

Antagonistic Activity of *Lactobacillus acidophilus* Against *Acinetobacter baumannii* Isolated from Feline Conjunctivitis in Baghdad, Iraq

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Abstract:

Background:

Acinetobacter baumannii is an opportunistic pathogen associated with nosocomial infections; it can cause ocular infections in animals and humans. In veterinary medicine, this bacterium has been increasingly reported in a variety of animal species, including companion animals, where it may lead to respiratory, conjunctivitis, urinary, and wound infections. In cats, *A. baumannii* has been implicated in conjunctivitis, often presenting with ocular discharge, redness, and discomfort. The growing concern lies in its ability to develop multidrug resistance, which limits therapeutic options. This situation underscores the need to investigate alternative antimicrobial strategies, such as the use of probiotic bacteria with potential inhibitory effects.

Aims:

This study aimed to isolate and identify *Acinetobacter baumannii* from feline conjunctivitis cases in Baghdad, Iraq, and to evaluate the antagonistic activity of *Lactobacillus acidophilus* against it in vitro

Results:

Out of 100 conjunctival swabs collected from cats between November 2024 and March 2025, *Acinetobacter baumannii* was isolated from 5 samples (5%). All isolates were oxidase-negative and catalase-positive, and identification was confirmed by the VITEK 2 system. *Lactobacillus acidophilus* obtained from commercial powder was successfully cultured and identified using the API 50 CHL system. Agar well diffusion assays revealed clear inhibition zones around the wells containing *L. acidophilus*, indicating a significant antagonistic effect against *A. baumannii*

Conclusions:

This study successfully achieved its primary objective of isolating *Acinetobacter baumannii* from cases of feline conjunctivitis. The findings further highlight the potential application of *Lactobacillus acidophilus* as a probiotic agent against multidrug-resistant *A. baumannii*.

Keyword: *Acinetobacter baumannii*, antagonistic activity, feline conjunctivitis, *Lactobacillus acidophilus* VITEK 2.



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Introduction:

Acinetobacter baumannii is a Gram-negative, aerobic, non-motile, oxidase-negative, catalase-positive, and non-fermentative coccobacillus belonging to the family **Moraxellaceae** (**Hussein & Mahdi, 2023; Noor Alhuda & Mahmood, 2024**). Its identification requires careful differentiation from other *Acinetobacter* species, often relying on molecular techniques such as 16S rRNA sequencing (**Owaid & Abood, 2024**). In veterinary medicine, *A. baumannii* has gained significance for its multidrug resistance and association with infections like conjunctivitis, urinary tract infections, and pneumonia (**van der Kolk et al., 2019**). This bacterium is particularly concerning due to its multidrug resistance and ability to persist in hospital and clinical environments. In recent years, alternative therapeutic strategies have gained attention, especially the use of natural growth promoters such as organic acids and probiotics, which can enhance host health and mitigate the impact of resistant pathogens (**Abdulwahhab & Baker, 2023**). Among probiotics, lactic acid bacteria (LAB) like *Lactobacillus acidophilus* are widely recognized for their antimicrobial and immunomodulatory properties. *L. acidophilus* is a Gram-positive, non-spore-forming rod that ferments carbohydrates into lactic acid, thereby creating an acidic environment that inhibits the growth of various pathogens (**Khudhir, 2014**). *L. acidophilus* contributes to gut health and can inhibit pathogenic bacteria by producing antimicrobial substances and enhancing host immunity (**Najim, 2013; Al-khafaji, 2013; al-Qayim, 2014**). This study was conducted in vitro in Baghdad and aimed to isolate *Acinetobacter baumannii* from cases of feline conjunctivitis and to evaluate the antagonistic activity of *Lactobacillus acidophilus* against the isolated strains.

Materials and Methods: Samples collection

Between November 2024 and March 2025, 100 conjunctival swabs were collected from domestic cats diagnosed with conjunctivitis in three areas of Baghdad: Al-Dawrah, Al-Adhamiya, and Zayona. The samples were collected aseptically using medium Amies transport swabs and transported immediately to the laboratory in the College of Veterinary Medicine/University of Baghdad under cold chain conditions for isolation and identification

Isolation of *Acinetobacter baumannii*

By using sterile loop samples were cultured on Blood Agar, MacConkey Agar, and HiCrome™ *Acinetobacter* Agar (Himedia/India). Colony morphology, including color, size, and shape, was documented after incubation at 37°C for 24 hours (*ALYais & Al-Shammmary, 2024; Atlas & Snyder, 2006*).

Identification of *Acinetobacter baumannii* by Biochemical tests (Oxidase and Catalase tests)

The oxidase test was performed using the filter paper method, where a colony from nutrient agar was transferred using a wooden stick onto filter paper moistened with tetramethyl-p-phenylenediamine. A color change to violet within 5–10 seconds indicated a positive result; absence of color indicated a negative result (*Yunfan et al., 2023*). The catalase test was conducted using the slide method. A small portion of the colony was placed on a microscope slide, followed by the addition of 3% hydrogen peroxide. Immediate bubble formation indicated a positive catalase reaction (*Mustafa, 2014*).

Identification of *Acinetobacter baumannii* using the VITEK 2 System

All five Isolates were subjected to biochemical identification using the VITEK 2 Compact System. Colonies were suspended in sterile saline (0.5–0.63 McFarland standard) and inoculated into VITEK 2 GN cards. The system processed 64 biochemical reactions and provided results within 4–6 hours (*Pincus, 2006*).

Preparation of *Lactobacillus acidophilus*

Twenty milligrams of commercial *Lactobacillus acidophilus* powder (Swanson/USA) were inoculated into Man-Rogosa-Sharp broth (MRS) containing "Tween 80" and incubated under CO₂-enriched conditions for 72 hours. Colonies were confirmed by subculturing on MRS agar (*Abdul-Ratha et al., 2024*).

Microscopy and Identification of *L. acidophilus*

The colonies were collected with a sterile loop, transferred to a microscope slide, and then Gram-stained after fixation. The Gram reaction, cell arrangements, and shapes were observed using a light microscope with an oil immersion lens (100X) (*Padmavathi et al., 2018*).

Detection of *Lactobacillus acidophilus* by Analytical Profile Index 50 Carbohydrates for Lactic acid bacteria (API50 CHL test)

According to the manufacturer's instructions (Biomérieux, Marcy l'Étoile, France), *Lactobacillus acidophilus* and similar genera strips are identified using API 50 CHL in conjunction with API 50 Carbohydrates for Lactic acid bacteria (CHL) media (*Ghanbari et al, 2009*). (*Ozgun & Vural, 2011*).

Antagonistic Activity Evaluation:

The antagonistic effect was tested using the agar well diffusion method. A single colony of *A. baumannii* was picked from a fresh nutrient agar plate and suspended in sterile normal saline. The bacterial suspension was adjusted to a turbidity equivalent to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) using a spectrophotometer or by visual comparison and inoculated onto Mueller-Hinton Agar. Wells (6 mm) were created and filled with 100 μ L of *L. acidophilus* culture. After pre-incubation at 4°C for 2 hours to allow the diffusion of antimicrobial substances from *L. acidophilus* into the agar before bacterial growth begins, plates were incubated at 37°C for 24 hours, and inhibition zones were measured (Abbasi et al., 2024)

Ethical Approval

Ethical approval for this study was obtained from the Local Committee on Animal Care and Use at the College of Veterinary Medicine, University of Baghdad (Approval No. 581, dated 3/11/2024). Participation in the study required prior informed consent from the cat owners, who were provided with a clear explanation of the study's objectives. Verbal consent was also obtained before sample collection.

Results:

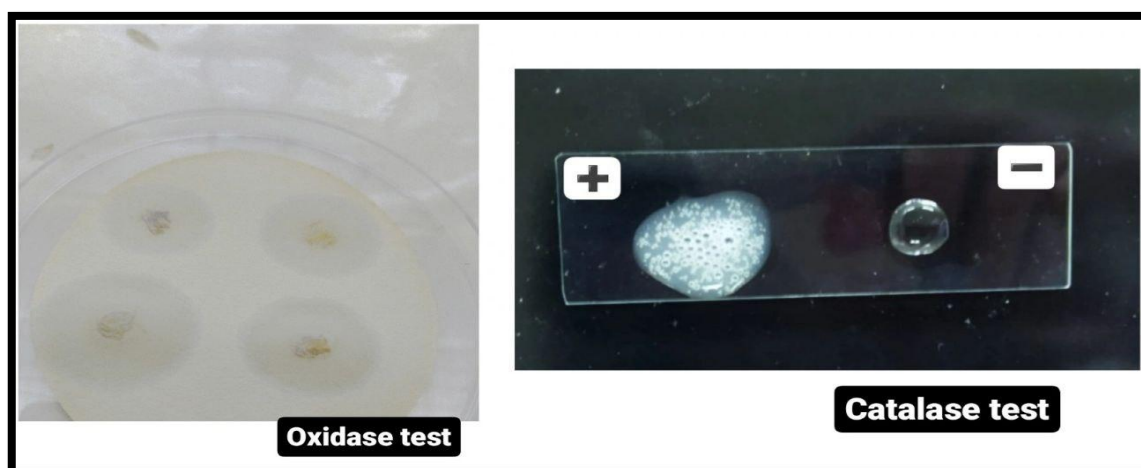
On blood agar, *A. baumannii* formed convex, gray to white colonies without hemolysis, indicating the absence of hemolysin production, on MacConkey agar, all isolates produced pale, round, non-lactose fermenting colonies and, on HiCrome agar, colonies appeared convex, circular, smooth, and light purple with a surrounding halo after 24 hours at 37°C, As shown in Figure (1)



"Figure (1)": culture of *A. baumannii* on a-blood agar, b-MacConkey agar, c-HiCrom agar

Biochemical test

All five (100%) *A. baumannii* isolates exhibited positive results for the Catalase test, while they all tested negative for the oxidase test. Figure(2)



"Figure (2)": Catalase and Oxidase tests of *A. baumannii*

Confirmation of *Acinetobacter baumannii* by VITEK 2

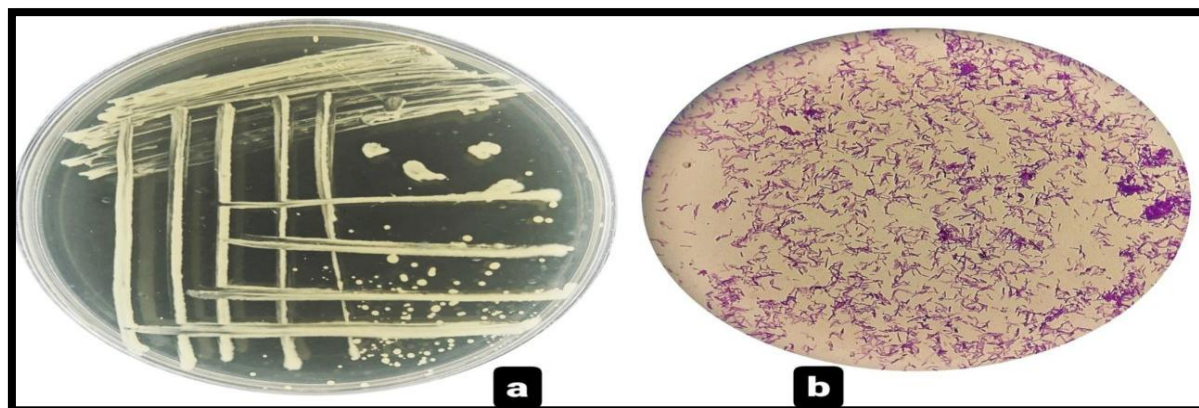
The VITEK 2 system accurately identified all five *A. baumannii* isolates, with an accuracy rate of up to 99%. The Gram-Negative Bacteria (GNB) identification card includes 64 biochemical tests, enabling precise profiling of Gram-negative specie Figure (3)

bioMérieux Customer:		Microbiology Chart Report		Printed November 10, 2024 4:41:36 PM AST	
Patient Name: Ayat Ali.				Patient ID: 7011	
Location:				Physician:	
Lab ID: 7011				Isolate Number: 1	
Organism Quantity:					
Selected Organism : <i>Acinetobacter baumannii</i> complex					
Source:				Collected:	
Comments:					
Identification Information		Analysis Time: 5.82 hours		Status: Final	
Selected Organism		99% Probability		<i>Acinetobacter baumannii</i> complex	
ID Analysis Messages		Bionumber:		0241010103500210	
Biochemical Details					
2	APPA	-	3	ADO	-
10	H2S	-	11	BNAG	-
17	BGLU	-	18	dMAL	-
23	ProA	-	26	LIP	-
33	SAC	-	34	dTAG	-
40	ILATk	+	41	AGLU	-
46	GlyA	-	47	ODC	-
58	O129R	+	59	GGAA	-
4	PyrA	-	5	IARL	-
12	AGLTp	+	13	dGLU	+
19	dMAN	-	20	dMNE	+
27	PLE	-	29	TyrA	+
35	dTRE	+	36	CIT	+
42	SUCT	+	43	NAGA	-
48	LDC	-	53	IHISa	-
61	IMLTa	-	62	ELLM	-
7	dCEL	+	9	BGAL	-
14	GGT	-	15	OFF	-
21	BXYL	-	22	BAlap	-
31	URE	-	32	dSOR	-
37	MNT	+	39	SKG	-
44	AGAL	-	45	PHOS	-
56	CMT	+	57	BGUR	-
64	ILATa	-			

"Figure (3)": VITEK® 2 Compact identification results showing 99% probability of identification as *Acinetobacter baumannii*

Cultural and Microscopic Characteristics of *Lactobacillus acidophilus*

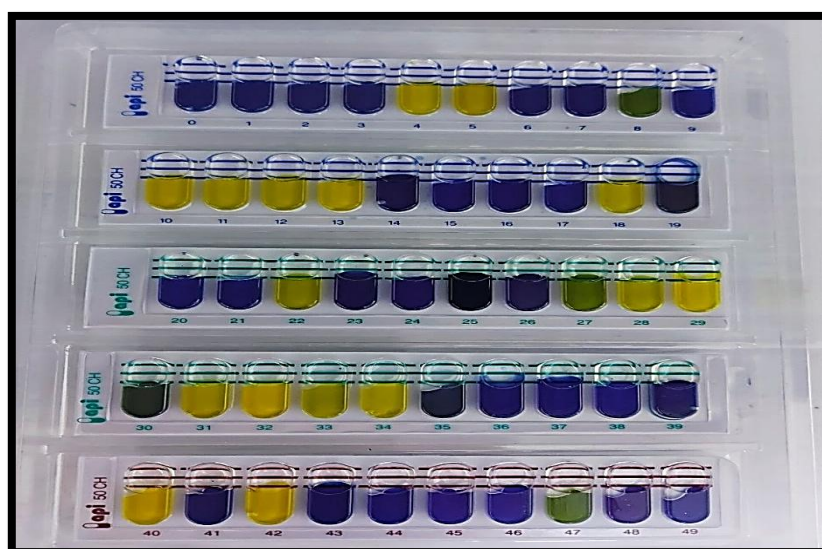
L. acidophilus isolates cultured on MRS agar under 5–10% CO₂ at 37°C for 24 hours formed white to milky, circular, smooth, and shiny colonies. Microscopic examination using Gram staining revealed Gram-positive bacilli, appearing violet to blue, arranged singly or in pairs.



"Figure (4)" : (a) Colonies of *L. acidophilus* on MRS agar; (b) Gram-positive bacilli-shaped *L. acidophilus* under light microscope(100X).

Confirmation of *L. acidophilus* by API50CHL (Carbohydrate Fermentation Profile System)

After 48 hours of incubation at 37°C under microaerophilic conditions, the isolate showed positive fermentation of several carbohydrates such as glucose, fructose, mannose, lactose, and maltose, while most sugar alcohols and polysaccharides yielded negative results. The profile matched the standard characteristics of *L. acidophilus*, with a high confidence level exceeding 99.9%.



"Figure (5)": API 50 CHL of *Lactobacillus acidophilus*

Antagonistic Effect of *Lactobacillus acidophilus* Against *Acinetobacter baumannii* in vitro

The agar well diffusion method was employed to assess the antagonistic activity of *Lactobacillus acidophilus* against *Acinetobacter baumannii*. Distinct and consistent inhibition zones were observed around the wells containing *L. acidophilus* across all tested plates (15-18mm), indicating its reliable antibacterial effect. The absence of *A. baumannii* growth in proximity to the wells suggests that *L. acidophilus* produces potent antimicrobial compounds capable of effectively inhibiting this pathogen. As illustrated in Figure 6



" Figure (6)": Inhibitory effect of *Lactobacillus acidophilus* against the growth of *Acinetobacter baumannii* in Muller Hinton agar.

Discussion

The results of this study demonstrated the successful isolation and identification of *Acinetobacter baumannii* from feline conjunctivitis cases using conventional culture media, biochemical tests, and the automated VITEK 2 system. The colonies displayed morphological and biochemical characteristics consistent with previous reports (Saikia *et al.*, 2024; Alyais & Al-Shammary, 2024), including oxidase negativity and catalase positivity (Anton *et al.*, 2008; Abdulhussein & Mahdi, 2023). The isolation rate of *A. baumannii* (5%) highlights its presence as an emerging ocular pathogen in companion animals, particularly in urban environments such as Baghdad. This finding aligns with (Holmström *et al.*, 2022), who emphasized the increasing detection of the *A. baumannii* in veterinary settings and its clinical importance due to multidrug resistance. Additionally, the API 50 CHL system confirmed the identity of *L. acidophilus*, supporting its reliability for characterizing lactic acid bacteria in veterinary microbiology (Ozgun & Vural, 2011).

In evaluating the probiotic potential of *Lactobacillus acidophilus*, the study showed significant inhibitory zones against *A. baumannii* in the agar well diffusion assay. This antagonistic effect is attributed to the production of antimicrobial metabolites, such as organic acids and bacteriocins, consistent with findings by (Al-Haik et al., 2023) and (Stanbro et al., 2020), who reported similar effects of *L. acidophilus* supernatants on multidrug-resistant bacteria. These findings support the potential use of *L. acidophilus* as a natural antimicrobial agent in veterinary ophthalmology, offering an alternative to conventional antibiotics in managing drug-resistant pathogens

Conclusions:

This study demonstrated that *Lactobacillus acidophilus* exhibits significant in vitro antagonistic activity against *Acinetobacter baumannii* isolates recovered from cases of feline conjunctivitis in Baghdad. The observed inhibition zones highlight the probiotic's potential as a natural antibacterial agent. These findings suggest that *L. acidophilus* could serve as a promising alternative or complementary approach to combat multidrug-resistant pathogens in ocular infections.

Recommendations:

Due to its promising in vitro antagonistic effect, *Lactobacillus acidophilus* may be included in upcoming veterinary treatments to manage eye infections in companion animals. Moreover, veterinary practitioners are advised to consider probiotic-based options as supportive or preventative treatments, especially in cases involving ocular pathogens resistant to multiple medications. In addition, regular microbiological testing for *A. baumannii* should be incorporated into diagnostic regimens for feline conjunctivitis, particularly in metropolitan regions with a high concentration of pets and frequent human–animal interaction. Such measures could enhance early detection and improve treatment outcomes in resistant infections.

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Conflict of Interest:

There is no Conflict of interest

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Authors Contributions:

There are no Author's Contributions

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