

Isolation, Identification and Determination of Antimicrobial Susceptibility of *Arcobacter Butzleri* Isolated from Chicken Carcass in Tonekabon

Zahra Pourabbasgholi¹, Hami Kaboosi^{1*}, Masood Ghane², Rahem Khoshbakht³, Mehdi Ghiyamirad⁴

1- Department of Microbiology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran

* Corresponding author (h.kaboosi@iauamol.ac.ir)

2- Department of Microbiology, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran

3- Department of Bacteriology, Faculty of Veterinary Medicine, University of New Technologies, Amol, Iran

4- Department of Microbiology, Faculty of Basic Sciences, Ahar Branch, Islamic Azad University, Ahar, Iran

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Abstract

Arcobacter butzleri, is the most common genus of the *Campylobacteriaceae* family, known as an emerging zoonotic pathogen. The aim of this study was to isolate, identify and determine the antimicrobial susceptibility of *Arcobacter butzleri* strains to antibiotics used in the treatment of infectious diseases in humans and animals. Therefore, 297 samples of chicken carcasses were collected in slaughterhouses of Tonekabon city. Suspected colonies were isolated and identified using biochemical test and polymerase chain reaction (PCR) technique was used to confirm the isolates. The pattern of antibiotic resistance of *Arcobacter Butzleri* to 16 antibiotics was determined by disk diffusion method and the minimum inhibitory concentration of the strains to tetracycline, erythromycin and gentamicin was determined by Broth Macrodilution (Tube) method. All of the 36 strains which were isolated and identified were resistant to penicillin 100%, ampicillin 100%, oxacillin 100% and also to resistance to trimethoprim/sulfamethoxazole 94.4%, ciprofloxacin 94.4%, nalidixic acid 91.7%, azithromycin 91.7% and amoxicillin 80.6% were evaluated. Of the 36 isolates tested, all isolates were sensitive to gentamicin 100%. 72% of strains had MIC \geq 128 (g/mL) and MBC \geq 256 (μ g/mL) for tetracycline antibiotics. There were also 10 MDR strains (27.77%) and 24 XDR strains (66.66%). The findings indicate the presence of *Arcobacter butzleri* in chicken carcasses and the high prevalence of antimicrobial resistance to various antibiotics in this area.

Keywords: *Arcobacter butzleri*, Antimicrobial resistance, Minimum concentration of growth inhibitor

Introduction

Arcobacter spp. are S-shaped, gram-negative, spiral, motile, non-spore forming and fastidious microorganisms that belong to the *Campylobacteraceae* family. Their germination ability at 15 ° C is the most significant distinctive feature of *Arcobacter* spp. that distinguishes them from the *Campylobacter* spp. (Kabeya *et al.*, 2003). Recently, the *Arcobacter* genus has been gaining increasing importance in community health due to its zoonotic potential, the appearance of new species and the fact that several species are emerging enteropathogens (Collado & Figueras, 2011). Further, the genus has been classified as a serious hazard to human health by the International Commission on Microbiological Specifications. The

presence of *Arcobacter* spp. has been demonstrated in foods of animal origin, various animal faeces (such as cattle, sheep, dog, rabbit, and chicken), the environment and water in many studies (Kabeya *et al.*, 2003; Van Driessche *et al.*, 2003; Vandenberg *et al.*, 2006). The prevalence of antibiotic resistance among environmental pathogens has increased in recent decades (Noh *et al.*, 2012; Shah *et al.*, 2013; Suelam, 2013). The aim of this study was to isolate, identify and determine the antimicrobial susceptibility of *Arcobacter Butzelri* strains to antibiotics used in the treatment of infectious diseases in humans and animals.

Materials and methods

In order to isolate *Arcobacter butlerzi* from carcasses of slaughterhouse in Tonekabon county, 297 samples were collected in different seasons of 1399. Samples collected in sterile containers next to ice were transferred to the laboratory and examined for the detection of *Arcobacter Butzelri*. Method of isolation and identification of bacteria from the collected samples after transferring the samples to tubes containing Preston culture medium, they were incubated at 25 °C for 24-48 h. After the desired time, the bacteria grown with the help of sterile loop on CAMP medium (Merck-Germany), enriched with defibrillated sheep blood containing antibiotics such as vancomycin 2 mg/mL, Polymyxin was 0.05 mg/mL, trimethoprim was 1 mg/mL, linear culture was performed. The culture media were then placed in a 25 ° incubator for 24-72 h. After the warm-up period, the plates were evaluated to identify *Arcobacters*. On the basis culture medium, the bacterial colony was convex, smooth, transparent, and colorless to worm size of 2-4 mm as a suspected colony of *Arcobacter*. These colonies were subjected to microbial tests such as gram staining, catalase, oxidase and glucose fermentation and motility tests for the initial identification of *Arcobacters*. By observing gram-negative, motile, oxidase-positive curved bacilli and negative glucose fermentation test, it is possible to be very sure of the isolation and identification of the genus *Arcobacter*. In the next step, phenotypic tests introduced by Atabay *et al.* (1998), which included tests for urease production, growth at 37 °C and microaerophilic conditions, and growth on MacConkey agar, were used. Confirmation of culture results by PCR technique In order to perform PCR, DNA extraction from the target colonies was performed using a kit (Kiagen Teb Sadra). To confirm the DNA extraction, light absorption in the range of 260 and 280 nm was examined by a biophotometer and the rest of the samples were placed at -20 °C. In order to perform PCR, forward and reverse primers were prepared for replication of 16SrRNA specific for *Arcobacter Butzelri* by Tag Copenhagen (Denmark), (Table 1). The reaction mixture consisted of: 5 µL of bacterium's extracted DNA, 10 µL of Mastermix (Takara-Japan), 1 µL of forward primer, 1 µL of reverse primer, 3 µL of sterile distilled water was poured into a microtube, spun and placed in a thermocycler (Eppendorf, Germany) to perform the PCR process. To perform the polymerization process, the thermocycler was placed at 94 °C for 4 min for initial denaturation. Then 35 PCR cycles including 54 and 94 °C for 45 s, 72 °C for 90 s were performed. Finally, the final elongation operation was performed for 10 min at 72 °C (Table 2).

Table 1. Sequence of forward and reverse primers for *Arcobacter* gene

Primer name	primer	5' → 3'	Product size
<i>Arco 1</i>	Forward	AGAGATTAGCCTGTATTGTATC	1200
<i>Arco 2</i>	Reverse	TAGCATCCCCGCTTCGAATGA	

Table 2. Thermal and timing plan of PCR in order to reproduce 16SrRNA gene of the *Arcobacter*

94°C- 4'	Pre denaturation	35 cycle
94°C- 45"	Denaturation	
54°C- 45"	Annealing	
72°C- 90"	Extention	
72 °C – 10'	Final extention	

Results and discussion

From 297 samples collected during different seasons, based on culture tests, 36 isolates of 12.12% *Arcobacters* were isolated and identified. It is noteworthy that the highest separation rate was 50% in spring and the lowest separation rate was 5.5% in winter. In spring 18 strains 50%, in summer 3 strains 8.33%, in autumn 13 strains 36.11% and in winter 2 strains 55.5% *Arcobacter butzelri* were isolated from chicken carcasses. Using PCR technique, phenotyping results were confirmed and with the help of Arco1 and Arco2 specific primers, the band was observed in the 1200 region (Fig. 1).

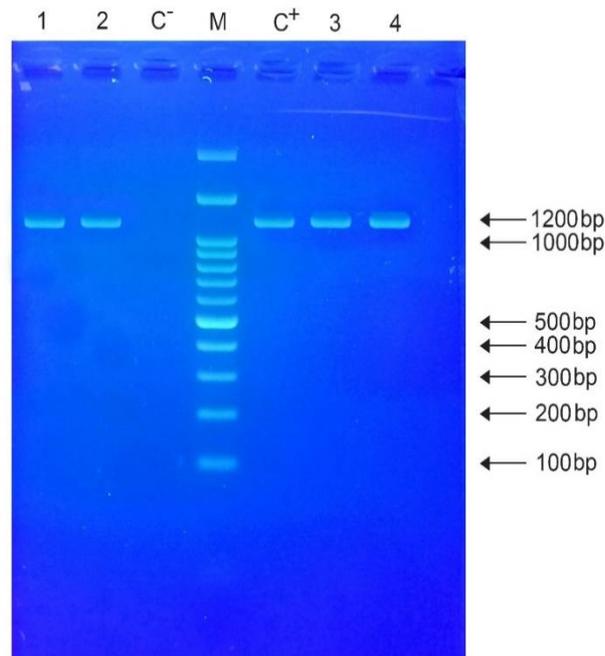


Fig. 1. The result of polymerase chain reaction on 1.5% agarose gel (M molecular ruler 1 kilobase, C+ (positive control) with DNA obtained from the pure culture of *Arcobacter butzelri*, 1, 2, 3 and 4 isolated *Arcobacter* samples and C- (control) negative (with no DNA).

All isolates were resistant to 100% penicillin, 100% ampicillin and 100% oxacillin. Also, the resistance to trimethoprim/sulfamethoxazole was 94.4%, ciprofloxacin 94.4%, nalidixic acid 91.7%, azithromycin 91.7% and amoxicillin 80.6%. Of the 36 isolates tested, all isolates were 100% sensitive to gentamicin and 97.2, 75 and 86.1% sensitive to amikacin, erythromycin and nitrofrontoin, respectively. They also showed 94.4% sensitivity to amoxicillin/clavunic acid, 88.9% to chloramphenicol, 72.2% to tetracycline and 71.4% to cephalothin. The results of the test for determining the minimum concentration of tetracycline growth inhibitor in this study showed that 26 strains of 72% *Arcobacter* butler were highly resistant ($MIC \geq 128 \mu\text{g/mL}$) and 27 strains were 75% resistant to erythromycin.

Over the past few decades, there has been growing concern about the increasing prevalence of food-borne pathogens and their associated antimicrobial resistance (Sousa, 2017). Food-borne pathogens can enter the food chain at any time. Millions of people around the world suffer from foodborne illness every year, and this has created social and economic pressures in developing countries (Okeke *et al.*, 2005). The results of our study showed that the antibiotics nalidixic acid, ciprofloxacin, and azithromycin could be used as the first line of antibiotic therapy to treat infections caused by gram-negative bacteria, including *Arcobacter butzelri*, but increased resistance to penicillin. Ampicillin, oxacillin, and trimethoprim/sulfomethoxazole, nalidixic acid, ciprofloxacin, and azithromycin are among the most common isolates of *Arcobacter butzelri*. In our study, resistance to ciprofloxacin was

91.7%, nalidixic acid was 91.7%, azithromycin was 91.7%, and these drugs should not be used alone as first-line drug therapy. The increase in resistance in our country is due to the overuse of these antibiotics. Widespread spread of antibiotic resistance in *Arcobacter* strains through different media may play a role in the spread of resistance. Data on antimicrobial resistance are important because these antimicrobial agents can be used as the first line in the treatment of disease (Ferreira *et al.*, 2019). The antibiotic susceptibility data obtained in this study can also be used when designing the environment to isolate these bacteria. This study shows that strains of *Arcobacter butzelri* differ in susceptibility to different types of antibiotics.

Conclusions

The study findings indicate the critical role of environmental resources as reservoirs of *Arcobacter butzelri* contamination. The study showed that 12.12% of *Arcobacter butzelri* isolates were isolated and identified from 297 samples collected during various seasons according to culture tests. It must be noted that the highest rate of isolation was 50% in spring and the lowest rate 5.5% in winter. Additionally, the study findings have reported the highest rate of antibiotic resistance in the case of penicillin, ampicillin and oxacillin antibiotics with a frequency of 100%. The high level of prevalence of antibiotic resistance among *Arcobacter butzelri* strains from environmental samples shows the excessive use of antibiotics and heavy pollution in the area under discussion. Hence, careful attention needs to be paid to the strict monitoring of the health and food system. It is obvious that the indiscriminate and unsupervised use of antibiotics to treat or control infection in humans or as growth factors in animal feed is one of the reasons for the spread of antibiotic-resistant bacteria. The antibiogram technique and the tube dilution method were used in this study given the status quo and the limitation of time and facilities in the study. Hence, in spite of newer and more accurate techniques, it is suggested use other techniques available ETEST technique.

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