] and feces of healthy farm animals [7]. Furthermore, the majority of isolated *Arcobacters* belong to one of the three species *A. butzleri*, *A. cryaerophilus* or *A. skirrowii* [8].

Raw meat is considered as a source of *Arcobacter* infection in human [1]. Rabbit meat production is developing and in Egypt, rabbit meat represents 2.9% of the total meat consumption [9]. *Arcobacter* species was previously studied in different sources other than rabbits in Egypt [10, 11]. *Arcobacter* species were isolated from rabbit meat in a prevalence of 10% in Spain [12]. *Arcobacter* species was recorded in the most fecally contaminated groundwater wells that provide potable water to the public [13].

Different methods have been applied for distinguishing one strain of *Arcobacter* from another, for studying transmission routes or for tracing sources of outbreaks, including several PCR methods, one of them the enterobacterial repetitive intergenic consensus (ERIC-PCR) [14], the most used typing technique which has been successfully applied for investigating outbreaks [15]. The genetic diversity of *Arcobacter* species was previously studied using ERIC-PCR profiling [16].

**Corresponding Author:** Iman I.A. Suelam, Diagnostic Laboratory, Veterinary Hospital, Zagazig University, Egypt.
The objective of this research was to study the prevalence of Arcobacter species in rabbits’ meat, feces and their drinking water. Moreover, genotypic characterization of the most common isolated Arcobacter species was carried out.

**MATERIALS AND METHODS**

**Sample Collection:** A total of 50 different samples was collected from the rabbit farm of Zagazig University, Zagazig in January 2012 including rabbits’ meat (n=20), feces (n=20) and drinking water (n=10). The samples were immediately transported to the laboratory in a cool box and processed within 2-4 h of sampling.

**Isolation Media:** Arcobacter enrichment broth (AEB) was prepared using Arcobacter enrichment basal media (Oxoid, CM965, Hmpshire, UK) with cefoperazone-amphotericin-teicoplanin (CAT) selective supplement (Oxoid, SR174E) as described previously [17]. Blood agar was prepared by adding 5% (v/v) defibrinated sheep blood in blood agar base.

**Samples Preparation and Isolation Procedures**

**Rabbit Carcass Samples:** Each carcass sample was rinsed with sterile distilled water by thorough shaking for approximately 1 min. One ml portion of well mixed samples was inoculated into 9 ml portion of AEB supplemented with CAT.

**Fecal Samples:** Each sample (1 g) was homogenized in a sterile saline. One ml of each suspension was then inoculated into 9 ml of AEB containing CAT supplement.

**Drinking Water Samples:** Tap water samples (1 ml from each sample) were added to 9 ml of AEB containing the CAT supplement.

**Method of Isolation:** Isolation of Arcobacter species by microfilter technique [18] using sterile, individually packaged filter-AC (Sartorius Goettingen, 82122-001-51) by picking up the membrane filter together with the yellow protective disc using sterile forceps, fixed onto the blood agar plates, then the protective disc was removed.

One hundred and twenty µl portions were taken from each previously enriched homogenate, dropped onto this microfilter membrane (pore size 0.45 µ) and incubated aerobically at 30°C for one hour to be allowed for passive filtration. The filters were removed and the plates were incubated aerobically at 30°C until visible colonies were obtained (up to 7 days).

**Identification of Presumptive Arcobacter Isolates:** Suspected colonies were picked, purified by subculture onto blood agar (BA) and identified according to Atabay et al. [18]. Isolates were preserved for molecular characterization.

**Extraction of Genomic DNA:** Arcobacter isolates were grown on BA at 30°C for 48-72 h under microaerophilic conditions. After incubation, one or two colonies of each strain grown on BA plate was suspended in 1 ml of sterile distilled water and centrifuged for 5 min at 13000 RPM and the supernatant was discarded. DNA extract were prepared by re-suspending the cell pellets in 1 ml of sterile distilled water and boiling the suspension for 10 min and centrifugation, the supernatant was used as DNA templates in PCR.

**ERIC PCR Technique:** Ten isolated Arcobacter species were genotyped using enterobacterial repetitive intergenic consensus (ERIC) PCR technique [14]. ERIC-PCR was carried out in Department of genetics, Faculty of Agriculture, Zagazig University. The concentration of each DNA template was determined at A260 and adjusted to 25 ng/µl.

Each 50 µl PCR mixture was composed of 5 µl of 10X PCR buffer (Invitrogen), 5 U of Taq DNA polymerase and a mixture of each dNTP at 0.2 mM. The primers ERIC 1R and ERIC 2 designed by Versalovic et al. [19] (Table 1) were each used at concentrations of 25 pmol. The PCR consisted of 40 cycles of 94°C for 1 min, 45°C for 1 min and 72°C for 2 min, prior to cycling, samples were heated at 94°C for 5 min. The PCR products were size separated by electrophoresis of 8 µl portions of the reaction mixtures in ethidium bromide-stained 2% agarose gels with 1X TBE buffer for 2.5 h at 100 V. The DNA profiles were visualized by UV transilluminator and photographed. Patterns with at least one different band were considered as different genotypes.

**Table 1: Primers used in ERIC-PCR fingerprinting for Arcobacter species strains**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5’ to 3’</th>
<th>Gene</th>
<th>Reference</th>
</tr>
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<td>ERIC 1R</td>
<td>ATGTAAGCTCCTGGGATTAC</td>
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<td>[19]</td>
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<td>ERIC 2</td>
<td>AAGTAAGTGACTGGGTGAGGC</td>
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RESULTS

The occurrence of *Arcobacter* species in the examined rabbit and drinking water samples are shown in Table 2. From 50 samples including 20 rabbit meat, 20 rabbit stool and 10 drinking waters, a total number of 35 (70%) *Arcobacter* species strains were isolated. Recovered *Arcobacter* species isolates from the examined meat, stool and water samples were 15, 20 and 0 light figures/?? with the percentages of 75, 100 and 0, respectively. Concerning, *A. butzleri*, it was isolated from 2(10%) of meat samples and 3(15%) of stool samples. *A. skirrowii* was isolated from meat and stool with the frequency of 6(30%) and 8(40%), respectively. *A. cryaerophilus* was isolated from 7(35%) and 9(45%) of meat and fecal samples, respectively.

The reproducibility of ERIC-PCR for the tested 10 clones of *Arcobacter* species are shown in Table 3 and figure 1. ERIC-PCR discriminated the examined 10 isolates of *Arcobacter* species based on the presence or absence of the major amplified bands (A-M) which ranged from >2072 to 200 bp. The molecular weights of the conservative bands in all isolates were 300 and 400 bp bands. It was found that there are both intra-species and interspecies molecular diversity of examined strains.

Table 2: Occurrence of *Arcobacter* species in the examined rabbit farm samples

<table>
<thead>
<tr>
<th>Source (No)</th>
<th>Meat (20)</th>
<th>Stool (20)</th>
<th>Water (10)</th>
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<tr>
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<td>No</td>
<td>%</td>
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<td><em>A. skirrowii</em></td>
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<td><em>A. cryaerophilus</em></td>
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<td>35</td>
<td>9</td>
<td>45</td>
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<td>Total</td>
<td>15</td>
<td>75</td>
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Table 3: ERIC-PCR product reproducibility of *Arcobacter* species isolated from apparently healthy rabbits

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<th>Amplicon bp</th>
<th>Band code</th>
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<th>5/7</th>
<th>6/8</th>
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Fig. 1: ERIC-PCR fingerprints of the 10 Arcobacter species isolates. The lane numbers correspond to the isolate numbers shown in table 3. Lane 1 contains 100 bp marker. Lane 2 contains master mix without template DNA as a control.

DISCUSSION

Due to the lack of available literature concerning the role of rabbit as a source for arcobacterioses, the present study was carried out to isolate and identify Arcobacter species from rabbit meat, stool and their drinking water. The isolation rate (75%) of Arcobacter species in rabbit meat in the present study is higher than that recorded in Spain [12] which was (1/10)10%. Difference in isolation rate of Arcobacter from examined rabbit samples may be attributed to several factors such as hygienic conditions during the processing and sensitivity of the isolation method used [20].

The genus Arcobacter has gained increased attention as an emergent waterborne and foodborne enteropathogens. A. butzleri, A. cryaerophilus and A. skirrowii have been associated with gastrointestinal disease and bacteremia in humans and with abortion and diarrhea in animals [21]. A. butzleri is the most commonly isolated species and has been classified as a serious hazard to human health by ICMSF [22]. Contamination of rabbit carcasses with Arcobacters poses a risk for both human and animal's infection. The presence of Arcobacter in the feces of healthy livestock at slaughter constitutes a significant risk of carcass and meat contamination [23].

Detection of several different Arcobacter strains may suggest multiple source of infection. In this study no Arcobacter was detected in drinking water. The obtained result agrees with previous studies [16, 24, 25] which could not find any Arcobacter in drinking water samples.

The absence of Arcobacter in the examined drinking water samples may be due to proper disinfection practices as Arcobacter are sensitive to chlorine [26]. Nevertheless, water has a significant role in the transmission of Arcobacter species both to human and animals and it has been estimated that 63% of A. butzleri infection in humans is from the consumption of or contact with contaminated water [27].

Because of the biochemical inertness of Arcobacters, the applications of these tests are often not adequate to differentiate Arcobacter spp. properly at the species level [28]. Therefore, DNA-based methods have been established for rapid and correct identification and/or differentiation of Arcobacter spp. at the species level [29].

The variant genotypes of the examined 10 clones of Arcobacter species were shown in table 3 and figure 1. Van Driessche et al. [30] found that individual pig could excrete up to 7 A. butzleri, 10 A. cryaerophilus and 6 A. skirrowii genotypes. Similar results were recorded by Van Driessche et al. [31] who recorded shedding of 6 A. cryaerophilus and 2 A. skirrowii genotypes in cows by using ERIC-PCR. Moreover, Houf et al. [32] characterized A. cryaerophilus and A. butzleri clones by ERIC-PCR and reproduced banding patterns genotypes ranged from 100-2072 bp. The extreme genetic diversity of
Arcobacter species on the carcasses of the same flock can be explained by cross-contamination within one flock and from flocks of different farms [33]. The genetic diversity 90% in the present results is near to that found by Collado et al. [16] who recorded genetic diversity of Arcobacter species range from 11 to 58.6% for isolates of A. butzleri and from 43.2 to 100% for the isolates of A. cryaerophilus. It was found that ERIC-PCR fingerprinting profiling of Arcobacter species is reproducible and discriminative. It could help in tracing the sources of infections. The circulating Arcobacter strains are diverse as the detected genotypes were 9 clones per 10 strains.

In conclusion, rabbit meat and stool may be a potential source of arcobacterioses in both human and animal niches. Further molecular epidemiological studies are needed to trace the different sources of Arcobacter infection at national level. ERIC-PCR is an efficient method to detect the molecular diversity of Arcobacter species.

ACKNOWLEDGEMENT

The author is indebted to Dr. Ahmed Mansour, Department of Genetics, Faculty of Agriculture, for his kind help in PCR.

REFERENCES


