Iranian Journal of Microbiology

Volume 12 Number 6 (December 2020) 531-536

ORIGINAL ARTICLE

The incidence and antimicrobial resistance of *Arcobacter* species in animal and poultry meat samples at slaughterhouses in Iran

Sepideh Khodamoradi[1*], Ramin Abiri[2]

[1]Department of Microbiology, Shahr-e-Qods Branch- Islamic Azad University, Tehran, Iran
[2]Department of Microbiology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

Received: September 2020**, Accepted:** October 2020

ABSTRACT

Background and Objectives: *Arcobacter* species are food-borne and zoonotic enteropathogens. Defined breakpoints for the investigation of antimicrobial resistance of *Arcobacter* are missing.

Materials and Methods: The study was performed to investigate the incidence and antimicrobial resistance of *Arcobacter* species in animals and poultry meat samples procured from slaughterhouses in Iran. To investigate the prevalence of antimicrobial resistance, samples were collected from cattle (n=100), sheep (n=100), goat (n=100), broiler chicken (n=100), turkey (n=100) and quail (n=100). *Arcobacter* isolates of meat samples were isolated, investigated by PCR method and antibiotic resistance was also investigated. The susceptibility was assessed by Kirby-Bauer disc diffusion.

Results: The results showed that 52 samples (8.66%) were positive for *Arcobacter* spp. The most prevalence were observed in broiler chickens (26%, n=26 samples), quail (13%, n=13 samples), turkey (8%, n=8), cattle (3%, n=3), sheep (1%, n=1) and goat (1%, n=1). *Arcobacter butzleri* had highest prevalence among *Arcobacter* species. All the isolates showed sensitivity to gentamicin, streptomycin and tetracycline.

Conclusion: Poultry meat is a potential source of infection with *Arcobacter* that must be considered in slaughterhouses in Iran. *Arcobacter* species showed sensitivity for a broad spectrum of antibiotics that can be used during infection with *Arcobacter* species.

Keywords: *Arcobacter*; Broiler chicks; Iran; Prevalence; Antimicrobial susceptibility

INTRODUCTION

Arcobacters are foodborne and zoonotic enteropathogens and known as food-borne enteropathogens (1). *Arcobacter* species are commonly isolated from animal source foods (2). They cause bacteraemia, endocarditis, peritonitis, gastroenteritis in human, and diarrhea in both animals and humans (3). *Arcobacter* genus belongs to *Campylobacteraceae* family, the class *Epsilonproteobacteria* of the *phylum Proteobacteria* (4). *Arcobacter* species of *A. butzleri, A. cryaerophilus, A. thereius,* and *A. skirrowii* infect hosts and habitats and are mainly transmitted by water routes (5), such as rivers and lakes (6), drinking water (7), groundwater and recreational water (8). Infections induced by *A. butzleri* are mainly transmitted by water routes (9).

Slaughterhouses are sources for spreading disease (5). Arcobacters are commonly isolated from healthy cattle, sheep and pigs (3). Infections induced by *Arcobacter* species commonly occur during slaughter process (10). Increased trade of meat products between developing and industrialized countries increases the risk of the animal-associated pathogens in all over world (11). It is a major challenge in coun-

*Corresponding author: Sepideh Khodamoradi, Ph.D, Department of Microbiology, Shahr-e-Qods Branch- Islamic Azad University, Tehran, Iran.
Tel: +98-9183333817
Fax: +98-21-44861889
Email: Sepideh.khodamoradi2020@gmail.com

tries without surveillance systems and/or sites that pathogens can cause contamination.

Identification of *Arcobacter* by different biochemical tests is difficult as these organisms are metabolically inert (8). Culture media was previously used for diagnosis of *Arcobacter*. Cultural differentiation is difficult between *Arcobacter* and *Campylobacter* due to their phenotypic similarity. Molecular methods are useful for diagnosis of *Arcobacter*. PCR is a common and rapid technique to investigate *Arcobacter* in meat samples. Several antibacterial drugs are used for the treatment of infections with *Arcobacter*, such as erythromycin, fluoroquinolones and ciprofloxacin (13). Utilization of antibiotics has faced with forbidden, because they cause antimicrobial resistance. It was reported *A. butzleri* as more resistant compared to *A. cryophilus* and *A. skirrowi* (14, 15).

Iranian people raise animals and poultry for meat consumption. Shirzad Aski et al. (16) showed the prevalence of the *Arcobacter* spp. was 9% for slaughterhouse samples. With regards to *Arcobacter* species, their contamination rate in animals meat, and providing an effective strategies for alleviation of the both the infections induced by *Arcobacter* species in humans and animals, this study investigates the incidence and antimicrobial resistance of *Arcobacter* species in animal and poultry meat samples at slaughterhouses in Iran.

MATERIALS AND METHODS

Samples. Six-hundred samples were collected from cattle (n=100), sheep (n=100), goat (n=100), broiler chicken (n=100), turkey (n=100) and quail (n=100) during 2019 year. The samples were immediately transferred to laboratory. The samples in weight of 50 g were collected from different parts of animal meat at slaughterhouse and five samples were collected for each animal.

Isolation and identification of *Arcobacter* species. *Arcobacter* species were isolated as reported by Maruyama et al. (17). To identify and differentiate of *Arcobacter* species, DNA was extracted from meat samples using DNA kit as reported by Cinnagen Company (Tehran-Iran). The used primers were as follows;

1. *(Arcobacter)*: TTCGCTTGCGCTGCATCAT
2. *(A. butzleri)*: AGCGTTCTATTCAGCGTAGAAGATGT
3. *(A. cryaerophilus)*: ACCGAAGCTTTAGATTCGAATTTATTCA
4. *(A. skirrowii)*: CGAGGTCACGGATGGAAGTG

Amplification was conducted in a thermal cycler (Master Cycle Gradient, Eppendorf, Germany) as follows: initial denaturation and denaturation at 94°C, and annealing at 64°C. The final elongation was conducted at 72°C for 7 min. The quality and quantity of extracted DNA was determined by measurement of the concentration. Test method and thermal program were as reported by Son et al. (18). Purity was investigated by a UV spectrophotometer (NanoDrop™ 1000). Absorption was investigated at 260 nm (A260) and 280 nm (A280). DNA purities were calculated through calculating the A260/A280 ratios. Samples containing A260/A280 ratios of 1.7-2.0 were considered as pure samples, free from protein and other impurities (19). The samples were held at 4°C until the PCR products were analyzed. Agarose gel 1.5% was used to trace the products. The amplified DNA products were electrophoresed on 1.5% agarose gels at 90 V for 6 h using 1× TBE (0.89 *M* Tris borate, 0.02 *M* EDTA, pH 8.3) as the running buffer, and then stained with ethidium bromide. Gels were visualized using a UV gel documentation system. A set of molecular weight standards was included on each gel. DNA samples from reference strains were considered as positive controls. Negative controls in that DNA was replaced with sterile distilled water were included in all assays. Negative controls were prepared from American Type Culture Collection. Species were identified by 16SrRNA using specific described primers. Product size was 298 bp, 214 bp, and 421 bp for *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*, respectively.

Antibiotic resistance. Kirby–Bauer disc methods was used for assessing antimicrobial susceptibility testing in Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India, MV1084) enriched with 5% defibrinated sheep blood as reported by Rahimi (19). *Arcobacter* isolates of meat samples were investigated for antibiotic resistance for chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), penicillin (10 µg), streptomycin (30 µg), tetracycline (15 µg), azithromycin (10 µg), and nalidixic Acid (30 µg) by disc method as reported by rahimi (19).

Statistical analysis. The data were analyzed by Chi-square and Fisher tests using SPSS software. The data were reported as mean and frequency (%).

RESULTS

The results for prevalence of *Arcobacter* species in animals and poultry are shown in Table 1. The results showed that 52 samples (8.66%) were positive for *Arcobacter* spp. The most prevalence were observed in broiler chickens (26%, n=26), quail (13%, n=13), turkey (8%, n=8), cattle (3%, n=3), sheep (1%, n=1) and goat (1%, n=1). In all positive samples, *A. butzleri* species were positive (Fig. 1). Fig. 2 shows electrophoresis gel for the samples.

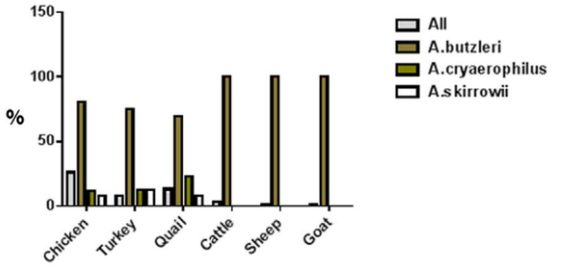

Fig. 1. *Arcobacter* species in animal and poultry meat samples

The results for antimicrobial resistance are shown in Table 2. The results showed that all the species were sensitive to gentamicin, streptomycin and tetracycline. Higher resistance rate were observed for chloramphenicol (41/52=78.84%), azithromycin (41/52=78.84%) and clindamycin (36/52=69.23%). Among species, *A. butzleri* showed high resistance to chloramphenicol (36/41=87.80%) and azithromycin (32/41=78.04%). *A. cryaerophilus* showed highest resistance to azithromycin (6/7=85.71%), clindamycin (5/7=71.42%) and erythromycin (5/7=71.42%). However, *A. skirrowii* showed the highest resistance to *Azithromycin* (3/4=75.00%).

DISCUSSION

Arcobacter prevalence is increasing in food and most species show antimicrobial resistance. Increased in the prevalence and resistance pushed us to conduct this study. This study investigated the incidence and antimicrobial resistance of *Arcobacter* species in animal and poultry meat samples at slaughterhouses in Iran. The results showed that 52 samples (8.66%) were positive for *Arcobacter* spp. The most prevalence were observed in broiler chickens (26%,

Table 1. Prevalence of *Arcobacter* species in animal and poultry meat samples

		***Arcobacter* species**			
Samples	**Number**	**positive samples**	***A. butzleri***	***A. cryaerophilus***	***A. skirrowii***
Broiler	100	26 (26.00%)	21 (80.76%)	3 (11.53%)	2 (7.69%)
Turkey	100	8 (8.00%)	6 (75.00%)	1 (12.50%)	1 (12.50%)
Quail	100	13 (13.00%)	9 (69.23%)	3 (23.07%)	1 (7.69%)
Cattle	100	3 (3.00%)	3 (100%)	-	-
Sheep	100	1 (1.00%)	1 (100%)	-	-
Goat	100	1 (1.00%)	1 (100%)	-	-

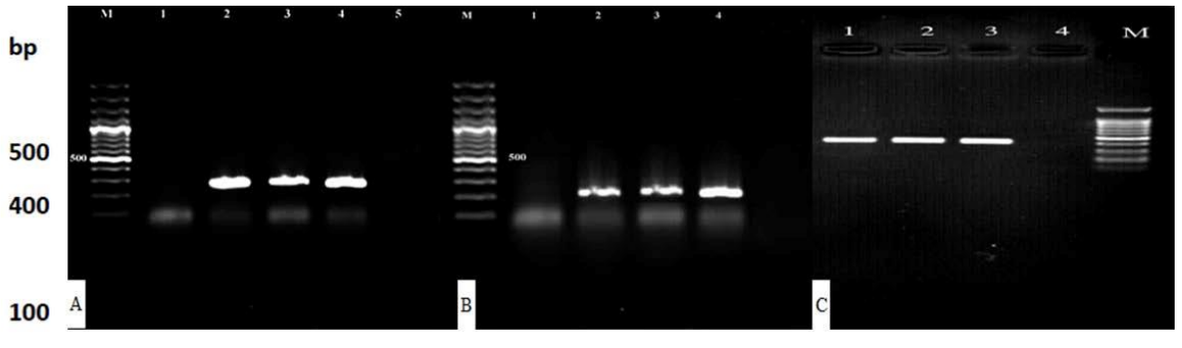

Fig. 2. Agarose gel electrophoresis of *A. butzleri* (A), *A. cryaerophilus* (B) and *A. skirrowii* (C). Lane M: Trackit 100 bp ladder

Table 2. Antibiotic resistance of *Arcobacter* species isolated from birds

Antibiotics	*Arcobacter* (n=52)	*A. butzleri* (n=41)	*A. cryaerophilus* (n=7)	*A. skirrowii* (n=4)
Gentamicin	-	-	-	-
Chloramphenicol	41 (78.84%)	36 (87.80%)	3 (42.85%)	2 (50.00%)
Clindamycin	36 (69.23%)	29 (70.73%)	5 (71.42%)	2 (50.00%)
Erythromycin	25 (48.07%)	20 (48.78%)	5 (71.42%)	-
Streptomycin	-	-	-	-
Tetracycline	-	-	-	-
Azithromycin	41 (78.84%)	32 (78.04%)	6 (85.71%)	3 (75.00%)
Nalidixic Acid	30 (57.69%)	26 (63.41%)	2 (28.57%)	2 (50.00%)

n=26 samples), quail (13%, n=13 samples), turkey (8%, n=8), cattle (3%, n=3), sheep (1%, n=1) and goat (1%, n=1). In contrast to our findings, Shirzad Aski et al. (16) investigated the incidence of *Arcobacter* spp. in animal meat samples at slaughterhouses in Southern Iran and reported 9% and 14% positive samples for cattle and sheep, respectively. The most prevalence was observed for broiler chicks meat samples. Similar to our findings, Atabay et al. (20) reported a significant contamination (65.3%) for broiler chickens. These findings confirm that broiler chicks are sensitive to *Arcobacter* species. The results showed 36.25% contamination in samples procured from turkey meat. Aydin et al. (21) reported rates of 68% and 4% for contamination of chicken and turkey meat samples, respectively. Bogantes et al. (22) reported prevalence rate to be 36% in duck meat sample which is lower than the present study (54%). In the current study, in the animals, prevalence rate was very low that may be attributed to geographical condition and animal types that influence bacterial growths. Merga et al. (23, 24) reported the incidence of 43% and 40% from feces of cattle and sheep in the United Kingdom. A difference between our findings and Merga et al. findings could be attributed to sampling types (meat vs feces). The results also showed that in all the positive samples, *A. butzleri* species were positive. Similar to our findings previous studies also have reported *A. butzleri* as most prevalent species (7, 26, 27). *A. butzleri* is a prevalent organism in dairy farms compared with other *Arcobacter* species, because it survives in different environmental conditions (27). Badilla-Ramırez et al. (28) reported that *A. butzleri* could grow at 4°C and 10°C. It was reported that *A. butzleri* species could survive in different storage temperatures, and they contaminate poultry meat samples (29). Our findings for contamination with *A. butzleri*, *A. skirrowii* and *A. cryaerophilus* that are parallel with the results reported by Verma et al. (30). The results showed that *Arcobacter* species were sensitive to gentamicin, streptomycin and tetracycline. The data for antimicrobial resistance are important, because the agents can be used as first-line drugs for the treatment of infection induced by *Arcobacter* (31). The results showed that *Arcobacter* species showed sensitivity to tetracycline. Yesilmen et al. (26) showed that the acquired resistance for *Arcobacter* species against tetracycline and ampicillin antibiotics. Several studies have reported high susceptibility of *Arcobacter* species to tetracycline (14, 20, 32). Shah et al. (33) reported that *Arcobacter* species are susceptible to gentamicin. Unver et al. (34) showed that *A. skirrowii* and most of the *A. cryaerophilus* isolates were resistant to chloramphenicol, erythromycin, and amoxicillin. Our results for the effect of tetracycline on *Arcobacter* species are consistent with previous results that showed *Arcobacter* species are susceptible to tetracycline (19). In sum, the results suggest that gentamicin, streptomycin and tetracycline are efficient agents for the treatment of infections induced by *Arcobacter* spp. It means that some antibiotics do not kill them, due to bacterial structure.

In conclusion, poultry meat was more sensitive to *Arcobacter* species that might be attributed to nutrient nature of poultry meat samples. In all the positive samples, *A. butzleri* species were positive. *Arcobacter* species were sensitive to tetracycline. Since high levels of contamination with *Arcobacter* spp. can occur in poultry slaughterhouses, considering hygiene in slaughterhouses is an essential principle for alleviating the risk of contamination and the use of tetracycline as an efficient agent for the treatment of contamination with *Arcobacter* species is suggested.

ACKNOWLEDGEMENTS

This study was conducted in the form of an internal study opportunity at Islamic Azad University Sanandaj Branch (Kurdistan, Iran). The authors thank the Dr. Ali Olfati for all efforts to improve the written language of the paper.

REFERENCES

1. Çelik C, Ikiz S. The investigation of the presence and antimicrobial profiles of *Arcobacter* species in sheep carcasses and feces. *Acta Vet Eurasia* 2019; 45:42-49.
2. Aydin F, Yağiz A, Abay S. Prevalence of *Arcobacter* and *Campylobacter* in beef meat samples and characterization of the recovered isolates. *J Consum Prot Food Saf* 2020; 15:15-25.
3. Ferreira S, Queiroz JA, Oleastro M, Domingues F. Insights in the pathogenesis and resistance of *Arcobacter*: a review. *Crit Rev Microbiol* 2016; 42:364-383.
4. Fanelli F, Pinto AD, Mottola A, Mule G, Chieffi D, Baruzzi F, et al. Genomic characterization of *Arcobacter butzleri* isolated from shellfish: novel insight into antibiotic resistance and virulence determinants. *Front Microbiol* 2019; 10: 670.
5. Collado L, Figueras MJ. Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. *Clin Microbiol Rev* 2011; 24:174-192.
6. Park S, Jung YT, Kim S, Yoon JH. *Arcobacter acticola* sp. Nov isolated from seawater on the east sea in south Korea. *J Microbiol* 2016; 54:655-659.
7. Chieffi D, Fanelli F, Fusco V. *Arcobacter butzleri:* Up-to-date taxonomy, ecology, and pathogenicity of an emerging pathogen. *Compr Rev Food Sci Food Saf* 2020; 19:2071-2109.
8. Collado L, Levican A, Perez J, Figueras MJ. *Arcobacter defluvii* sp. nov., isolated from sewage samples. *Int J Syst Evol Microbiol* 2011; 61:2155-2161.
9. Lee C, Agidi S, Marion JW, Lee J. *Arcobacter* in Lake Erie beach waters: an emerging gastrointestinal pathogen linked with human-associated fecal contamination. *Appl Environ Microbiol* 2012; 78:5511-5519.
10. De Smet S, De Zutter L, Van Hende J, Houf K. *Arcobacter* contamination on pre- and post-chilled bovine carcasses and in minced beef at retail. *J Appl Microbiol* 2010; 108:299-305.
11. Van Driessche E, Houf K, Van Hoof J, De Zutter L, Vandamme P. Isolation of *Arcobacter* species from animal feces. *FEMS Microbiol Lett* 2003; 229:243-248.
12. Dekker DD, Eibach KG, Boahen C, Wiafe Akenten Y, Pfeifer AE, Zautner E, et al. Fluoroquinolone-resistant *Salmonella enterica*, *Campylobacter* spp., and *Arcobacter butzleri* from local and imported poultry meat in Kumasi, Ghana. *Foodborne Pathog Dis* 2019; 16: 352-358.
13. Skirrow MB (2000). Clinical aspects of *Campylobacter* infection, in: I.Nachamkin, M.J. Blaser (Eds.), Campylobacter, second ed., ASM Press, Washington, DC. 69-88.
14. Kabeya H, Maruyama S, Morita Y, Ohsuga T, Ozawa S, Kobayashi Y, et al. Prevalence of *Arcobacter* species in retail meats and antimicrobial susceptibility of the isolates in Japan. *Int J Food Microbiol* 2004; 90:303-308.
15. Abay S, Kayman T, Hizlisoy H, Aydin F. *In vitro* antibacterial susceptibility of *Arcobacter butzleri* isolated from different sources. *J Vet Med Sci* 2012; 74:613-616.
16. Shirzad Aski H, Tabatabaei M, Khoshbakht R, Raeisi M. Occurrence and antimicrobial resistance of emergent *Arcobacter* spp. isolated from cattle and sheep in Iran. *Comp Immunol Microbiol Infect Dis* 2016; 44:37-40.
17. Kabeya H, Maruyama S, Morita Y, Ohsuga T, Ozawa S, Kobayashi Y, et al. Prevalence of *Arcobacter* species in retail meats and antimicrobial susceptibility of the isolates in Japan. *Int J Food Microbiol* 2004;90:303-308.
18. Son I, Englen MD, Berrang ME, Fedorka-Cray PJ, Harrison MA. Prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. *Int J Food Microbiol* 2007; 113:16-22.
19. Rahimi E. Prevalence and antimicrobial resistance of *Arcobacter* species isolated from poultry meat in Iran. *Br Poult Sci* 2014; 55:174-180.
20. Atabay HI, Aydin F. Susceptibility of *Arcobacter butzleri* isolates to 23 antimicrobial agents. *Lett Appl Microbiol* 2001; 33:430-433.
21. Aydin F, Gümüşsoy KS, Atabay HI, Iça T, Abay S. Prevalence and distribution of *Arcobacter* species in various sources in Turkey and molecular analysis of isolated strains by ERIC-PCR. *J Appl Microbiol* 2007; 103:27-35.
22. Bogantes EV, Fallas-Padilla KL, Rodriguez-Rodriguez CE, Jaramillo HF, Echandi MLA. Zoonotic species of the genus *Arcobacter* in poultry from different regions of Costa Rica. *J Food Prot* 2015; 78:808-811.
23. Merga JY, Leatherbarrow A, Winstanley C, Bennett M, Hart CA, Miller WG, et al. Comparison of *Arcobacter* isolation methods, and diversity of *Arcobacter* spp. in Cheshire, United Kingdom. *Appl Environ Microbiol* 2011; 77:1646-1650.
24. Merga JY, Williams NJ, William G, Miller WG, Leatherbarrow AJH, Bennett M, et al. Exploring the diversity of *Arcobacter butzleri* from cattle in the UK using MLST and whole genome sequencing. *PLoS One* 2013;

8(2): e55240.
25. Levican A, Alkeskas A, G€unter C, Forsythe SJ, Figueras MJ. Adherence to and invasion of human intestinal cells by *Arcobacter* species and their virulence genotypes. *Appl Environ Microbiol* 2013; 79:4951-4957.
26. Yesilmen S, Vural A, Erkan ME, Yildirim IH. Prevalence and antimicrobial susceptibility of *Arcobacter* species in cow milk, water buffalo milk and fresh village cheese. *Int J Food Microbiol* 2014; 188:11-14.
27. Giacometti F, Lucchi A, Di Francesco A, Delogu M, Grilli E, Guarniero I, et al. *Arcobacter butzleri, Arcobacter cryaerophilus* and *Arcobacter skirrowii* circulation in a dairy farm and sources of milk contamination. *Appl Environ Microbiol* 2015; 81:5055-5063.
28. Badilla-Ramırez Y, Fallas-Padilla KL, Fernandez-Jaramillo H, Arias-Echandi ML. Survival capacity of *Arcobacter butzleri* inoculated in poultry meat at two different refrigeration temperatures. *Rev Inst Med Trop Sao Paulo* 2016; 58:22.
29. Ramees K, Kumaragurubaran K, Ramswaroop S, Ashok K, Mani Sa, Ruchi T, et al. *Arcobacter*: an emerging food-borne zoonotic pathogen, its public health concerns and advances in diagnosis and control-a comprehensive review. *Vet Q* 2017; 37:136-161.
30. Verma M, Joshi N, Rathore RS, Mohan HV. Detection of *Arcobacter* spp. in poultry, pigs, their meat and environmental samples by conventional and PCR assays. *Indian J Anim Sci* 2015; 85:954-957.
31. Vandenberg O, Houf K, Douat N, Vlaes L, Retore P, Butzler JP. Antimicrobial susceptibility of clinical isolates of non-jejuni/coli campylobacters and Arcobacters from Belgium. *J Antimicrob Chemother* 2006; 57:908-913.
32. Zacharow I, Bystron J, Waecka-Zacharska E, Podkowik M, Bania J. Prevalence and antimicrobial resistance of *Arcobacter butzleri* and *Arcobacter cryaerophilus* isolates from retail meat in Lower Silesia region. *Pol J Vet Sci* 2015; 18:63-69.
33. Shah AH, Saleha AA, Zunita Z, Murugaiyah M, Aliyu AB, Jafri N. Prevalence, distribution and antibiotic resistance of emergent *Arcobacter* spp. from clinically healthy cattle and goats. *Transbound Emerg Dis* 2013; 60:9-16.
34. Unver A, Atabay HI, Sahin M, Celebi €O. Antimicrobial susceptibilities of various *Arcobacter* species. *Turk J Med Sci* 2013; 43:548-552.