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*Research Article*

# DETERMINATION OF ANTIBIOTIC RESISTANCE PROFILES IN ACINETOBACTER SPECIES ISOLATED FROM TANK MILK

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**ABSTRACT.** The aim of this study was to determine the occurrence of *Acinetobacter* spp. in tank milk samples (TMS) in Hatay province and to analyze their antimicrobial susceptibility profile. In this study, a total of 60 TMS were collected between April and December 2016. *Acinetobacter* spp. was identified by using Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry (MS). The identified *Acinetobacter* isolates were tested to determine the antimicrobial resistance profiles towards 23 antibiotics using the disc diffusion method. A total of 13 *Acinetobacter* isolates were obtained from TMS (21.6%). Species distribution were as follow; *Acinetobacter baumannii* (n=9), *Acinetobacter ursingii* (n=3) and *Acinetobacter iwoffii* (n=1). Resistance to aztreonam, chloramphenicol, amoxicillin and clavulanate, cefoxitin, cefpodoxime gentamicin, cefuroxime, florphenicol was 53.8%, 15.3% 15.3%, 15.3%, 15.3%, 7.7%, 7.7%, and 7.7%, respectively. However, none of the isolates was resistant to nalidixic acid, ciprofloxacin, enrofloxacin, levofloxacin, norfloxacin, penicillin, tetracycline, sulfamethoxazole/trimethoprim, ampicillin, imipenem, colistin, cephalothin, and ceftazidime. As a result, the TMS were found to be contaminated with different species of *Acinetobacter*. Another important finding of this study is the antimicrobial resistance characteristics of these strains, which might pose a public health problem. Therefore, it may be said that TMS would be better to be monitored for this potentially pathogenic bacterium.

**Keywords:** *Acinetobacter, tank milk, antibiotic resistance.*

## INTRODUCTION

Milk is of great importance in nutrition with its basic nutritional components [1]. The fact remains that, milk from a healthy udder contains many bacteria that can multiply during processing [2]. With its unique composition, milk also provides an excellent environment for bacterial growth. Therefore, it is an important source of bacterial infection in terms of consumption. Pathogenic bacteria in milk threaten human health and bacterial infection is responsible for approximately 90% of the diseases related to milk consumption [3]. There are many types of cheese prepared and offered for consumption in the world and Turkey without pasteurization [4]. Carra cheese, which is widely consumed especially in the Hatay region and produced from raw milk, is one of these examples. Therefore, raw milk is an important vector for the spread of community-acquired antibiotic-resistant bacteria. Major pathogens found in milk: *Staphylococcus*

*aureus, Salmonella* spp., *Listeria monocytogenes, Escherichia coli* O157:H7, and *Campylobacter* [5]. However, in recent years, there have been studies showing that other pathogenic bacterial species, in particular *Acinetobacter* spp., have also been isolated from raw milk samples [1, 4, 6]. *Acinetobacter* spp. is of a great concern due to their rapid development of resistance to a wide variety of antimicrobials, their recognition as an important nosocomial pathogen, and their prolonged persistence in the environment [7]. In addition, the fact that they are transmitted through person-to-person contact, water, and food contamination increases the concern even more [8]. Although *Acinetobacter baumannii* (*A. baumannii*) is considered a nosocomial pathogen, it can cause community-acquired infections [9, 10]. The rate of antimicrobial resistance among human clinical *A. baumannii* isolates has reached a significant level, which has been clearly demonstrated by the fact that this bacterial species is at the top of the list of resistant bacteria requiring new antibiotic discovery published by the World Health Organization in 2018 [11]. The Centers for Disease Control and Prevention (CDC) published 7300 *Acinetobacter* infections and 500 deaths annually in 2018 [12]. Therefore, detection of this potentially pathogenic bacteria in foods, including samples of bovine milk, goat's milk, and dairy products, is particularly important [13, 14].

*Acinetobacter* species are heat-resistant, psychotropic bacteria. The heat-stable, proteolytic and lipolytic enzymes produced by these bacteria can cause deterioration in milk even after the pasteurization step [15]. These enzymes can be easily produced in raw milk stored in the cold. These bacteria can continue their activities due to extracellular enzymes [16].

Since *Acinetobacter* spp. remains a significant pathogen worldwide, we aimed to determine the prevalence of *Acinetobacter* spp. and the antibiotic resistance of the isolated strains in raw tank milk samples (TMS) in our region.

## MATERIALS AND METHODS

### *Milk Samples*

In this study, 60 milk samples were collected from the milk collection tanks of the Hatay Province Cattle Breeders Association between April and December 2016. Milk samples were taken into sterile sample containers and samples were transferred to the laboratory quickly for microbiological assessment.

### *Preparation of Milk Samples for Analysis*

Tank milk samples were enriched with alkaline peptone water (1/9; v/v) and at incubated 37 °C for 24 h. Enriched samples were inoculated on MacConkey agar at 37 $^{o}$C for overnight. Non-lactose fermenting colonies were selected and initially characterized by phenotypic tests including motility, oxidase, and catalase. The genus *Acinetobacter*, their identification at the species level was done using the Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry (MS) (Bruker Daltonik GmbH, Leipzig, Germany) device. As specified by the manufacturer's guideline, identification scores of ≥2000 and between 1700 and 1900 were accepted for reliable identification to the species and genus level, respectively, while identification scores of 1700 were considered unreliable and omitted in this study.

### *Determination of Antibiotic Susceptibility Profile*

Antibiotic susceptibility tests were investigated using the Kirby-Bauer disk diffusion method according to Clinical Laboratory Standards Institute (CLSI, 2010) using Mueller-Hinton agar (Becton Dickinson, Sparks, MD). The antimicrobial drugs (n=23) tested were chloramphenicol (30µg), amoxicillin-clavulanate (20/10µg), cefoxitin (30µg), aztreonam (30µg), florphenicol (30µg), cefpodoxime (30µg), cefuroxime (30µg), nalidixic acid (30µg), ciprofloxacin (5µg), enrofloxacin (5µg), levofloxacin (5µg), norfloxacin (10µg), penicillin (30µg), tetracycline (30µg), sulfamethoxazole/trimethoprim (1.25/23.75µg), ampicillin (10µg), imipenem (10µg), colistin (10µg), cephalothin (30µg), ceftazidime (30µg). Because no *Acinetobacter* spp. reference strain was available, *E. coli* ATCC was used as the standard strain.

## RESULTS AND DISCUSSION

A total of 13 milk samples (21.6%) were found to be contaminated with *Acinetobacter* spp. (Table 1). These *Acinetobacter* strains (n=13) were identified as *Acinetobacter baumannii* (n=9), *Acinetobacter ursingii* (n=3) and *Acinetobacter iwoffii* (n=1) (Table 1).

The highest resistance rate in isolates was found to be 53.8% against aztreonam. The resistance rate against chloramphenicol, amoxicillin/clavulanate, cefoxitin, and cefpodoxime with 15.3% (2/13), and cefuroxime, florfenicol and gentamicin 7.7% (1/13) were lower than the rates of other (Table 2). None of the isolates was resistant to nalidixic acid, ciprofloxacin, enrofloxacin, levofloxacin, norfloxacin, penicillin, tetracycline, sulfamethoxazole/trimethoprim, ampicillin, imipenem, colistin, cephalothin, ceftazidime.

*Acinetobacter* spp. is of great concern due to their rapid development of resistance to a wide variety of antimicrobials and their prolonged persistence in the environment. In addition, transmission through person-to-person contact, water and food contamination are the other causes for concern [8]. Antimicrobial-resistant *Acinetobacter* spp. in raw milk could be potential reservoirs of resistance genes, as they may survive pasteurization due to their ability to resist heat [15].

In this study, *Acinetobacter* spp. was isolated from 21.3% of the tank milk samples. To the best of our knowledge, this is the first report of prevalence of *Acinetobacter* spp. in TMS in the south region of Turkey. In a study conducted by Gurung et al. [6] in Korea, *Acinetobacter* spp. was detected in 7.7% of tank milk samples. In another study conducted in eastern South Dakota and western Minnesota by Jayarao and Wang [17], the isolation rate of *Acinetobacter* spp. was found to be 1.3%. The contamination rate of *Acinetobacter* spp. in dairy goat milk samples in Kenya was reported to be 5% [18]. However, a much lower isolation rate (0.3%) was also reported from cow udder samples with mastitis in Korea [19]. The reason for such a wide range of results may be due to the fact that the studies were conducted in different regions.

*Acinetobacter* spp. including *A. baumannii* commonly occurs in raw tank milk samples [1,6,20]. In this study: in 60 TMS, 9 *A. baumannii* isolates (15%) were identified. In a study conducted in 2012 by Gurung et al [6] in Korea, Asinetobacter baumani positivity was found at a rate of 32.4% in tank milk. Kim et al [21]; investigated 490 raw milk samples and found 136 samples contaminated with *A. baumannii* (27.8%) [21]. The study of Cho et al. [22] was reported that 89.4% of *Acinetobacter* isolates from tank milk were identified as *A. baumannii*.

The detection of high rates of *A. baummanni*, an important pathogen in previous studies, once again demonstrates that milk and dairy products marketed without

pasteurization can be a significant source of contamination. As stated above, it is necessary to prevent the spread of these bacteria in the community, since it is an important nosocomial pathogen, has widespread antibiotic resistance, is difficult to treat, and has a serious clinical picture (ischemia and pneumonia).

Acinetobacter species is naturally resistant to many antibiotics and has the potential to develop resistance to a wide range of antimicrobial agents easily [6]. Ten of the 13 Acinetobacter spp. isolates were resistant to at least one antimicrobial in our study. The highest antibiotic resistance rate was found against aztreonam (53.8%), followed by amoxicillin-clavulanate (15.3%), chloramphenicol (15.3%), cefoxitin (15.3%), cefpodoxime (15.3%), gentamicin (7.7%), florfenicol (7.7%) and cefuroxime (7.7%) (Table 2). A considerable number of the antibiotics we tested can be effective in the treatment of community-acquired Acinetobacter infections. However, due to the increased antibiotic resistance in Intensive Care Unit (ICU)-acquired Acinetobacter infections in recent years [23], the possibility of treating these infections with antibiotics whose tested in our study is low.

Antibiotics that are effective against these bacteria, such as carbapenems (except ertapenem), some third or fourth generation cephalosporins, piperacillin-tazobactam, tigecycline, or colistin, can be used in the treatment of ICU-acquired Acinetobacter infections [24]. Since the Acinetobacter strains isolated in the present study were community-acquired, the antibiotics that are effective in nosocomial infections have not been investigated.

Antibiotic resistance has become a global problem. Some bacteria resistant to three or more antibiotic groups (MDR) or even all antibiotics (PDR) have been isolated from hospitals and communities over the last two decades. In this study, only one isolate of *A. baumannii* was determined to be multidrug resistant. In the present study, MDR *A. baummanii* strain was resistant to antibiotic classes such as chloramphenicol, macrolide, penicillin, and cephalosporin. However, no resistance was found to nalidixic acid, ciprofloxacin, enrofloxacin, levofloxacin, norfloxacin, penicillin, tetracycline, sulfamethoxazole/trimethoprim, ampicillin, imipenem, colistin, cephalothin, ceftazidime in all the isolates. In comparison with a previous study conducted by Kim et al [21] in Korea: an antimicrobial susceptibility test revealed that 60 of the 136 isolates (44.1%) were antibiotic-resistant *A. baumannii* showing resistance against at least one antimicrobial [21]. The highest antibiotic resistance rate was found against tetracycline (30.8%), followed by cefotaxime (12.5%), ceftriaxone (4.4%), and gentamicin (2.9%). However, no resistance was found to imipenem, meropenem, and levofloxacin in all the isolates. In another study: Gurung et al [6], reported the resistance to amikacin, gentamicin, piperacillin, cefotaxime, tetracycline, trimethoprim-sulfamethoxazole, ceftazidime, and ampicillin-sulbactam against *Acinetobacter* spp. was 2.3, 7.4, 2.3, 4.0%, 17.6%,15.9%,10.8%, and 10.2%, respectively. The rate of antimicrobial resistance among *A. baumannii* from raw milk has significantly increased in the past 5 years in Korea, especially against tetracycline (5.3% to 30.8%) and cefotaxime (0% to 12.5%) [21].

We couldn't find any comparable data for *Acinetobacter* isolates from tank milk samples in Hatay/Turkey, hence we were able to compare Acinetobacter isolates resistance with the other food types. Kanaan et al [25] in Turkey detected multi-drug resistance *A. baumannii,* in 74 % of turkey and chicken raw meat isolates. We can state that, compared to tank milk samples, *Acinetobacter* rates are higher in other food types.

***Table 1.*** *Distribution of Acinetobacter isolated from milk by species*

| ***Acinetobacter* species** | **Number of isolates (n)** | **%** |
|---|---|---|
| *A. baumanni* | 9 | 15 (9/60) |
| *A. ursingi* | 3 | 5 (3/60) |
| *A. iwofii* | 1 | 1,6 (1/60) |
| Total | 13 | 21,6 (13/60) |

***Table 2.*** *Rates of antibiotic resistance of Acinetobacter* spp. *isolated from tank milk samples*

| **Antibiotics** | **Resistant-Isolate Number** | **Resistance Rate %** |
|---|---|---|
| Gentamicin | 1 | 7.7 |
| Chloramphenicol | 2 | 15.3 |
| Amoxicillin/Clavulanate | 2 | 15.3 |
| Cefoxitin | 2 | 15.3 |
| Aztreonam | 7 | 53.8 |
| Florfenicol | 1 | 7.7 |
| Cefpodoxime | 2 | 15.3 |
| Cefuroxime | 1 | 7.7 |

## CONCLUSION

In our study, it was found that there was *Acinetobacter* contamination in raw milk. Therefore, milk and dairy products should not be consumed raw, and raw dairy products, cheese, etc. can be an important source of contamination for antibiotic-resistant pathogenic bacteria. Resistance to important antibiotics preferred in community-acquired infections such as Aztreonam has been detected, so the use of these antibiotics in animal health should be closely monitored. Also, it is necessary to prevent the risk to public health by following the sanitation and hygiene rules in milk samples.

**Conflict of Interest.** The author declared that there is no conflict of interest.

**Authorship Contributions.** C.O.G collected samples, cultured the isolates, and performed the analyses. Also, C.O.G designed the study, wrote the manuscript.

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## REFERENCES

[1] Tamang, M. D., Gurung, M., Nam, H. M., Kim, S. R., Jang, G. C., Jung, S. C. (2014): Short communication: genetic characterization of antimicrobial resistance in Acinetobacter isolates recovered from bulk tank milk. J. Dairy Sci. 97, 704–709. DOI: 10.3168/jds.2013-7403.

[2] Nahla A. Abou El-Roos; Ebtesam M. Mazıd; Eman M. Zakary and Kayrı F. Abou El Yazıd. (2013): Molecular Characterızatıon of Pseudomonas Aerugınosa Isolated from Mılk. J. Assiut Vet. Med59, 14-22. DOI: 10.21608/avmj.2013.171897.

[3] Berhe, G., Wasihun, A.G., Kassaye, E., Gebreselasie, K. (2020): Milk-borne bacterial health hazards in milk produced for commercial purpose in Tigray, northern Ethiopia. BMC Public Health. 20:894. https://doi.org/10.1186/s12889-020-09016-6.

[4] Saad, N.M., Amın W.F., Mostafa, S.M. (2018): Detectıon of Acınetobacter Specıes in Mılk and Some Daıry Products. Assiut Vet. Med. J. Vol. 64 No. 156 2018, 34-40. DOI: 10.21608/AVMJ.2018.168683.

[5] Claeys W, Cardoen S, Daube G, De Block J, Dewettinck K, Dierick K. (2013): Raw or Heated Cow Milk Consumption: Review of Risks and Benefits. Food Control. 31:251e262. https://doi.org/10.1016/j.foodcont.2012.09.035.

[6] Gurung M., Nam H. M., Tamang M. D., Chae M. H., Jang G. C., Jung S. C., and Lim S. K. (2013): Prevalence and antimicrobial susceptibility of Acinetobacter from raw bulk tank milk in Korea J. Dairy Sci. 96:1997–2002. DOI: 10.3168/jds.2012-5965.

[7] Manchanda V., Sanchaita S., Singh NP. (2010): Resistant Acinetobacter significance and control. J Global Infect Dis. 2:291-304. DOI: 10.4103/0974-777X.68538.

[8] Doughari, H.J.; Ndakidemi, P.A.; Human, I.S. and Benade, S. (2011): The ecology, biology, and pathogenesis of Acinetobacter spp. an overview. Microbes and Environments/JSME, 26(2): 101-112. DOI: 10.1264/jsme2.me10179.

[9] Asif M., Alvi I.A., Rehman S.U. (2018): Insight into Acinetobacter baumannii: pathogenesis global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infect Drug Resist. 11:1249-60. DOI: 10.2147/IDR.S166750.

[10] Moreira Silva G, Morais L, Marques L, Senra V. (2012): Acinetobacter community-acquired pneumonia in a healthy child. Rev Port Pneumol. 18:96-8. DOI: 10.1016/j.rppnen.2011.12.005.

[11] Tacconelli E and Magrini N. (2018): Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. World Health Organization (WHO). DOI: 10.1016/S1473-3099(17)30753-3.

[12] Centers for Disease Control (CDC). (2018): Available from: www.cdc.gov/drugresistance/biggest_ treat.html.

[13] Amorim AM, Nascimento JD. (2017): Acinetobacter: an underrated foodborne pathogen? J Infect Dev Ctries. 11:111-4. DOI: 10.3855/jidc.8418.

[14] Ramos GLPA, Nascimento JS. (2019): Characterization of Acinetobacter spp. from raw goat milk. Cienc Rural. 49: e20190404. https://doi.org/10.1590/0103-8478cr20190404.

[15] Jain, R., and L. H. Danziger. (2004): Multidrug-resistant Acinetobacter infections: An emerging challenge to clinicians. Ann. Pharmacother. 38:1449–1459. DOI: 10.1345/aph.1D592.

[16] Gallego, L., and K. J. Towner. (2001): Carriage of class 1 integrons and antibiotic resistance in clinical isolates of Acinetobacter baumannii from northern Spain. J. Med. Microbiol. 50:71–77. DOI:10.1099/0022-1317-50-1-71.

[17] Jayarao, B. M., and L. Wang. (1999): A study on the prevalence of gram- negative bacteria in bulk-tank milk. J. Dairy Sci. 82:2620–2624. DOI: 10.3168/jds. S0022-0302(99)75518-9.

[18] Ndegwa E. N., Mulei C. M., and Munyua S. J. (2001): Prevalence of microorganisms associated with udder infections in dairy goats on small-scale farms in Kenya. J. S. Afr. Vet. Assoc. 72:97–98. DOI: 10.4102/jsava. v72i2.627.

[19] Nam H.M., Lim S.K., Kang H.M., Kim J.M., Moon J.S., Jang K.C., Kim J.M., Joo Y.S., Jung S.C. (2009): Prevalence and antimicrobial susceptibility of gram-negative bacteria isolated from bovine mastitis between 2003 and 2008 in Korea. J. Dairy Sci. 92:2020–2026. DOI: 10.3168/jds.2008-1739.

[20] Straley, B. A., Donaldson, S. C., Hedge, N. V., Sawant, A. A., Srinivasan, V., Oliver, S. P. (2006): Public health significance of antimicrobial-resistant gram- negative bacteria in raw bulk tank milk. Foodborne Pathog. Dis. 3, 222–233. DOI: 10.1089/fpd.2006.3.222.

[21] Kim C.U., Lee C. H., Kim J.H., Park Y.K., Shin S., Park K.T., Park H.Y. (2017): Prevalence and antimicrobial susceptibility of Acinetobacter baumannii in raw milk in Korea. Cheong Ung Kim et al. Appli Micro, 3:3 (Suppl) DOI: 10.4172/2471-9315-C1-009.

[22] Cho G.S., Li B., Rostalsky A., Fiedler G., Rösch N., Igbinosa E., Kabisch J., Bockelmann W., Hammer P., Huys G. (2018): Diversity and Antibiotic Susceptibility of Acinetobacter Strains from Milk Powder Produced in Germany. Front. Microbiol. 9:536. DOI: 10.3389/fmicb.2018.00536.

[23] Temel A, Eraç B. Küresel bir tehdit: Acinetobacter baumannii, antimikrobiyal dirençte güncel durum ve alternatif tedavi yaklaşımları. Turk Hij Den Biyol Derg, 2020; 77(3): 367-378. DOI: 10.5505/TurkHijyen.2019.04764.

[24] Savcı Ü., Özveren G., Yenişehirli G., Bulut Y., Özdaş S., (2020): Klinik örneklerden izole edilen Acinetobacter baumannii suşlarının in- vitro duyarlılık durumları. Turk Journal of Clin and Lab. 2015; 6:24-9. https://doi.org/10.18663/tjcl.42209.

[25] Kanaan Ghaffoori M.H., et al. (2020): Drug resistance and virulence traits of Acinetobacter baumannii from Turkey and chicken raw meat. Comparative Immunology, Microbiology, and Infectious Diseases 70, 101451. DOI: 10.1016/j.cimid.2020.101451.