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Prevalence and Distribution of *Arcobacter Butzelri* Virulence Genes in Poultry Slaughterhouse Effluent Samples in Tonekabon

Sara Fallahchay[1], Nima Bahador[2]*, Masood Ghane[3]

1- PhD. Student, Department of Microbiology, College of Sciences, Shiraz Branch, Islamic Azad University, Shiraz, Iran
2- Associate Professor, Department of Microbiology, College of Sciences, Shiraz Branch, Islamic Azad University, Shiraz, Iran
* Corresponding author (bahador@iaushiraz.ac.ir)
3- Associate Professor, Department of Microbiology, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran

Abstract

Arcobacter butzelri is known as the cause of enteritis, abdominal cramps, bacteremia, and appendicitis in humans, it is also the cause of enteritis and abdominal pain in animals. It has been introduced as the most dangerous species for human health by the International Committee on Food Microbiology and recently as an important zoonotic pathogen. The aim of this study was isolatation and characterization of *Arcobacter butzelri* based on pathogenic genes (*tlyA*, *ciaB*, *mviN*) in different seasons. Therefore, 238 samples were collected from the effluent of poultry slaughterhouse in Tonekabon, Iran. The suspected colonies were identified using biochemical test and then confirmed using the Polymerase chain reaction (PCR) technique. Out of 42 isolated of *A. butzleri*, the virulence genes were detected in 15 isolates. The results showed that *ciaB* gene was present in 13 strains with a frequency of 30.9%, *mviN* in 11 samples with a frequency of 26.2%, and *tlyA* gene in 9 samples with a frequency of 21.4%. The results also revealed that the highest frequency of *A. butzelri* was 8.4% in spring and the lowest rate was 1.2% in winter.

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Keywords
Arcobacter butzleri
Virulence gene
Polymerase chain reaction

Introduction

Arcobacter genus is a member of *Campylobacter* family and belongs to the class of *Epsilon proteobacteria* (Ellis, Neill, O'brien, Ferguson, & Hanna, 1977). *Arcobacteria* can cause infertility, miscarriage, and digestive disorders in animals, and bacterial gastroenteritis, endocarditis, and peritonitis in humans (Badilla-Ramirez, Fallas-Padilla, Fernandez-Jaramillo, & Arias-Echandi, 2016). They are widely found in water and the sources of animal foods. *Arcobacter butzelri* is the most common species which is usually isolated from a variety of environments (Figueras *et al.*, 2014; Jyothsna, Rahul, Ramaprasad, Sasikala, & Ramana, 2013; Levican, Collado, Yustes, Aguilar, & Figueras, 2014) Most studies on the prevalence of *Arcobacter* in foods are related to the poultry that have the highest prevalence, followed by pork and raw milk products (Collado, Guarro, & Figueras, 2009; Figueras *et al.*, 2014) The genus *Arcobacter* has become very important in recent years because its members are

considered as a potential zoonotic agent.

The global prevalence of *Arcobacter* infection is unclear since the usual diagnostic method is not applicable to this bacterium. There is no doubt that the *Arcobacter* species can cause disease in humans but more clinical observations are essential to determine their pathogenicity in human (Levican, Alkeskas, Günter, Forsythe, & Figueras, 2013). Preliminary studies on *A. butzelri* have shown that these bacteria have clinical and microbial characteristics similar to *Campylobacter jejuni* strain but they are more associated with severe and persistent diarrhea and less associated with *C. jejuni* (Vandenberg *et al.*, 2004; Wesley & Miller, 2010). In the recent decade, sequencing of the RM4018 genome of *A. butzelri* was an important step in the development of research on *Arcobacter*, in which 10 pathogenic genes were identified (Douidah *et al.*, 2012; Ferreira, Queiroz, Oleastro, & Domingues, 2016; Miller *et al.*, 2007). The pathogenicity of an organism is characterized according to the presence of virulence genes (Ghaju Shrestha, Tanaka, Sherchand, & Haramoto, 2019). The *cadF* and *cj1349* genes encode outer membrane proteins, which promote bacteria to cell contact by adherence to fibronectin (Dasti, Tareen, Lugert, Zautner, & Groß, 2010). *CiaB* gene promotes host cell invasion through the secretion system (Tabatabaei, Aski, Shayegh, & Khoshbakht, 2014). *MviN* gene is a vital protein required for peptidoglycan biosynthesis (Inoue *et al.*, 2008). *PldA* encodes the outer membrane phospholipase A (Grant, Belandia, Dekker, Richardson, & Park, 1997). *TlyA* gene encodes hemolysin which plays an important role of *C. jejuni* in adhesion to Caco-2 cells (Slater, Bailey, Tett, & Turner, 2008). The *hecA* gene encodes a protein belonging to a group of adhesives (Filamentous haemagglutinin family). The *hecB* encodes a related haemolysin activation protein (Miller *et al.*, 2007; Rojas, Ham, Deng, Doyle, & Collmer, 2002). *IrgA* gene encodes an outer membrane protein and the periplasmic enzyme of the *iroE* gene, both of which regulate iron (Mey *et al.*, 2002; Rashid, Tarr, & Moseley, 2006; Zhu, Valdebenito, Winkelmann, & Hantke, 2005). Although it remains unclear whether these virulence genes have similar functions to their homologs in different pathogenic bacteria, showed little or no invasion of cells by *Arcobacter* strains that lack virulence genes. Therefore, the detection and quantification of virulence genes of *Arcobacter* may elucidate its role in waterborne infections (Ghaju Shrestha *et al.*, 2019). *Arcobacter* can be considered as a relatively resistant bacteria which can survive in adverse conditions and affect the pathogenicity process in human (Ferreira *et al.*, 2016). Thus, the aim of this work was to isolate and identify this bacteria from wastewater of poultry slaughterhouse in Tonekabon to examine the presence of genes.

Materials and methods

Sample Collection and screening of the isolates

238 samples were collected from wastewater of poultry slaughterhouse in Tonekabon located in the geographical position of x=0494258 and y=4069580 (N=36.752036, E=50.929654) in different seasons. To isolate *A. butzelri*, 50 mL of the samples were transferred to sterile screw cap tubes and transferred to the microbiology laboratory. Then they were cultivated onto the Preston medium Broth enriched with supplements (Preston supplement, biomark) and incubated at 25 °C for 24 h. After enrichment, the membrane filtration technique was applied using 0.45 μm pore size nitrocellulose membrane filters. Then the samples transferred to Campylobacter Selective Agar enriched with defibrillated sheep blood and Campylobacter supplement, and

the plates were incubated at 25 °C for 48 to 72 h. Finally, the suspected colonies were identified using biochemical tests (Atabay & Corry, 1997).

Identification of *Arcobacter butzelri* and its pathogenic genes

All isolates were identified using polymerase chain reaction (PCR) techniques. For this purpose, DNA was extracted using Gene all kit (GeneAll Biotechnology Co., LTD.Korea, Cat.No. 106-152). The 16S r-RNA gene and virulence genes of *mvi*N, *cia*B, and *tly*A of *A. butzelri* were amplified using their special primers. Moreover, all PCRs were prepared with a final volume of 20 µL containing 10 µl of Mastermix (Hot Start *Taq* Polymerase, dNTP, buffer, Mgcl), 1 µL of forward primer, 1 µl of reverse primer, 3 µl of distilled water, and 5 µl of DNA To begin the process of polymerization of the 16S r-RNA gene, the thermocycler was set at 94 °C for 4 min for initial denaturation. Afterward, 25 cycles of PCR were performed including 94, 56, and 72 °C for 60s. The final extension was carried out at 72 °C for 7 min. In addition, the cycling condition for *mvi*N and *cia*B genes were placed in a thermocycler device at 94 °C for 3 min for initial denaturation. In the next stage, 35 PCR cycles were performed including 94, 56, and 72 °C for 45 s. The final extension was carried out at 72 °C for 10 min. To carry out the polymerization process of the *tly*A gene, the thermocycler device was set on 94 °C for 3 min for initial denaturation. Then, 35 PCR cycles were performed including 94, 55, and 72 °C for 45 s. Finally, the final extension was carried out at 72 °C for 10 min (Douidah *et al.*, 2012; Tabatabaei *et al.*, 2014).

Results and discussion

Isolation of *A. butzelri* from wastewater of a poultry slaughterhouse in Tonekabon

Out of 238 collected samples in 4 different seasons, 42 samples were positive for *A. butzelri* with a frequency of 17.6%. In this regard, the highest rate of isolation of *A. butzelri* was 8.4% in spring and the lowest rate was 1.2% in winter. In spring, 20 strains with a frequency of 8.4% in summer 8 strains with a frequency of 3.3% in fall, 11 strains with a frequency of 4.6%, and in winter, 3 strains of *Arcobacter* with a frequency of 1.2% were isolated from the slaughterhouse wastewater.

Identification of *A. butzelri*

The results of PCR and sequencing of 16S r-RNA specific genes confirmed the phenotypic results obtained in 42 isolated strains (Fig. 1).

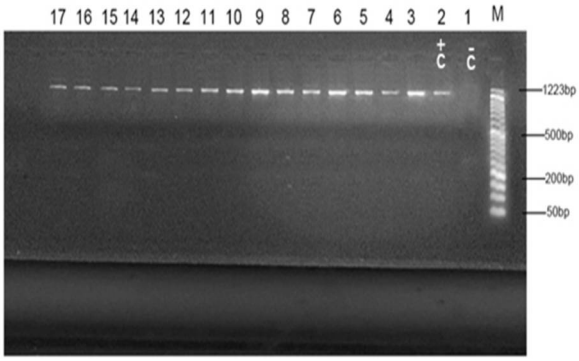

Fig. 1. PCR products on agarose gel (1.5%), Lane 1: negative control, Lane 2: positive control, Lanes 3-17: samples of isolated *A. butzleri*. M: Marker (1.5-k bp)

Evaluation of the presence of *A. butzelri* pathogenic genes

The presence of pathogenic genes of *tlyA*, *ciaB*, *mviN*, in *A. butzelri* was evaluated using polymerase chain reaction. According to the results obtained from this study indicated that 13 strains showed the presence of *ciaB* gene (frequency of 30.9%) (Fig. 2), 11 strains, *mviN* gene (frequency of 26.2%) (Fig. 3), and 9 strains showed *tlyA* gene (frequency of 21.4%) (Fig. 4). The results also revealed that out of 15 strains of *A. butzelri*, 9 strains with 60% frequency had all 3 pathogenic genes (Table 1).

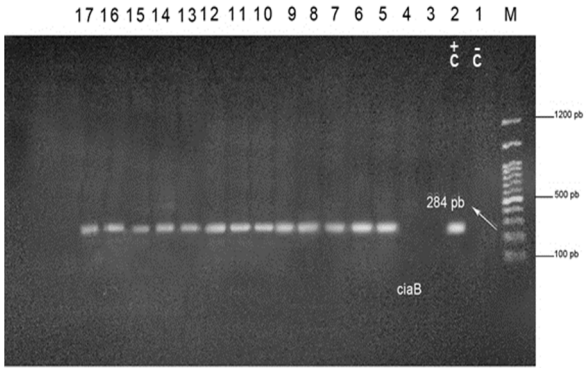

Fig. 2. PCR products on agarose gel (1.5%), Lane 1: negative control, Lane 2: positive control, Lane 3-17: samples of *A. butzleri* that contained the *cia*B gene. M: Marker (1-k bp)

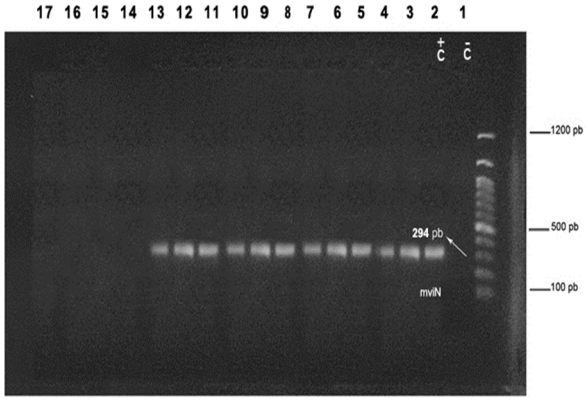

Fig. 3. PCR products on agarose gel (1.5%), Lane 1: negative control, Lane 2: positive control, Lane 3-17: samples of *A. butzleri* that contained the *mviN* gene. M: Marker (1-k bp)

Table 1. Selected isolates with the presence of the virulence genes

Number of strain	Selected genes		
	tlyA	*mviN*	*ciaB*
1	-	+	-
2	-	+	-
3	+	+	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
8	+	+	+
9	+	+	+
10	+	+	+
11	+	+	+
12	-	-	+
13	-	-	+
14	-	-	+
15	-	-	+
Sum of pathogens in each strain	9	11	13

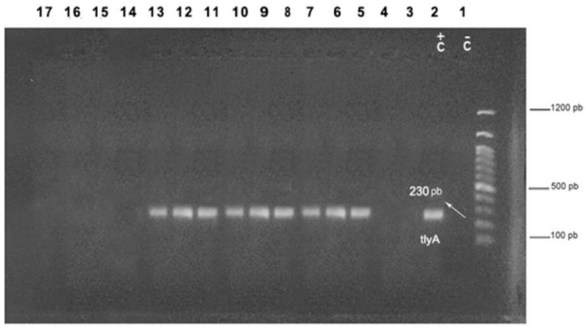

Fig. 4. PCR products on agarose gel (1.5%), Lane 1: negative control, Lane 2: positive control, Lane 3-17: samples of *A. butzleri* that contained the *tlyA* gene. M: Marker (1-k bp)

***A. butzelri* is listed as one of the most** Dangerous bacteria that threatens human health (Collado & Figueras, 2011). There are many reports on the presence of *Arcobacter* and its pathogenicity in animals as well as in human (Tabatabaei *et al.*, 2014). In 2002, the International Commission on Microbial Specification for Foods included *A. butzleri* in the list of microorganisms posing a serious risk to human health (Tompkin, 2002). However, many other *Arcobacter* species have been described in recent years. Although the number of diseases caused by *Arcobacter* spp. has increased in recent years, very little is known about their pathogenesis (Waite *et al.*, 2017). Moreover, the mechanisms of pathogenicity of the *A. butzleri* are still unknown (Ferreira *et al.*, 2016). Adhesion, invasion, and cytotoxicity have been previously examined *in vitro* using various cell lines_ (e.g., Caco-2, HeLa, HT-29, Hep-2, and IPEC-J2) (Ho *et al.*, 2007; Karadas *et al.*, 2013).

Arcobacter can be considered as an organism with a high ability to survive in adverse conditions, and it has shown to be highly antibiotic-resistant in multidrug therapy (Chieffi, Fanelli, & Fusco, 2020; Ferreira *et al.*, 2016).

Most studies on the prevalence of *Arcobacter* in foods are related to the poultry that has the highest prevalence (Collado *et al.*, 2009; Jribi *et al.*, 2020). There is still debate about the source of the contamination because some researchers believe that the slaughterhouse

environment was not the main source of excrement as *Arcobacter* could not be isolated from the feces. However, some researchers have shown that the bacterium can reside in chicken's intestines, suggesting that the age of the animals, the specimen, and the method used for its collection and identification affects the prevalence of the bacterium (Collado & Figueras, 2011). Consumption of contaminated food or water with *Arcobacter* is a route of transmission to humans and animals (Gobbi *et al.*, 2018). The association between *Arcobacter* species and their pathogenicity in human, in addition to their detection and prevalence in food and water, have shown that further studies should be performed on the survival of this microorganism. Thus, new therapies need to be explored to control and eliminate this bacterium.

In the present study, 42 samples of *A. butzelri* were isolated and identified by culture standard and based on 16S rRNA gene analyses. Eifert, Castle, Pierson, Larsen, & Hackney (2003) and Ghane, Moein, & Massoudian (2012) isolated *Arcobacter* species using culture and PCR techniques (Houf, De Zutter, Van Hoof, & Vandamme, 2002).

Snelling, Matsuda, Moore, & Dooley (2006) presented a technique enrichment step with filtration for isolation of *Arcobacter*, this method was used in this study and showed to be useful in the isolation of *Arcobacteria*. In our study, using the mentioned method was proved to be the most appropriate technique for isolation.

Although there are different types of specimen for isolation of *Arcobacter*, in this study, we attempted to isolate the organism from wastewater treatment of poultry slaughterhouse in Tonekabon, through which spreading bacteria into the environment and transmission to human can be prevented.

For this purpose, we considered seasonal changes for the prevalence of this bacterium and showed that the highest rate of isolation of *A. butzelri* was in spring with a frequency of 8.4%. Due to the high existing amount of organic matter in the effluent, the rate of isolation of *A.butzelri* was higher in slaughterhouse effluent. Rasmussen, Kjeldgaard, Christensen, & Ingmer (2013) suggested that the high prevalence of *Arcobacter* in slaughterhouses can be due to inadequate detoxification processes. The organism can potentially survive in 5% ethanol on culture and steel surfaces (Rasmussen *et al.*, 2013).

Furthermore, the results presented that the highest and the lowest prevalence of virulence genes belonged to *ciaB* (30.9%) and *tlyA* genes (21.4%), respectively. The invasive *ciaB* gene attacks the host cell through the secretion system (Tabatabaei *et al.*, 2014). It is an infection caused by *Arcobacteria* as a result of adhesion of this bacterium to the mucosal layers that protect epithelial cells (Wilson, Abner, Newman, Mansfield, & Linz, 2000). This phenomenon is often observed in infections caused by *Arcobacter* and the presence of *ciaB* gene. Hence, the invasive *ciaB* gene is one of the important pathogenic factors of this bacterium, which can increase its ability to survive in adverse environmental conditions, such as in poultry slaughter effluents and human transmission.

Collado, Jara, Vásquez, & Telsaint (2014), examined the presence of 9 pathogenic genes of *Arcobacter* using ERIC-PCR, PCR-RFLP, and PCR techniques in 106 strains of isolated *Arcobacter* from oysters. The results suggested that 62% of the isolated strains were *A. butzleri.* They identified *mviN* gene with the frequency of 83.8% *ciaB* gene with 82.8% and *tlyA* gene with 72.7% frequency in different strains of *Arcobacter*. They also found that the frequency of these 3 genes was higher than *cadF*, *pldA*, *hecB*, *hecA*, *irgA*, *cj1349* genes (Collado *et al.*, 2014), which is similar to our study. However, Girbau, Guerra, Martínez-Malaxetxebarria, Alonso, & Fernández-Astorga (2015) reported the presence of *ciaB* and *mviN* genes with

100% frequency, *tlyA* gene with 89.7%. Šilha, Vacková, & Šilhová (2018) showed the presence of *ciaB* gene with 98.8%, *mviN* gene with 97.5% and the *tlyA* gene with 95.0% frequency in 80 strains of *A. butzleri* isolated from water and clinical specimens using PCR technique, which is more than our results.

As virulence genes are associated with the pathogenic characteristics of *Arcobacter*, such as adhesion, invasion, and cytotoxicity in different cell lines. The detection of these genes in surface water and groundwater needs to be taken seriously (Ferreira *et al.*, 2016). Thus, the detection of virulence-associated genes along with *Arcobacter* in water samples may represent a high risk of waterborne disease transmission (Ghaju Shrestha *et al.*, 2019). More comprehensive studies on the survival of this microorganism are recommended considering the relationship between *Arcobacter* species and their pathogenicity in humans. Their detection and occurrence in food and water are also very important. Novel therapies should be developed for controlling and eliminating this bacterium (Cervenka *et al.*, 2008)

Conclusions

The results displayed that poultry slaughterhouse effluents are the source of *A. butzleri* isolates. According to these findings, pathogenic genes of *tlyA, mviN, ciaB* are present in all isolates *of A. butzleri* and the highest prevalence belonged to *ciaB* gene. The presence of pathogenic genes in the genome of *A. butzleri* can cause disease in humans. They also increase the environmental survival and the growth potential of this bacterium, increasing the likelihood of its transmission to humans and animals. Furthermore, blood infection caused by *A. butzleri* can be a serious threat to individuals with the impaired immune system and they could be life-threatening. Therefore, it is necessary to develop diverse methods to control or prevent the transmission of this bacterium.

Since most of the bacteria spend a big part of their whole life struggling with environmental tensions, more information about these tensions and responses to them are necessary to perceive the physiology of bacteria. Exploring the effects of environmental stress on the expression of *A. butzleri* virulence genes can be a new topic for biological and applied research. Also, with the introduction of new antimicrobial agents, appropriate strategies can be found to control and transmit this bacterium

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References

Atabay, H., & Corry, J. (1997). The prevalence of campylobacters and arcobacters in broiler chickens. *Journal of Applied Microbiology, 83*(5), 619-626.

Badilla-Ramirez, Y., Fallas-Padilla, K. L., Fernandez-Jaramillo, H., & Arias-Echandi, M. L. (2016). Survival capacity of Arcobacter butzleri inoculated in poultry meat at two different refrigeration temperatures. *Revista do Instituto de Medicina Tropical de São Paulo, 58*. doi:https://doi.org/10.1590/S1678-9946201658022

Cervenka, L., Kristlova, J., Peskova, I., Vytrasova, J., Pejchalova, M., & Brozkova, I. (2008). Persistence of Arcobacter butzleri CCUG 30484 on plastic, stainless steel and glass surfaces. *Brazilian Journal of Microbiology, 39*(3), 517-520. doi:https://doi.org/10.1590/S1517-83822008000300021

Chieffi, D., Fanelli, F., & Fusco, V. (2020). Arcobacter butzleri: Up-to-date taxonomy, ecology, and pathogenicity of an emerging pathogen. *Comprehensive Reviews in Food Science and Food Safety, 19*(4), 2071-2109. doi:https://doi.org/10.1111/1541-4337.12577

Collado, L., & Figueras, M. J. (2011). Taxonomy, epidemiology, and clinical relevance of the genus Arcobacter. *Clinical microbiology reviews, 24*(1), 174-192. doi:https://doi.org/10.1128/CMR.00034-10

Collado, L., Guarro, J., & Figueras, M. J. (2009). Prevalence of Arcobacter in meat and shellfish. *Journal of food protection, 72*(5), 1102-1106. doi:https://doi.org/10.4315/0362-028X-72.5.1102

Collado, L., Jara, R., Vásquez, N., & Telsaint, C. (2014). Antimicrobial resistance and virulence genes of Arcobacter isolates recovered from edible bivalve molluscs. *Food Control, 46*, 508-512. doi:https://doi.org/10.1016/j.foodcont.2014.06.013

Dasti, J. I., Tareen, A. M., Lugert, R., Zautner, A. E., & Groß, U. (2010). Campylobacter jejuni: a brief overview on pathogenicity-associated factors and disease-mediating mechanisms. *International Journal of Medical Microbiology, 300*(4), 205-211. doi:https://doi.org/10.1016/j.ijmm.2009.07.002

Douidah, L., De Zutter, L., Baré, J., De Vos, P., Vandamme, P., Vandenberg, O., . . . Houf, K. (2012). Occurrence of putative virulence genes in Arcobacter species isolated from humans and animals. *Journal of clinical microbiology, 50*(3), 735-741. doi:https://doi.org/10.1128/JCM.05872-11

Eifert, J., Castle, R., Pierson, F., Larsen, C., & Hackney, C. (2003). Comparison of sampling techniques for detection of Arcobacter butzleri from chickens. *Poultry science, 82*(12), 1898-1902. doi:https://doi.org/10.1093/ps/82.12.1898

Ellis, W., Neill, S., O'brien, J., Ferguson, H., & Hanna, J. (1977). Isolation of Spirillum/Vibrio-like organisms from bovine fetuses. *Veterinary Record, 100*(21), 451-452. doi:https://doi.org/10.1136/vr.100.21.451

Ferreira, S., Queiroz, J. A., Oleastro, M., & Domingues, F. C. (2016). Insights in the pathogenesis and resistance of Arcobacter: a review. *Critical reviews in microbiology, 42*(3), 364-383. doi:https://doi.org/10.3109/1040841X.2014.954523

Figueras, M., Levican, A., Pujol, I., Ballester, F., Quilez, M. R., & Gomez-Bertomeu, F. (2014). A severe case of persistent diarrhoea associated with Arcobacter cryaerophilus but attributed to Campylobacter sp. and a review of the clinical incidence of Arcobacter spp. *New microbes and new infections, 2*(2), 31-37. doi:https://doi.org/10.1002/2052-2975.35

Ghaju Shrestha, R., Tanaka, Y., Sherchand, J. B., & Haramoto, E. (2019). Identification of 16S rRNA and Virulence-Associated Genes of Arcobacter in Water Samples in the Kathmandu Valley, Nepal. *Pathogens, 8*(3), 110. doi:https://doi.org/10.3390/pathogens8030110

Ghane, M., Moein, F. G., & Massoudian, S. (2012). The first isolation of Campylobacter jejuni. *Advanced Studies in Biology, 4*(9), 407-418.

Girbau, C., Guerra, C., Martínez-Malaxetxebarria, I., Alonso, R., & Fernández-Astorga, A. (2015). Prevalence of ten putative virulence genes in the emerging foodborne pathogen Arcobacter isolated from food products. *Food microbiology, 52*, 146-149. doi:https://doi.org/10.1016/j.fm.2015.07.015

Gobbi, D. D., Spindola, M. G., Moreno, L. Z., Matajira, C. E., Oliveira, M. G., Paixão, R., . . . Moreno, A. M. (2018). Isolation and molecular characterization of Arcobacter butzleri and Arcobacter cryaerophilus from the pork production chain in Brazil. *Pesquisa Veterinária Brasileira, 38*(3), 393-399. doi:http://dx.doi.org/10.1590/1678-5150-pvb-4709

Grant, K. A., Belandia, I. U., Dekker, N., Richardson, P. T., & Park, S. F. (1997). Molecular characterization of pldA, the structural gene for a phospholipase A from Campylobacter coli, and its contribution to cell-associated hemolysis. *Infection and immunity, 65*(4), 1172-1180.

Ho, H. T., Lipman, L. J., Hendriks, H. G., Tooten, P. C., Ultee, T., & Gaastra, W. (2007). Interaction of Arcobacter spp. with human and porcine intestinal epithelial cells. *FEMS Immunology & Medical Microbiology, 50*(1), 51-58. doi:https://doi.org/10.1111/j.1574-695X.2007.00230.x

Houf, K., De Zutter, L., Van Hoof, J., & Vandamme, P. (2002). Assessment of the genetic diversity among arcobacters isolated from poultry products by using two PCR-based typing methods. *Applied and environmental microbiology, 68*(5), 2172-2178. doi:https://doi.org/10.1128/AEM.68.5.2172-2178.2002

Inoue, A., Murata, Y., Takahashi, H., Tsuji, N., Fujisaki, S., & Kato, J.-i. (2008). Involvement of an essential gene, mviN, in murein synthesis in Escherichia coli. *Journal of bacteriology, 190*(21), 7298-7301. doi:https://doi.org/10.1128/JB.00551-08

Jribi, H., Sellami, H., AMOR, S. B., Ducournau, A., Sifré, E., Benejat, L., . . . Gdoura, R. (2020). Occurrence and Antibiotic Resistance of Arcobacter Species Isolates from Poultry in Tunisia. *Journal of food protection, 83*(12), 2080-2086. doi:https://doi.org/10.4315/JFP-20-056

Jyothsna, T. S., Rahul, K., Ramaprasad, E., Sasikala, C., & Ramana, C. V. (2013). Arcobacter anaerophilus sp. nov., isolated from an estuarine sediment and emended description of the genus Arcobacter. *International journal of systematic and evolutionary microbiology, 63*(12), 4619-4625.

Karadas, G., Sharbati, S., Hänel, I., Messelhäußer, U., Glocker, E., Alter, T., & Gölz, G. (2013). Presence of virulence genes, adhesion and invasion of A rcobacter butzleri. *Journal of Applied Microbiology, 115*(2), 583-590. doi:https://doi.org/10.1111/jam.12245

Levican, A., Alkeskas, A., Günter, C., Forsythe, S. J., & Figueras, M. J. (2013). Adherence to and invasion of human intestinal cells by Arcobacter species and their virulence genotypes. *Applied and environmental microbiology, 79*(16), 4951-4957. doi:https://doi.org/10.1128/AEM.01073-13

Levican, A., Collado, L., Yustes, C., Aguilar, C., & Figueras, M. J. (2014). Higher water temperature and incubation under aerobic and microaerobic conditions increase the recovery and diversity of Arcobacter spp. from shellfish. *Applied and environmental microbiology, 80*(1), 385-391. doi:https://doi.org/10.1128/AEM.03014-13

Mey, A. R., Wyckoff, E. E., Oglesby, A. G., Rab, E., Taylor, R. K., & Payne, S. M. (2002). Identification of the Vibrio cholerae enterobactin receptors VctA and IrgA: IrgA is not required for virulence. *Infection and immunity, 70*(7), 3419-3426. doi:https://doi.org/10.1128/IAI.70.7.3419-3426.2002

Miller, W. G., Parker, C. T., Rubenfield, M., Mendz, G. L., Wösten, M. M., Ussery, D. W., . . . Wang, G. (2007). The complete genome sequence and analysis of the epsilonproteobacterium Arcobacter butzleri. *PloS one, 2*(12), e1358. doi:https://doi.org/10.1371/journal.pone.0001358

Rashid, R. A., Tarr, P. I., & Moseley, S. L. (2006). Expression of the Escherichia coli IrgA homolog adhesin is regulated by the ferric uptake regulation protein. *Microbial Pathogenesis, 41*(6), 207-217. doi:https://doi.org/10.1016/j.micpath.2006.07.006

Rasmussen, L. H., Kjeldgaard, J., Christensen, J. P., & Ingmer, H. (2013). Multilocus sequence typing and biocide tolerance of Arcobacter butzleri from Danish broiler carcasses. *BMC research notes, 6*(1), 1-7. doi:https://doi.org/10.1186/1756-0500-6-322

Rojas, C. M., Ham, J. H., Deng, W.-L., Doyle, J. J., & Collmer, A. (2002). HecA, a member of a class of adhesins produced by diverse pathogenic bacteria, contributes to the attachment, aggregation, epidermal cell killing, and virulence phenotypes of Erwinia chrysanthemi EC16 on Nicotiana clevelandii seedlings. *Proceedings of the National Academy of Sciences, 99*(20), 13142-13147.

Šilha, D., Vacková, B., & Šilhová, L. (2018). Occurrence of virulence–associated genes in Arcobacter butzleri and Arcobacter cryaerophilus isolates within the Czech Republic. *Acta Biochimica Polonica*.

Slater, F. R., Bailey, M. J., Tett, A. J., & Turner, S. L. (2008). Progress towards understanding the fate of plasmids in bacterial communities. *FEMS microbiology ecology, 66*(1), 3-13. doi:https://doi.org/10.1111/j.1574-6941.2008.00505.x

Snelling, W., Matsuda, M., Moore, J., & Dooley, J. (2006). Under the microscope: Arcobacter. *Letters in applied microbiology, 42*(1), 7-14. doi:https://doi.org/10.1111/j.1472-765X.2005.01841.x

Tabatabaei, M., Aski, H. S., Shayegh, H., & Khoshbakht, R. (2014). Occurrence of six virulence-associated genes in Arcobacter species isolated from various sources in Shiraz, Southern Iran. *Microbial Pathogenesis, 66*, 1-4. doi:https://doi.org/10.1016/j.micpath.2013.10.003

Tompkin, R. B. (2002). *Microbiological testing in food safety management* (Vol. 7): Springer Science & Business Media.

Vandenberg, O., Dediste, A., Houf, K., Ibekwem, S., Souayah, H., Cadranel, S., . . . Vandamme, P. (2004). Arcobacter species in humans. *Emerging infectious diseases, 10*(10), 1863-1867. doi:https://doi.org/10.3201/eid1010.040241

Waite, D. W., Vanwonterghem, I., Rinke, C., Parks, D. H., Zhang, Y., Takai, K., . . . Hanson, T. E. (2017). Comparative genomic analysis of the class Epsilonproteobacteria and proposed reclassification to Epsilonbacteraeota (phyl. nov.). *Frontiers in microbiology, 8*, 682. doi:https://doi.org/10.3389/fmicb.2017.00682

Wesley, I. V., & Miller, W. G. (2010). Arcobacter: an opportunistic human food-borne pathogen? In W. M. S. M. L. G. J. M. Hughes (Ed.), *Emerging infections 9* (pp. 185-212): Wiley Online Library.

Wilson, D. L., Abner, S. R., Newman, T. C., Mansfield, L. S., & Linz, J. E. (2000). Identification of ciprofloxacin-resistantcampylobacter jejuni by use of a fluorogenic PCR assay. *Journal of clinical microbiology, 38*(11), 3971-3978. doi:https://doi.org/10.1128/JCM.38.11.3971-3978.2000

Zhu, M., Valdebenito, M., Winkelmann, G., & Hantke, K. (2005). Functions of the siderophore esterases IroD and IroE in iron-salmochelin utilization. *Microbiology, 151*(7), 2363-2372.

شیوع و توزیع ژن‌های ویرولانس *آرکوباکتر بوتزلری* در نمونه‌های پساب کشتارگاه مرغ تنکابن

سیده سارا فلاح‌چای[۱]، نیما بهادور[۲*]، مسعود قانع[۳]

۱- دانشجوی دکتری، گروه میکروب‌شناسی، دانشکده علوم، واحد شیراز، دانشگاه آزاد اسلامی، شیراز، ایران

۲- استادیار، گروه میکروب‌شناسی، دانشکده علوم، واحد شیراز، دانشگاه آزاد اسلامی، شیراز، ایران

* نویسندهٔ مسئول (bahador@iaushiraz.ac.ir)

۳- دانشیار، گروه میکروب‌شناسی، واحد تنکابن، دانشگاه آزاد اسلامی، تنکابن، ایران

چکیده

آرکوباکتر بوتزلری درارتباط‌با انتریت، کرامپ‌شکمی، باکتریمی، آپاندیسیت در انسان و انتریت و درد شکمی در حیوانات شناخته می‌شود. این باکتری به‌عنوان خطرناک‌ترین گونه برای سلامت انسان براساس شاخص‌های کمیسیون میکروبیولوژی غذایی اعلام شده است. همچنین به‌عنوان پاتوژن مهم زئونوتیک معرفی شده است. هدف از این مطالعه جداسازی، شناسایی *آرکوباکتر بوتزلری* براساس ژن‌های بیماری‌زا (*ciaB*, *mviN*, *tlyA*) در فصول مختلف بود. ازاین‌رو، ۲۳۸ نمونه از پساب کشتارگاه مرغ شهرستان تنکابن جمع‌آوری شد. سپس کلنی‌های مشکوک با استفاده از تست بیوشیمیایی جداسازی و شناسایی شدند و از تکنیک واکنش زنجیره‌ای پلی‌مراز (PCR) جهت تأیید *آرکوباکترها* استفاده شد. از ۴۲ جدایهٔ *آرکوباکتر بوتزلری*، ژن‌های بیماری‌زا در ۱۵ جدایه ارزیابی شدند. نتایج به‌دست‌آمده نشان داد که ژن *ciaB* در ۱۳ سویه با فراوانی ۳۰/۹ درصد، ژن *mviN* در ۱۱ نمونه با فراوانی ۲۶/۲ درصد و ژن *tlyA* در ۹ نمونه با فراوانی ۲۱/۴ درصد وجود دارد. همچنین نتایج نشان داد که بالاترین میزان جداسازی *آرکوباکتر بوتزلری* ۸/۴ درصد در بهار و کمترین میزان آن ۱/۲ درصد در زمستان بود. همچنین نتایج نشان داد که بیشترین میزان جداسازی *آرکوباکتر بوتزلری* در فصل بهار با فراوانی ۸/۴ درصد و کمترین میزان جداسازی در فصل زمستان با فراوانی ۱/۲ درصد بوده است.

واژه‌های کلیدی: *آرکوباکتر بوتزلری*، ژن‌های ویرولانس، واکنش زنجیره‌ای پلی‌مراز