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Review Article

Probiotics as a Novel Approach Against *Acinetobacter baumannii* Urinary Tract Infections: A Mechanistic and Therapeutic Review

Dorna Rafighi[1*], Sina Samenezhad[2], Ali Khodadadeh Jigheh[3]

[1]Department of Microbiology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran
[2]Urology Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Department of Microbiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran

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***Corresponding author:**
Dorna Rafighi,
Email: dornarafighi2222@gmail.com

Abstract
Urinary tract infections (UTIs) have become a significant clinical problem, with *Acinetobacter baumannii* emerging as one of the major pathogens associated with UTIs. The increasing resistance of this organism to antibiotics has made treatment options considerably more difficult. This review examines probiotics as a potential preventive strategy against these infections. Existing literature describing the urinary microbiome remains limited and relies largely on studies of voided urine, which provide little clinical relevance, especially given that the urinary tract is colonized by a diverse microbiome, including lactobacilli, staphylococci, and streptococci, which serve as physical barriers to pathogens. However, multidrug-resistant *A. baumannii*, a gram-negative aerobic bacterium, has made current therapeutic approaches progressively ineffective. Probiotics are live microorganisms that confer protective effects via various mechanisms, such as competition for adhesion to the urinary tract mucosa and the production of antimicrobial substances. In vitro studies have demonstrated that certain strains of *Lactobacillus* and *Bifidobacterium* can also inhibit the growth and colonization of *A. baumannii*. However, clinical data are limited, and further studies are required to determine optimal doses, routes of administration, and the precise mechanisms involved. Taking all this information into account and bearing in mind the increasing drug resistance in *A. baumannii*, probiotic supplementation may represent a useful preventive and complementary therapeutic approach. These interacting factors may act synergistically to reduce the functional burden of these difficult-to-treat infections in clinical action.
Keywords: *Acinetobacter baumannii*, Urinary tract infections, Probiotics, Urinary tract microbial flora, Antibiotic resistance

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Introduction

Infection is a process in which harmful agents invade the body's tissues, leading to disease due to interactions between the invading agents and the host's defense mechanisms (1,2). Bacterial infections of the respiratory tract trail only behind urinary tract infections (UTIs), which affect approximately 150 million people worldwide each year. Studies have confirmed that UTI cases reach massive numbers annually, demonstrating why this infection ranks as a serious global health issue (3). The clinical presentation of UTIs varies according to the site of involvement. Apart from physical problems, these infections have significant adverse effects on patients' social and personal lives, reducing their quality of life (4-6). Members of the Enterobacteriaceae family are significant pathogenic agents in UTIs (7). Gram-negative pathogens with antibiotic resistance, including carbapenem-resistant Enterobacteriaceae strains, show a higher prevalence in healthcare facilities compared with community settings (8).

The aerobic Gram-negative bacterium *Acinetobacter baumannii* causes diverse clinical infections affecting the urinary tract, bloodstream, and soft tissues (9). There is an urgent medical need for effective therapeutic options because this bacterium is one of the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), a group characterized by mounting antimicrobial resistance. *A. baumannii* strains resistant to carbapenems were classified by the World Health

Organization (WHO) in 2018 as a top-priority pathogen in need of new antibiotic development (10,11). The spread of resistance genes among bacterial strains occurs through several genetic mechanisms, including plasmid transfer, integrons, transposons, and other mobile genetic elements (12,13). Multidrug resistance in *A. baumannii* has evolved progressively, substantially complicating antibiotic treatment approaches. Consequently, healthcare professionals increasingly use combination therapies because this microorganism has developed resistance to many antimicrobial drug classes. However, studies have demonstrated limited therapeutic success with these treatment approaches and an inability to significantly decrease patient mortality (13).

The urinary tract includes four major parts: the kidneys, ureters, bladder, and urethra. The urethra contains a primarily bacterial profile that incorporates members of the Lactobacillaceae family, coagulase-negative *Staphylococcus* species, viridans and non-hemolytic *Streptococcus* strains, *Lactobacillus* species, diphtheroid bacteria, commensal *Neisseria*, coccobacilli, *Mycoplasma*, select anaerobic organisms, and occasional *Saccharomyces* yeast populations (14-16). These resident microbial communities, particularly *Lactobacillaceae*, *Lactobacillus*, *Staphylococcus*, and *Gardnerella* species, play a crucial protective role in bladder health by inhibiting pathogenic overgrowth and maintaining urinary system equilibrium. Contemporary investigations are actively examining dynamic alterations in urinary microbiota composition, with particular focus on the transition between balanced (eubiosis) and imbalanced (dysbiosis) microbial states. Such research aims to elucidate the microbiota's involvement in the pathogenesis of UTIs, as well as its potential connections with various urological conditions, including incontinence and malignancies (17).

To qualify as probiotic bacterial strains, the Food and Agriculture Organization (FAO) outlines key requirements, including viability within the intestine, proliferative ability, and beneficial host effects derived from metabolism and growth, while exhibiting no toxicity or pathogenicity, no harmful pathogen inhibition mechanisms, and resistance to antibiotic gene transfer. Current probiotic research aims to produce innovative formulations through purposeful utilization of mobile genetic elements to obtain strains with improved functionality (18). The primary use of probiotics in modern practice relies on specific lactic acid bacteria (LAB) strains from *Lactobacillus* and *Bifidobacterium* genera, while researchers have begun to investigate *Saccharomyces* yeasts for novel applications. The protective effects of immunomodulatory probiotic strains, known as immunobiotics, act by regulating both mucosal and systemic immune mechanisms to enhance disease resistance (18,19). Three major protective mechanisms by which probiotics support urinary tract health include (*i*) antimicrobial compound production, (*ii*) modulation of host defense systems, and (*iii*) competition with uropathogens for attachment sites on the urinary tract epithelium (20).

This comprehensive analysis evaluates recent advances in the use of probiotics to prevent *A. baumannii*-related UTIs. The analysis is structured around four investigative dimensions, starting with modification of the urinary microbiota through probiotic interventions, followed by their antimicrobial mechanisms, available clinical evidence supporting therapeutic efficacy, and prospective future clinical applications. This work evaluates the potential benefits of probiotic use in improving urological health while reducing the severity of multidrug-resistant (MDR) *A. baumannii* infections.

Urinary Tract Microbiota

Scholarly investigation into the urinary microbiota, comprising diverse microbial communities present in bladder urine, continues to expand (21,22). Scientific understanding of human urinary microbial communities remains insufficient, as much of the existing published research focuses on differences between male and female microbial profiles. Comprehensive research involving large-scale studies of urinary microbiome profiles in the general population and in clinical patients, including those with systemic disorders, requires carefully controlled study designs to advance understanding in this field. The microbial content of urine is significantly lower than that observed in other body sites, notably the gastrointestinal tract (21). Taxonomic analyses using 16S rRNA sequencing demonstrate that males and females display similar phylum-level distributions, with Firmicutes prevailing in males (65%) and females (73%). The urinary microbiome also contains Actinobacteria at 15% in males and 19% in females, Bacteroidetes at 10% in males and 3% in females, and Proteobacteria at 8% in males and 3% in females, as depicted in Figure 1 (23).

Bacterial genera show extensive overlap between sexes within the urinary microbiome, as they persist in both male and female populations. Several bacterial community members, including *Prevotella*, *Escherichia*, *Enterococcus*, *Streptococcus*, and *Citrobacter*, represent common taxa shared between male and female subjects. Men tend to exhibit higher levels of *Pseudomonas* infections more exclusively. Urobiome composition shows major sex-based variations by affecting bacterial population distributions instead of altering the presence or absence of specific microorganisms. The male urinary microbiota is dominated by *Corynebacterium* and *Streptococcus* species, whereas *Lactobacillus* species are present at higher levels in female urinary ecosystems (24).

In healthy women, the most prevalent genus within the urinary microbiome is *Lactobacillus*, but its association with health varies depending on the species present. For example, *Lactobacillus crispatus* is associated with urinary health, while *Lactobacillus gasseri* has been linked to pathological conditions such as urge urinary incontinence (21). A reduction of *Lactobacillus* populations is associated

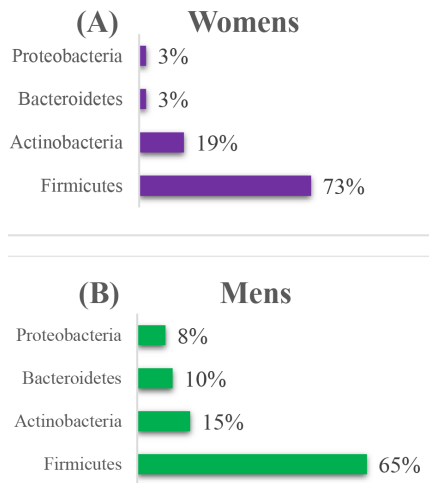

Figure 1. Comparative Urine Microbiome Composition at the Phylum Level in Men and Women. A comparative bar graph illustrating urine microbiome composition at the phylum level in men and women. *Firmicutes* was the most abundant phylum, both in men and women. Other phyla, including *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*, differed in men when compared to women. Overall, the urine microbiome composition of men and women is largely similar, with only slight differences observed

with pathological status, as this decline creates ecological space for pathogenic colonization by uropathogens (25). *Gardnerella*, the second most common genus in the urinary microbiome after *Lactobacillus*, is characterized by *Gardnerella vaginalis*. Some strains of this species are pathogenic and can induce UTIs, which are more frequently observed in women (26). Various urotypes have been identified in the urinary microbiome, which are named based on the dominant genus, including *Prevotella*, *Sneathia*, *Gardnerella*, *Atopobium*, *Lactobacillus*, *Shigella*, *Escherichia*, *Enterococcus*, *Streptococcus*, and *Citrobacter*. Dominant urotypes such as *Lactobacillus crispatus*, *Gardnerella vaginalis*, and *Atopobium vaginae* are specifically associated with healthy women, while other urotypes are linked to diseases (21, 27).

The male urinary microbiome, especially in healthy individuals, is considerably less characterized than the female urinary microbiome. Early studies described the male urinary microbiome using midstream urine samples and urethral swabs from individuals who were either positive or negative for sexually transmitted infections. Nevertheless, the application of 16S rRNA gene sequencing has allowed more precise characterization of the male urinary microbiome in healthy populations, as well as the identification of disease-related dysbioses (21, 28). Urotypes such as *Prevotella*, *Shigella*, *Enterococcus*, *Streptococcus*, and *Citrobacter*, which are prevalent in the female urinary microbiota, have also been reported in men (27). Although *Lactobacillus* is the most dominant genus in the female urinary microbiota, the most common genera in the male urinary microbiota are *Corynebacterium* and *Streptococcus* (21,24). The male urinary microbiota, dominated by *Corynebacterium* and *Streptococcus,* also includes the genera *Lactobacillus* and *Pseudomonas* (29). *Staphylococcus haemolyticus* is a group that is commonly found in the urinary flora of healthy males (30).

Acinetobacter baumannii and Urinary Tract Infections

Patients admitted to the intensive care unit represent the primary group affected by *A. baumannii*, which is recognized as a significant nosocomial Gram-negative coccobacillus pathogen. The growing incidence of MDR infections requires detailed microorganism studies to develop targeted management approaches that will help effective patient treatment (31). Morphologically, *Acinetobacter* species are pleomorphic, non-motor, non-spore-forming organisms that appear as cocci and bacilli, stain Gram-negative, necessitate strict aerobic conditions for growth, and exhibit catalase positivity but oxidase negativity. The cytosine-guanine content of their genomes ranges from 39% to 47% within these microorganisms. When cultured on blood agar and trypticase soy agar plates at 37 ˚C, microorganisms produce colonies with a mucoid characteristic, smooth texture, and grayish-white color (32). Clinical evaluations of *A. baumannii* routinely identify the pathogen in samples obtained from different body sites, including skin injuries, respiratory specimens, bloodstream infections, and cerebrospinal fluid. The pathogen primarily manifests in healthcare-related settings, where it causes postoperative wound infections, ventilator-associated pneumonia, and catheter-linked UTIs (33,34). The emergence of MDR *A. baumannii* strains exhibiting multiclass antimicrobial resistance constitutes a major concern, as these strains are associated with severe clinical outcomes as well as heightened mortality worldwide (35).

Microorganisms develop antimicrobial resistance through three main mechanisms: membrane modification or increased efflux transporter activity that limits antibiotic entry, genetic modifications or enzymatic alterations of antibiotic target sites, and enzyme-dependent or chemical inactivation of antimicrobial agents (10,36). *A. baumannii* executes these resistance mechanisms with remarkable efficiency due to its exceptional genomic adaptability, as illustrated in Figure 2. This evolutionary advantage facilitates both rapid chromosomal mutations and horizontal acquisition of resistance determinants through mobile genetic elements, especially insertion sequences (37,38).

Probiotics and Their Mechanisms of Action

Probiotics are live bacterium-containing dietary supplements that, when used appropriately, exert beneficial effects on the host. Except a limited number of yeasts, such as *Saccharomyces boulardii*, they are most commonly composed of LAB, including *Lactobacillus* and *Bifidobacterium* species. Probiotics are considered beneficial, among other reasons, for their capability to prevent and treat UTIs through multiple mechanisms. By competing with pathogenic bacteria for adhesion sites on the urinary tract mucosa, they help keep *A. baumannii* in check. Probiotics also produce substances such as

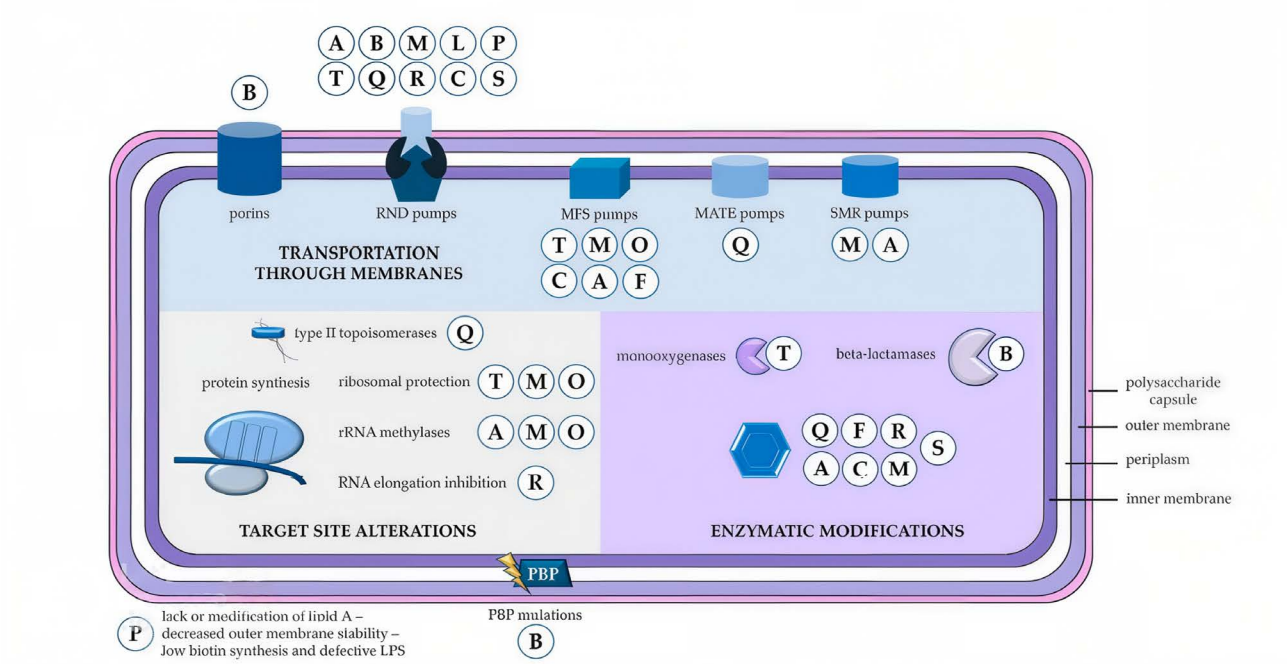

Figure 2. Mechanisms of *Acinetobacter baumannii* Antibiotic Resistance. Antibiotic resistance in *Acinetobacter baumannii* may result primarily from three mechanisms: (1) blocking the entry of an antibiotic into the cell due to membrane alteration (reduced porin permeability or enhanced efflux), (2) alteration of antibiotic target structures, and (3) enzymatic inactivation of the antibiotic. *Note.* A: Aminoglycosides; B: Beta-lactams; C: Chloramphenicol; F: Fosfomycin; L: Lincosamides; M: Macrolides; MATE: Multidrug and toxic compound extrusion; MFS: Major facilitator superfamily; O: oxazolidinones; P: Polymyxins; PBP: Penicillin-binding protein; Q: Fluoroquinolones; R: Rifampicins; RND: Resistance nodulation cell division; S: Diaminopyrimidines and sulfonamides; SMR: Small multidrug resistance family; T: Tetracyclines (10)

hydrogen peroxide, organic acids, and bacteriocins that not only suppress the multiplication of these disease-causing bacteria but also help maintain the microbial ecosystem balance of the urinary tract (39).

Modulation of the host immune system through increasing innate and acquired immune responses, along with increased synthesis of anti-inflammatory cytokines and immunoglobulin A (IgA), strengthens urinary tract mucosal defense against infection. Strengthening of the mucosal barrier through increased mucin synthesis prevents invasion of pathogenic bacteria into underlying tissues. Shaping the urinary tract microbiota by increasing its balance and diversity favors the development of beneficial bacteria and inhibits the formation of pathogenic bacteria. Additionally, competition for substrate and pH buffering within the urinary tract further inhibits infection. However, the effectiveness of probiotics is strain-specific, dose-dependent, and mode-dependent, and the selection of appropriate strains with desired characteristics is critical to achieving maximum benefit (40).

Probiotics and the Prevention of Urinary Tract Infections Caused by *Acinetobacter baumannii*

UTIs due to *A. baumannii* are increasingly common in hospital settings. Antibiotic treatment is further complicated by the biofilms formed by these bacteria. Probiotics are a complementary and alternative medicine approach that is being intensively studied due to their diverse and safe mechanisms of action. Laboratory data indicate that bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium* can inhibit the growth of *A. baumannii* by competing for receptors on urinary bladder epithelial tissues and producing antimicrobial agents. Research indicates that probiotics may interact with *A. baumannii* by reducing its binding to host substrates, decreasing biofilm formation, and increasing bacteriocin production. Small-scale clinical trials also suggest that probiotic use may reduce chronic UTIs caused by *A. baumannii* in hospitalized patients. These studies demonstrate that vaginal or oral probiotic administration may lower infection risk by altering urinary tract microbial flora and modulating mucosal immunity (41,42).

Oral probiotics, particularly strains from the *Lactobacillus* and *Bifidobacterium* genera, represent a promising approach for preventing UTIs, including those caused by MDR pathogens such as *A. baumannii*. These probiotics influence the interconnected gut-vagina-urinary axis, thereby modulating the urogenital microbiota. Following oral administration, probiotic strains initially colonize the gastrointestinal tract and subsequently migrate to the vaginal and urethral mucosa via the perineal route. This enables them to compete with uropathogens for adhesion sites on mucosal epithelial cells, thereby preventing pathogen attachment and colonization. Additionally, probiotics produce antimicrobial metabolites, including organic acids such as lactic acid, hydrogen peroxide, and bacteriocins, which lower the local pH and create an environment unfavorable for bacterial growth, while also disrupting biofilm formation, a key factor in persistent

A. baumannii infections. Furthermore, oral probiotics modulate host immune responses by enhancing both innate and adaptive immunity; they promote the production of anti-inflammatory cytokines, secretory IgA, and mucins, thereby reinforcing mucosal barrier integrity and facilitating pathogen clearance. Although clinical evidence remains limited, emerging studies suggest that oral probiotic regimens can reduce UTI recurrence by restoring microbial balance (eubiosis) in the urogenital tract and may serve as adjuncts to conventional therapies. Nonetheless, further research is needed to optimize strain selection, dosing, and delivery methods to improve prevention strategies against resistant uropathogens (43).

Probiotics, with their diverse mechanisms of action, can be used as a novel strategy in the prevention of *A. baumannii* UTI. The mechanisms of action of probiotics against *A. baumannii* include direct competition for adhesion, production of specific antimicrobial substances, inhibition of biofilm formation, and immunomodulation of the host. However, the probiotic dose and route of administration for preventing such infections have yet to be defined, and further studies are required to delineate effective doses and administration routes. Clinical efficacy trials evaluating the prevention of *A. baumannii*-induced UTIs are limited, and probiotic efficacy is strain-, dose-, and route-dependent, with mechanisms of action against *A. baumannii* still not fully understood. Nonetheless, given the increasing antibiotic resistance of *A. baumannii* and the modest success of conventional therapies, the potential use of probiotics as a prophylaxis and adjunct therapy is extremely promising. Additional research is needed to establish appropriate probiotic strains, dosages, administration modes, and mechanisms of action against *A. baumannii* (13,44).

Conclusion

From a comprehensive analysis of the application of probiotics in preventing UTIs caused by *A. baumannii*, it can be concluded that this novel approach shows great promise in combating this drug-resistant pathogen. Probiotics, through different mechanisms including competition for adhesion to urinary tract epithelial cells, production of antimicrobial compounds, inhibition of biofilm formation, and modulation of the host immune system, can inhibit the growth and colonization of *A. baumannii*.

However, it should be noted that clinical trials in this area remain limited, and further studies are needed to determine the optimal dosage and mode of administration of probiotics and to identify effective strains. In addition, the precise mechanisms of action of probiotics against *A. baumannii* are not yet fully understood.

Despite these limitations, due to the increasing antibiotic resistance of *A. baumannii* and the lack of conventional treatments, the use of probiotics as a prophylactic and adjuvant therapy appears promising. Future studies focusing on appropriate probiotic strains, dosage, and route of administration, as well as investigation into their precise mechanisms of action against *A. baumannii*, will be essential to effectively utilize this new technique in the prevention and treatment of UTIs caused by this pathogen.

Authors' Contribution

Conceptualization: Dorna Rafighi.
Data curation: Sina Samenezhad.
Formal analysis: Sina Samenezhad.
Funding acquisition: Dorna Rafighi.
Investigation: Dorna Rafighi, Ali Khodadadeh Jigheh.
Methodology: Dorna Rafighi.
Project administration: Ali Khodadadeh Jigheh.
Resources: Ali Khodadadeh Jigheh.
Software: Sina Samenezhad.
Supervision: Ali Khodadadeh Jigheh.
Validation: Dorna Rafighi, Sina Samenezhad.
Visualization: Sina Samenezhad.
Writing–original draft: Dorna Rafighi.
Writing–review & editing: Dorna Rafighi, Ali Khodadadeh Jigheh.

Competing Interests

The authors declare no conflict of interests.

Ethical Approval

Not applicable.

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