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# Probiotics in Goat Milk: A Promising Solution for Management of Drug-Resistant Acinetobacter baumannii

**Leila Fozouni[1]*,Maryam Yaghoobpour[2], Ania Ahani[3]**

1. Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran.
2. Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran.
3. Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran.

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***Correspondence:***

Leila Fozouni

Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran

lili_kia@yahoo.com

## Abstract

***Background and objectives:*** Acinetobacterisa genus of opportunistic pathogens that are commonly found in the environment. Given the unique ability of these bacteria to survive in the hospital, they are considered as one of the main causes of hospital-acquired infections.The emergence of multidrug-resistant Acinetobacter spp., particularly Acinetobacter baumannii has become a major health threat worldwide. In this study, we investigate antibacterial effects of probiotic isolates from goat milk on clinical isolates of A. baumannii.

***Methods:*** In this study, 100 clinical specimens were taken from patients hospitalized in six hospitals in the Golestan Province, north of Iran. Following isolation and identification of A. baumannii strains, antibiotic resistance patterns of the isolates were investigated using the Kirby-Bauer method according to the Clinical and Laboratory Standards Institute (CLSI-2015) guidelines. Probiotic bacteria in goat milk were isolated and identified by culture in MRS and M17 media and carbohydrate fermentation tests. Antibacterial effects of the probiotic bacteria against resistant A. baumannii isolates were evaluated using the agar well diffusion method.

***Results:*** Overall, 55% of the isolates were identified as A. baumannii. The highest resistance rates were observed against tobramycin (76.3%), mezlocillin (74.5%) and cefotaxime (74.5%). Resistance to levofloxacin, tetracycline, imipenem and minocycline was detected in 72.7%, 72.7%, 70.9% and 29.1% of the isolates, respectively. The most common probiotic isolates were Lactobacillus plantarum and Lactococcus piscium (30% each). The highest and lowest effects were exerted by Lactococcus lactis (34.54%) and Lactobacillus bulgaricus (3.63%), respectively.

***Conclusion:*** Our results demonstrate that the prevalence ofdrug-resistantA. baumannii strains is high in the hospitals. Given the promising antimicrobial effects of the isolated probiotic bacteria, goat milk can be recommended as an adjuvant therapy or an alternative to common antibiotics for improving treatment outcome of infections caused by drug-resistantA. baumannii.

**Keywords:** Acinetobacter, Goat milk, Probiotic, Nosocomial infection

## Introduction

Emergence of antibiotic-resistant microorganisms that cause nosocomial infections has become a major healthcare challenge (1). Acinetobacter is a genus of gram-negative coccobacilli from the Moraxellaceae family. Acinetobacter species, including Acinetobacter baumannii, are opportunistic pathogens that can cause both community-acquired and nosocomial infections, especially in intensive care unit and high-dependency unit patients (2,3). These bacteria have been isolated from various infections including ventilator-associated pneumonia, endocarditis, meningitis, skin and soft-tissue infections, urinary tract infection and prosthesis-related infections. A. baumannii has been isolated from diverse animal, human and environmental sources, but the control of its presence in healthcare settings is most importance (4,5). Until three decades ago, A. baumannii infections were treated effectively with conventional antibiotics (6,7), but, the recent emergence of multi-drug resistant A. baumannii strains has made them difficult to control (8). Therefore, much attention has been paid to discovery of novel, effective and non-toxic alternatives to conventional antibiotics. Probiotics are an example of such compounds that lack the side effects of antibiotics. Probiotics create holes in the phospholipid bilayer of bacteria by disrupting the cytoplasmic membrane and generating the proton motive force. Also, they prevent the probable growth and metastasis of bacteria through immunologic and non-immunologic mechanisms (9,10). The purpose of this study was to investigate in vitro antibacterial activity of goat milk as probiotics source against A. baumannii.

## Materials and Methods

*Sampling and bacterial isolation*

This descriptive study was performed on 100 samples (blood, burn wound and trachea) from six hospitals in the Golestan Province, Iran. Before sampling, written informed consent was taken from all patients. Thesamples were cultured on blood agar and MacConkey agar. A. baumannii strains were identified by colony morphology, gram staining and biochemical tests including oxidase, catalase, citrate, motion, TSI, indole, methyl red, Voges–Proskauer andcarbohydrate fermentation test.

*Antimicrobial susceptibility testing*

Antimicrobial susceptibility was evaluated using the disc diffusion method (Kirby-Bauer method). First, a suspension (equivalent to a 0.5 McFarland standard) was prepared from an overnight culture of Acinetobacter spp. isolates. After incubation at 37°C, the isolates were cultured on Mueller Hinton agar (Merck, Germany) using sterile swabs. Levofloxacin (5 µg), minocycline (30 µg), cefotaxime (30 µg), tobramycin (10 µg), mezlocillin (75 µg), imipenem (10 µg) and tetracycline (30 µg) disks were purchased fromPadtan Teb Co. (Tehran, Iran). The antibiotics disks were placed on the culture medium. After 18-24 hours of incubation at 37 °C, results were interpreted as resistant, intermediate and susceptible by measuring the diameter of inhibition zone according to the Clinical and Laboratory Standards Institute guidelines (CLSI-M100-S25) (11).

*Isolation of probiotic bacteria*

Milk samples were collected from 1-3 years old Pakistani-Turkmen goats. The milk samples were cultured on MRS agar and M17 agar (Merck, Germany) and incubated at 37 °C

for 48 hours under anaerobic conditions. Bacterial species were identified based on colony morphology, gram staining, catalase test and ability to ferment cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, melezitose, ribose and trehalose.

In order to isolate antimicrobials, several colonies of lactic bacteria isolated from goat milk were inoculated into tubes containing M17 broth and MRS broth. After addition of paraffin to the media, the tubes were incubated at 37 °C for 4 days. Next, paraffin was removed and the tubes were centrifuged at 3500 rpm for 10 minutes. Sediment was removed under sterile conditions and supernatant containing bacterial metabolites was stored for future use.

*Evaluation of antimicrobial activity of probiotic isolates*

The agar well diffusion method was used to evaluate antimicrobial activity of the probiotic isolates. First, a suspension (equivalent to a 0.5 McFarland standard; 1.5 × 108 CFU/mL) from all antibiotic-resistant A. baumannii isolates was prepared in physiological saline solution and then cultured on Muller Hinton agar. Next, wells (7mm diameter) were created on the culture medium and 100 µl of the bacterial suspension were added to each well. A well containing distilled water was considered as negative control. The plates were incubated at 37 °C for 24 hours. A growth inhibition zone diameter of ≥15 and ≤12 mm indicated susceptibility and resistance, respectively. Moreover, A. baumannii ATCC 19606 was used as a positive control.

## Results

Overall, 55% of the isolates were identified as A. baumanni. The frequency of A. baumanni isolates was highest in men (76.4%) and patients aged 50 years and older (60%). The rate of antibiotic resistance among A. baumanni was highest against tobramycin (76.3%) and lowest against minocycline (29.1%) (Figure 1).

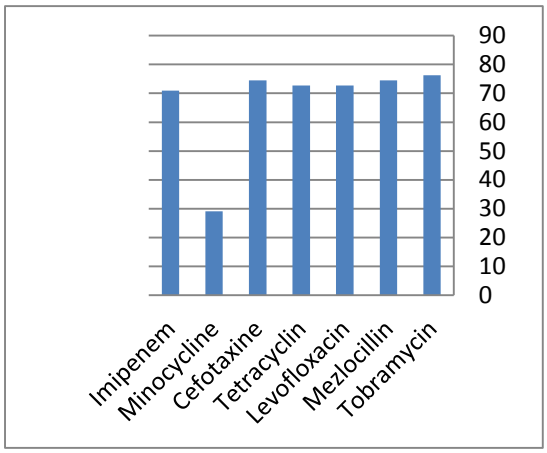


**Figure 1.** Relative frequency of antibiotic resistance among A. baumanni isolates

According to the results of the carbohydrate fermentation tests, Lactobacillus plantarum and Lactococcus piscium were identified as the probiotic strains in milk samples from three-year-old goats. Lactobacillus bulgaricus and Lactococcus lactis were identified as the probiotic strains in milk samples from two-year-old goats. L. plantarum, Lactococcus raffinolactis and L. piscium were identified as the probiotic strains in milk samples from one-year-old goats (Figure 2).

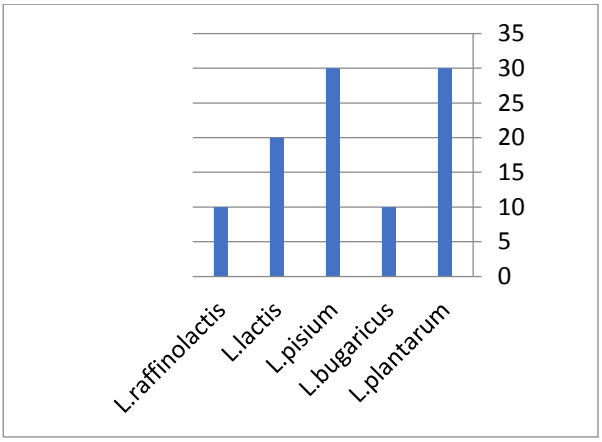


**Figure 2.** Relativeabundance of the probiotic strains isolated from goat milk**.**

According to results of the agar well diffusion assay, L. lactis exerted the strongest antibacterial activity against A. baumanni isolates (Table 1).

**Table 1**. Antibacterial activity of the probiotic isolates from goat milk against drug-resistant A. baumanni

| | *L. lactis* | | *L. piscium* | | *L. raffinolactis* | | *L. plantarum* | | *L. bulgaricus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % | No | % |
| **Grown** | 36 | 65.45 | 44 | 80 | 49 | 89.09 | 46 | 83.63 | 53 | 96.36 |
| **Inhibited** | 19 | 35.45 | 11 | 20 | 6 | 10.90 | 9 | 16.36 | 2 | 3.63 |
| **Total** | 55 | 100 | 55 | 100 | 55 | 100 | 55 | 100 | 55 | 100 |
| **P-Value** | 0.01 | | 0.01 | | 0.04 | | 0.036 | | 0.05 | |

**Table 2.** Mean diameter of growth inhibition zones caused by probiotic isolates around drug-ResistantA.baumanii[a,b]

| Probiotics | Mean ±SD |
|---|---|
| *L. bulgaricus* | 15.5±4.01 |
| *L. plantarum* | 16.3±3.90 |
| *L. lactis* | 18.6±0.03 |
| *L. piscium* | 17.4±0.00 |
| *L.raffinolactis* | 15.8±0.00 |
| [a] Diameterof theInhibitionZone,mm. [b] Pvalue< 0.01. | |

## Discussion

Management of nosocomial infections caused by Acinetobacter species has become challenging due to the recent emergence and spread of drug-resistant strains. A. baumannii is one of the most clinically important Acinetobacter species that can cause a wide range of infections (12). The purpose of our study was to evaluate the antagonistic activity of probiotic isolates from goat milk against drug-resistant A. baumannii isolates. Of 100 clinical specimens collected from patients in intensive care and high dependency units, 55 were identified as A. baumannii. In the antibiotic susceptibility test, we observed high resistance rates against tobramycin (76.3%), mezlocillin (74.5%), cefotaxime (74.5%), levofloxacin (72.7%), tetracycline (72.7%), imipenem (70 .9%) and minocycline (29.1%).

In a previous study in Iran, the prevalence of imipenem- and ciprofloxacin-resistant A. baumannii isolates was 40.9% and 77.7%, respectively (13). A more recent study in Iran reported high rates of resistance against cefotaxime (93%), cefipime (91%), norfloxacin (87 %), imipenem (86%) and tobramycin (67%) among A. baumannii clinical isolates (14). These results indicate the rising prevalence of resistant A. baumannii strains in recent years. According to a systematic review, the frequency of carbapenem-resistant A. baumannii strains increased in 2010-2013, while the rate of resistance to aminoglycosides did not change significantly (15). A 10-year survey on the prevalence of drug-resistant A. baumannii strains between 2004 and 2014 demonstrated a 64% increase in the frequency of imipenem-resistant strains and a 49% increase in the frequency of extensively drug-resistant strains in 2014 (16). These findings highlight the eminent need for discovery of novel antimicrobial agents against drug-resistant bacteria. In this regard, probiotics have been suggested as promising and economically feasible alternatives to conventional antibiotics. In the present study, we isolated and identified L. bulgaricus, L. plantarum, L. raffinolactis, L. piscium and L. lactisfrom from Pakistani-Turkmen goat milk samples In 2004, a study in Algeria reported Streptococcus thermophilus, Lactobacillus helveticus, L. plantarum, Lactobacillus delbrueckii and Lactobacillus lactis as the most common lactic acid bacteria in raw milk samples from four goat breeds (17).

In a study in 2015, Lactobacillus fermentum (48%), Lactobacillus acidophilus (34%), Lactobacillus viridescens (8%), Lactobacillus brevis (5%) and Lactobacillus gasseri (4%) were detected in 40 milk samples collected from the Aarey Milk Colony in India (18). The results of these studies indicate the great diversity of probiotic bacteria in goat milk.

In the present study, L. bulgaricus and L. lactis exhibited the highest and lowest antagonistic activity against resistant A. baumannii isolates, respectively. Similar to our findings, a study in Mexico demonstrated the inhibitory activity of bacteriocinogenic probiotic bacteria from goat cheese against Staphylococcus aureus, Bacillus cereus, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Shigella flexneri, Serratia marcescens, Enterobacter cloacae and Klebsiella pneumoniae (19).

In 2004, a study confirmed antimicrobial activity of lactic acid bacteria against a standard strain of S. aureus (20). Among the probiotic bacteria isolated from goat milk, we

observed that Lactococcus spp. could exert a more promising antibacterial activity compared to Lactobacillus isolates against resistant A. baumannii strains.

## Conclusion

Our findings revealed the alarmingly high prevalence of drug resistance among A. baumannii clinical isolates from hospitals of the Golestan Province, Iran. Given the favorable antimicrobial activity of goat milk-derived probiotics against resistant A. baumannii isolates, we suggest conducting future studies on the potential antimicrobial activity of these probiotics or their metabolites against other clinically important bacteria.

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***Conflict of interest:***
The authors declare that there is no conflict of interest.

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