Original Article

Isolation and Molecular Characterization of Multidrug-resistant *Acinetobacter baumannii* From Cat Conjunctivitis in Baghdad Province, Iraq

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How to Cite This Article Hussein, A. A., & Hayyawi, S. M. (2026). Isolation and Molecular Characterization of Multidrug-resistant *Acinetobacter baumannii* From Cat Conjunctivitis in Baghdad Province, Iraq. *Iranian Journal of Veterinary Medicine, 20*(3), 553-562. http://dx.doi.org/10.32598/ijvm.20.3.1005861

doi http://dx.doi.org/10.32598/ijvm.20.3.1005861

ABSTRACT

Background: *Acinetobacter baumannii* is an emerging opportunistic pathogen implicated in various infections in both animals and humans. Its role in feline ocular infections, particularly conjunctivitis, has not been extensively studied, especially in Iraq.

Objectives: This study aimed to isolate and characterize *A. baumannii* from conjunctival swabs of cats with conjunctivitis in Baghdad Province, Iraq, and to assess the antimicrobial susceptibility of the isolates.

Methods: From November 2024 to March 2025, conjunctival swabs were collected from clinically infected cats presented to veterinary clinics specializing in pet care. Samples were cultured on HiCrome™ Acinetobacter Agar (M1938) supplemented with MDR selective supplement vials (FD271) for selective and differential isolation. Additional culturing was done on MacConkey and blood agar. Preliminary identification was performed using standard biochemical tests and confirmed using the VITEK 2 system. Antibiotic susceptibility was tested, and molecular confirmation was performed on selected multidrug-resistant (MDR) isolates using polymerase chain reaction (PCR) targeting the *16S rRNA* and *blaOXA-51*-like genes.

Results: Out of 100 conjunctival swab samples, 5(5%) yielded positive results for *A. baumannii*. Among them, two isolates exhibited higher resistance to commonly used antibiotics and were further confirmed by molecular detection methods.

Conclusion: This study reports the isolation and characterization of *A. baumannii* from feline conjunctivitis cases for the first time in Iraq. The findings highlight the presence of MDR strains in companion animals and emphasize the importance of antimicrobial stewardship in veterinary practice.

Keywords: *Acinetobacter baumannii*, Conjunctivitis, polymerase chain reaction (PCR), *16S rRNA*, *blaOXA-51*-like, Antimicrobial resistance

Article info:
Received: 07 Nov 2025
Accepted: 10 Jan 2026
Publish: 01 May 2026

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Introduction

Acinetobacter baumannii is the most common species within the *Acinetobacter* genus. It is a gram-negative, non-fermenting, coccobacillus bacterium with a genomic DNA (G+C) content ranging from 39% to 47% (Noor Alhuda & Mahmood, 2024; Wareth et al., 2019). Members of the *Acinetobacter* genus, belonging to the Moraxellaceae family, are widely distributed in various environments, such as soil, freshwater, oceans, and sediments (van der Kolk et al., 2019). *A. baumannii* is aerobic, non-motile, oxidase-negative, catalase-positive, indole-negative, and citrate-positive (AL-Juboori & Muhammad, 2025). This organism has emerged as a clinically significant pathogen due to its remarkable ability to acquire multidrug resistance (MDR) determinants and cause a wide range of infections in animals and humans. It is classified among the ESKAPE pathogens—*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species—known for their ability to "escape" the effects of antibiotics and complicate treatment (Hussein & Mahdi, 2024). The global rise of MDR pathogens, including *A. baumannii*, has created an urgent need for alternative therapeutic strategies (Lafta & Sadeq, 2024). *A. baumannii* is associated with nosocomial infections, including meningitis, septicemia, endocarditis, and wound and burn infections (Sehree et al., 2023). Its pathogenicity is supported by multiple virulence factors, such as adhesion molecules, outer membrane proteins, biofilm formation, and iron acquisition mechanisms, enabling adherence to host tissues and evasion of immune responses (Momtaz et al., 2015; Peleg et al., 2008). In recent years, the prevalence of MDR *A. baumannii* has raised global public health concerns.

Studies have highlighted the presence of antibiotic resistance genes and antiseptic resistance, which reduce the effectiveness of treatment (Ghasemzadeh-Moghaddam et al., 2025). The emergence of plasmid-mediated resistance, such as colistin and fosfomycin resistance in Enterobacteriaceae and non-fermenting bacteria, illustrates the potential for horizontal transfer of resistance determinants (Falsafi et al., 2024). These findings underscore the importance of monitoring resistance profiles and the role of *A. baumannii* as a reservoir of clinically relevant resistance genes.

Moreover, natural compounds have been evaluated as potential alternatives for combating resistant pathogens. For instance, *Leucas aspera* leaf extracts exhibited antimicrobial and anti-inflammatory activities against MDR bacteria, reflecting interest in novel therapeutic approaches to tackle antimicrobial resistance (Sungar et al., 2024).

Despite extensive research on *A. baumannii* in human medicine, information regarding its distribution, virulence, and antimicrobial resistance in veterinary medicine is limited. Reports of *A. baumannii* in companion animals, including cats, are scarce, although its role in conjunctivitis and other infections has been suggested (van der Kolk et al., 2019). This gap highlights the necessity to study *A. baumannii* in veterinary settings, particularly in pet-associated infections, and to characterize its antimicrobial resistance and genetic determinants. Therefore, this study aimed to isolate and molecularly characterize *A. baumannii* from cases of conjunctivitis in cats in Baghdad Province, Iraq, and to determine the antimicrobial susceptibility profile of the recovered isolates.

Materials and methods

Sample collection and bacterial isolation

Conjunctival swabs were collected from 100 cats diagnosed with conjunctivitis, representing both sexes (48 females, 52 males) and different breeds: 22 Himalayan, 30 Sherazi, 23 Indigenous, 1 Siamese, 2 Scottish, 11 Persian, 6 British, 3 Calico, and 2 Angora. These samples were obtained from veterinary clinics located in three areas: Al-Dawrah, Al-Adhamiya, and Zayona, between November 2024 and March 2025. The specimens were transported to the laboratory using Amies transport media and cultured on selective *A. baumannii* media (HiCrome™ agar), blood agar, and MacConkey agar, and then incubated at 37 ˚C for 24 hours under aerobic conditions (Athanasiou, et al., 2018; Rebekah & Christie, 2023). All microbiological and molecular experiments were conducted at the Postgraduate Laboratory, Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, ensuring accurate identification and further analysis of the isolates.

Identification

Cultural characteristics and microscopy

The cells were examined microscopically using oil immersion to observe the staining reaction and cell arrangement (Karen C & Patel, 2015).

Biochemical tests

A. baumannii Identification using conventional biochemical tests: oxidase test, urease test, motility test, oxidation–fermentation test (O-F test), catalase test (Jasim & Hayyawi, 2025), IMViC test (indole production test, methyl red test, Voges-Proskauer test, citrate utilization test), and triple sugar iron test (Hasso, 2018) were prepared according to previous studies (Fischbach & Dunning, 2009; MacFaddin, 2000). The VITEK2 compact system identified the suspected isolates and accurately identified each bacterial isolate with a 99% confidence level (Biomerieux, France).

Antimicrobial susceptibility test

Following the Kirby-Bauer method for assessing antibiotic susceptibility, five *A. baumannii* isolates were tested for their resistance against 10 discs for all classes of antibiotics Bioanalyse (Turkey) on Muller Hinton Agar, and were incubated at 37 °C for 24 hours. Antibiotics were chosen from the appropriate table of agents outlined in the relevant Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2023) document for testing against *Acinetobacter* spp., emphasizing those suitable for use in veterinary medicine. The following antimicrobial agents were employed in the study: amikacin (30 µg), ampicillin-Sulbactam (10 µg), cefepime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), colistin (10 µg), gentamycin (10 µg), levofloxacin (5 µg), and tobramycin (30 µg) (Lysitsas et al., 2023)especially during the last 30 years. Recent research in veterinary medicine also supports its emergence as an animal pathogen. However, relevant data are limited. In this study, we obtained 41 *A. baumannii* isolates from clinical samples of canine and feline origin collected in veterinary clinics in Greece between 2020 and 2023. Biochemical identification, antimicrobial susceptibility testing, molecular identification and statistical analysis were performed. Most of the samples were of soft tissue and urine origin, while polymicrobial infections were recorded in 29 cases. Minocycline was the most effective in vitro antibiotic, whereas high resistance rates were detected for almost all the agents tested. Notably, 20 isolates were carbapenem resistant and 19 extensively drug resistant (XDR).

Molecular identification

The molecular characterization of *A. baumannii* isolates was achieved through polymerase chain reaction (PCR), targeting the specific genes *16s rRNA* and *bla-OXA-51*-like (Qader, 2021).

DNA extraction

Genomic DNA was extracted using a commercially available purification kit from Favorgen Biotech Corp in Korea. The DNA retrieved from the *A. baumannii* isolates was assessed through spectrophotometric analysis, particularly utilizing a NanoDrop spectrophotometer (Razzaq, 2017).

Primers used to detect *16S rRNA* and *blaOXA-51-like*

The primers utilized for identifying *A. baumannii* using PCR, specifically targeting these genes, are found in Table 1.

Amplification of *blaOXA-51*-like and *16S rRNA* using PCR

A specific set of primers, detailed in Table 1, was employed to identify *A. baumannii* by targeting the *bla-OXA-51-like* and *16S rRNA* genes. DNA was extracted from *A. baumannii*, and a reaction mixture with a total volume of 25 µL was prepared for each gene. This mixture consisted of 1 µL of forward primer, 1 µL of reverse primer, and 5 µL of MasterMix. To reach the final volume of 25 µL, 1.5 µL of DNA was combined with 16.5 µL of nuclease-free water. The amplified fragments were subsequently separated using electrophoresis in a 1.5% agarose gel at 70 V for 2 hours. Finally, the fragments were stained with ethidium bromide and visualized under UV light. The PCR program was executed using a Thermal Block, as outlined in Table 2.

Table 1. Primers of *16S rRNA* and *BlaOxa51* genes with sequencing used to detect *A. baumannii*

Target Gene	Primer	Sequence (5'–3')	Annealing Temperature (°C)	Product Size (bp)
16s RNA	27F 1492R	AGAGTTTGATCCTGGCTCAG GGTTACCTTGTTACGACTT	56.92 52.20	1250
blaOXA-51-like	F R	TAATGCTTTGATCGGCCTTG TGGATTGCACTTCATCTTGG	56.48 56.01	353

Statistical analysis

SPSS software, was used for data analysis. The chi-square test was used to find significant differences at P<0.05 and P<0.01.

Results

Isolation and identification of *A. baumannii* from cats suffering from conjunctivitis

Between November 2024 and March 2025, a total of 100 conjunctival samples were collected from cats of various breeds and both sexes presenting with conjunctivitis. *A. baumannii* was successfully isolated from 5 cases. All five isolates were confirmed through culture and conventional biochemical tests. Among these, the two isolates exhibiting the highest resistance to multiple antibiotics were further confirmed using PCR molecular detection, ensuring accurate identification at the genetic level.

Bacterial culturing and colony morphology

After transportation to the laboratory, the samples were cultured on selective media specific for *A. baumannii*. On HiCrome agar, the bacterial colonies appeared circular, convex, smooth, and translucent to opaque, with a light purple coloration surrounded by a halo, following 24 hours of incubation at 37 ˚C. On MacConkey agar, all five *A. baumannii* isolates exhibited pale, round, non-lactose fermenting colonies. On blood agar, the colonies appeared convex and grayish or whitish, with no evidence of hemolysis, indicating the absence of hemolysin enzyme activity.

Microscopic examination

The microscopic examination of the bacterial smears showed gram-negative coccobacilli grouped in pairs or singly.

Table 2. Amplification program of primers

Phase	Temperature (˚C)	Time	No. of Cycles
Initial denaturation	95	5 min	1
Denaturation 2	95	45 sec	35
Annealing of *blaOXA-51-like* Annealing of *16S rRNA*	54 56	45 sec	
Extension 1	72	1 min	
Extension 2	72	5 min	1

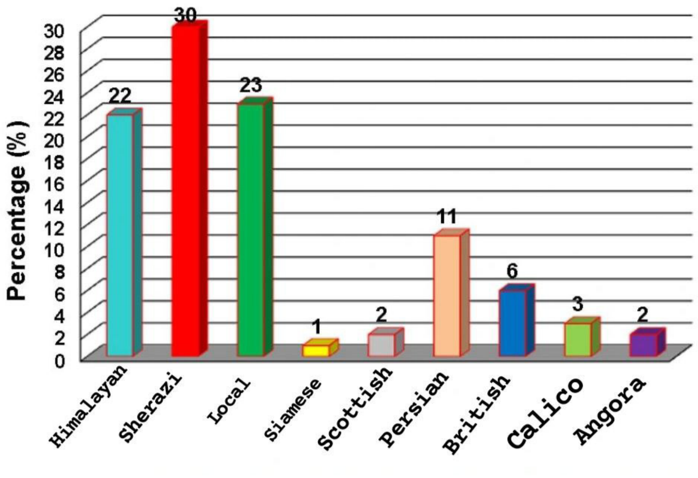

Figure 1. Percentage distribution of cat breeds included in the study

Note: The highest prevalence was observed in Shirazi cats (30%), followed by Local breeds (23%), Himalayan (22%), British (11%), Calico (6%), Scottish and Persian (2% each), and Angora (2%), while Siamese cats represented the lowest proportion (1%).

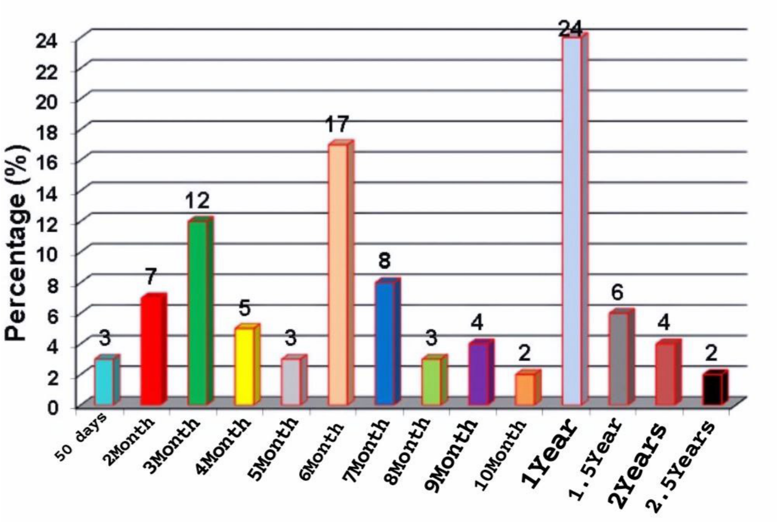

Figure 2. The percentage distribution of samples collected from clinically diagnosed cats with conjunctivitis according to age groups

Note: The most represented age group was cats aged 1 year (24%), and the lowest percentages were observed in the 10-month and 2.5-year age groups, each accounting for 2%.

Biochemical characterization

All five *A. baumannii* isolates tested positive for the catalase test, as evidenced by the formation of bubbles upon the addition of hydrogen peroxide (H_2O_2), while all were negative for the oxidase test. Furthermore, biochemical analyses revealed that none of the isolates produced indole, and both the Voges-Proskauer (VP) and methyl red (MR) tests yielded negative results. In contrast, all isolates showed positive results in the Simmons citrate test. Triple sugar iron (TSI) agar slants exhibited an alkaline over alkaline (K/K) reaction, indicating no glucose fermentation, along with the absence of gas and hydrogen sulfide (H_2S) production. The urease test yielded negative results, as indicated by the yellow coloration of the medium. The sulfide-indole-motility (SIM) test was also negative for all isolates, confirming the absence of H_2S production, indole formation, and bacterial motility. In the oxidative-fermentative (O/F) glucose test, the isolates only demonstrated oxidative metabolism, confirming their aerobic utilization of glucose.

Molecular diagnosis of isolates using *blaOXA-51-like* and *16S rRNA* genes

The two *A. baumannii* isolates exhibiting the highest antimicrobial resistance were selected for sequencing. The partial sequences of the *16S rRNA* gene were obtained using Sanger dideoxy sequencing. The resulting sequences were analyzed using the BLAST tool against the NCBI GenBank database to confirm species identity. Both sequences were submitted to and annotated in GenBank, receiving the following accession numbers: PQ269693.1 for isolate Ay.Sa.1 and PQ269694.1 for

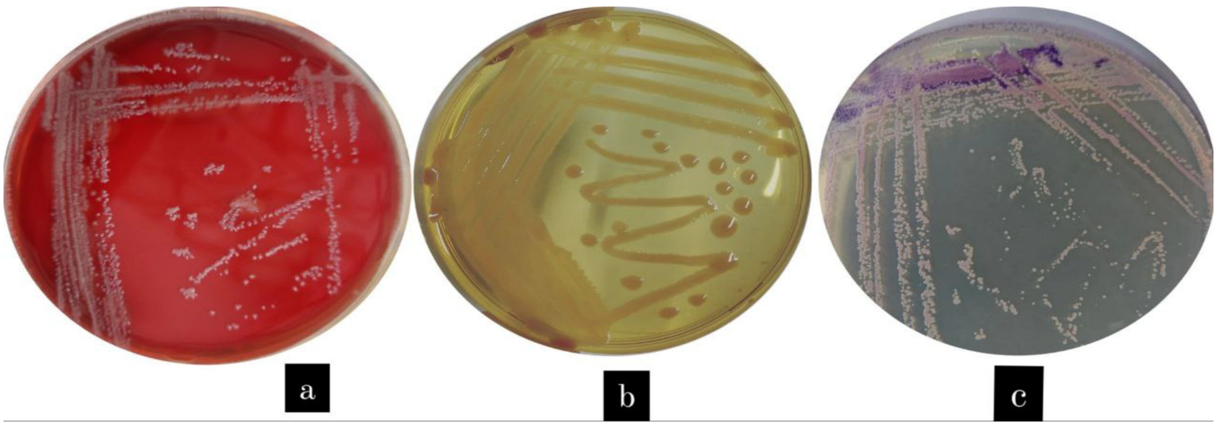

Figure 3. *A. baumannii* on (a) blood agar, (b) MacConkey agar, and (c) HiCrome™ acinetobacter agar (M1938)

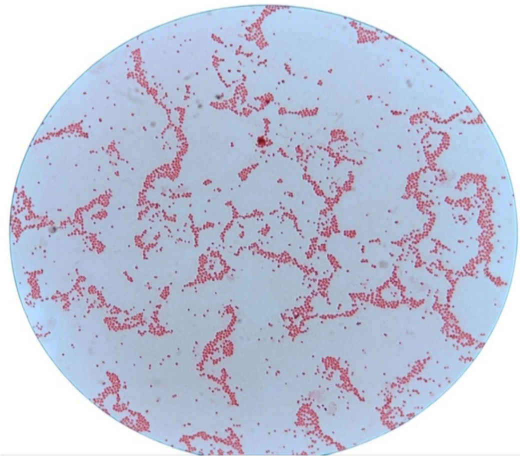

Figure 4. *A. baumannii* under a light microscope (×100)

isolate Ay. Sa.2, thereby confirming the reliability of the biochemical tests. Additionally, the PCR results for the *blaOXA-51*-like gene indicated that this gene was present in both isolates identified as *A. baumannii*. These findings suggest that molecular diagnostics may offer a more accurate and sensitive detection method compared to HiCrome agar, *Acinetobacter*, and conventional biochemical tests.

Antimicrobial susceptibility testing

The antimicrobial resistance patterns of the isolates revealed worrisome trends. All five isolates (100%) were completely resistant to ceftazidime, cefepime, ceftriaxone, colistin, and ampicillin-sulbactam, showing no zone of inhibition around the antibiotic discs. Furthermore, 2 out of 5 isolates (40%) showed resistance to ciprofloxacin, tobramycin, gentamicin, amikacin, and levofloxacin. The inhibition zones for these antibiotics in the resistant isolates were below the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2023) interpretive criteria for susceptibility. Table 5 illustrates the resistance patterns in detail, confirming that a significant portion of the isolates were MDR. This indicates the possible role of veterinary environments as reservoirs for resistant strains and the selective pressure exerted by overuse or misuse of antibiotics in pets.

Discussion

The detection of *A. baumannii* in 5% of feline conjunctivitis cases is a noteworthy epidemiological observation, underscoring the pathogen's capacity to act as an

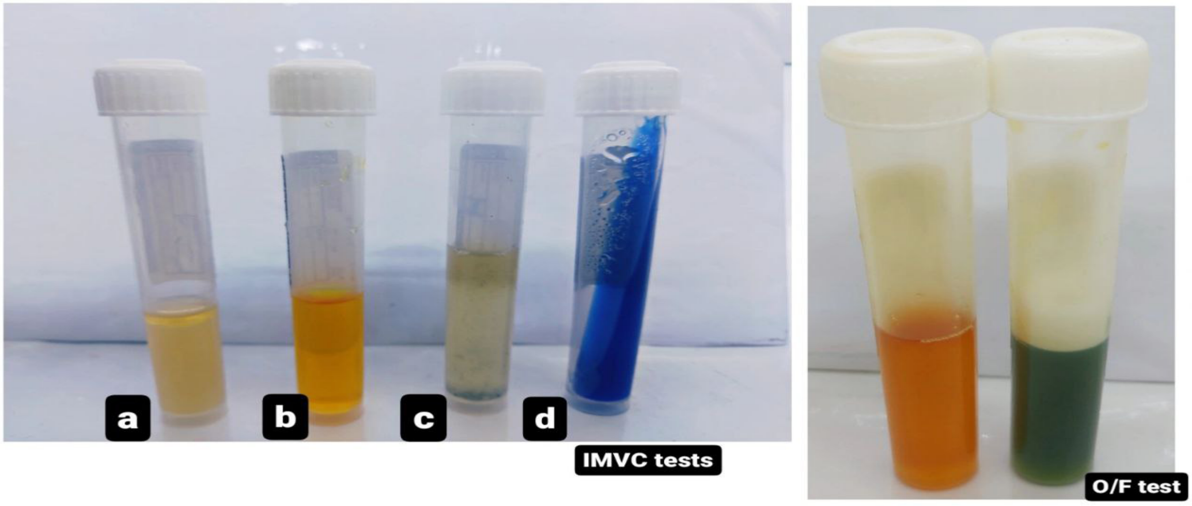

Figure 5. IMViC (a) Indole, (b) methyl red, (c) Voges-Proskauer, and (d) Simmons citrate) and oxidative fermentative (O/F) tests of *A. baumannii*

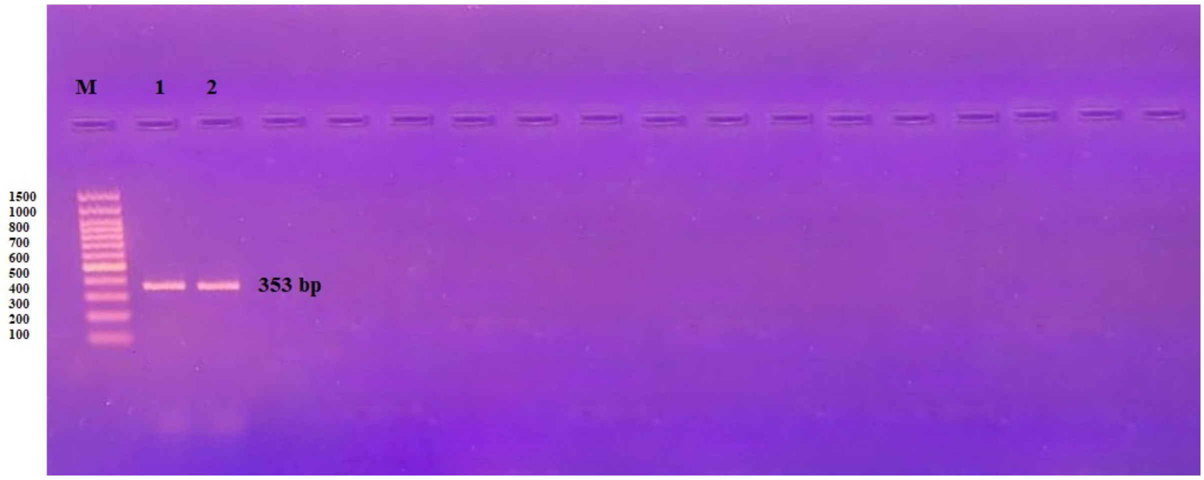

Figure 6. Ethidium bromide-stained agarose gel (1.5%) of PCR-amplified products from the extracted DNA of *A. baumannii* isolates and amplified with primers of the *16S rRNA* gene

Note: Lane M: DNA molecular size marker (l00 bp ladder). Lanes 1 and 2 show a positive result with positive bands of 1250 bp (5 V for 1.5 hr).

opportunistic infectious agent in the ocular environment of companion animals. This finding aligns with prior investigations, such as those conducted by Holmström et al. (2022), who documented the presence of *A. baumannii* in domestic animals, indicating its ecological versatility and ability to colonize non-human hosts. The observed colonial characteristics—namely, pale-to-pink colonies on HiCrome agar, lactose non-fermenting colonies on MacConkey agar, and the absence of hemolysis on blood agar—are consistent with standard morphological profiles previously described for *A. baumannii* (Alyais & Al-Shammary, 2024; Alshawi et al., 2019; Saikia et al., 2024). These phenotypic traits reinforce the reliability of conventional culturing techniques when coupled with accurate interpretation. Microscopically, the isolates were characterized as gram-negative, nonmotile coccobacilli exhibiting catalase positivity and oxidase negativity—features that concur with taxonomic descriptions provided by previous research (Peleg et al., 2008; Abdulhussein & Mahdi, 2023). The biochemical profiles, including an alkaline slant/alkaline butt (K/K) reaction on TSI agar, and negative results for urease, indole, and motility, further validate the identity of the isolates as *A. baumannii* (Holmström et al., 2022; Lal et al., 2019). These results emphasize the diagnostic utility of phenotypic methods as a foundational step in bacterial identification.

To enhance diagnostic certainty, molecular confirmation was conducted using PCR assays targeting both the *16S rRNA* gene and the intrinsic *blaOXA-51*-like carbapenemase gene. The detection of *blaOXA-51*-like, a chromosomally encoded gene highly conserved within *A. baumannii* serves as a robust molecular signature for species

Figure 7. Ethidium bromide-stained agarose gel (1.5%) of PCR-amplified products from the extracted DNA of *A. baumannii* isolates and amplified with primers of the *blaOXA-51*-like gene

Note: Lane M: DNA molecular size marker (l00 bp ladder). Lanes 1- 2 show a positive result with positive bands of 350 bp (5 V for 1.5 hr).

Table 5. Distribution of samples by antibiotic

Antibiotic	No. (%)			P
	Resistance	Intermediate	Sensitive	
Ciprofloxacin (CIP 5)	2(40)	0(0)	3(60)	0.0392*
Ceftazidime (CAZ 30)	5(100)	0(0)	0(0)	0.0084**
Cefepime (CPM 30)	5(100)	0(0)	0(0)	0.0084**
Ceftriaxone (CTR 30)	5(100)	0(0)	0(0)	0.0084**
Tobramycin (TOB 30)	2(40)	0(0)	3(60)	0.0392*
Ampicillin -sulbactam (AMP 10)	5(100)	0(0)	0(0)	0.0084**
Gentamycin (GEN 10)	2(40)	0(0)	3(60)	0.0392*
Colistin (CL 10)	5(100)	0(0)	0(0)	0.0084**
Amikacin (AK 30)	2(40)	0(0)	3(60)	0.0392*
Levofloxacin (LE 5)	2(40)	0(0)	3(60)	0.0392*
P	0.04227*	NS	0.04194*	---

*P<0.05, **P<0.01

confirmation (Qader, 2021) and (Ewers et al., 2017). The integration of genotypic techniques strengthens diagnostic precision, especially in the context of MDR or phenotypically ambiguous isolates.

A. baumannii isolates recovered from feline conjunctivitis exhibited high levels of resistance to most tested antibiotics. Notably, 100% resistance was observed against ceftazidime, cefepime, ceftriaxone, ampicillin-sulbactam, and colistin, with highly significant P-values (P=0.0084). These findings reflect the global trend of *A. baumannii* becoming a MDR pathogen, particularly resistant to beta-lactams and polymyxins. These findings are in concordance with those of Lysitsas et al. (2023) who reported extensive resistance patterns in *A. baumannii* of animal origin, and also Leelapsawas et al. (2022) who associated antimicrobial overuse in veterinary practices with the propagation of resistant strains. Similar patterns were reported by other researchers (Hamidian & Nigro, 2019 ; Peleg et al., 2008) who highlighted the emergence of extended spectrum β-lactamases (ESBLs) and carbapenemases and resistance to last-line antibiotics in *Acinetobacter baumannii* clinical isolates.

Fluoroquinolones (ciprofloxacin and levofloxacin) and aminoglycosides (gentamycin, tobramycin, and amikacin) showed partial efficacy, with 60% of isolates being sensitive and 40% resistant (P=0.0392). This finding is supported by earlier research (Aliakbarzade et al., 2014). According to reports, resistance to older aminoglycosides is common, but amikacin remains more effective because it is less affected by aminoglycoside-modifying enzymes, like aacC1 and aphA6. The complete resistance to colistin, a last-resort antibiotic, is of particular concern. While earlier research (Li et al., 2006) reported preserved activity of colistin against *A. baumannii*, more recent research (Olaitan et al., 2014) reported emerging plasmid-mediated colistin resistance via mcr genes and lipid A modifications. This resistance threatens therapeutic outcomes in veterinary and human medicine alike. The variation in antibiotic responses (P<0.05) shows diverse resistance mechanisms among isolates, highlighting the necessity for improved diagnostics and careful antibiotic use. Partial sensitivity to amikacin and fluoroquinolones limits treatment options, making susceptibility testing vital. From a veterinary medicine perspective, ampicillin-sulbactam and gentamicin are considered first-line agents, whereas amikacin, tobramycin, fluoroquinolones, and advanced cephalosporins are regarded second-line and should be used restrictively. Importantly, colistin is classified as a last-resort antibiotic in human medicine and must not be applied in veterinary practice. This emphasizes the need for prudent antimicrobial stewardship to avoid further dissemination of resistant *A. baumannii* strains across animal and human populations

In regions, such as Iraq, where pet ownership is widespread and often unregulated, the implications of these findings are particularly consequential. The intimate cohabitation between humans and domestic animals increases the risk of interspecies transmission of MDR organisms. *A. baumannii* may act as a silent reservoir of resistance genes, with potential horizontal gene transfer to human-associated pathogens. This risk is exacerbated among immunocompromised individuals, children, and veterinary personnel. Therefore, these findings underscore the necessity for implementing structured antimicrobial stewardship programs, routine microbiological surveillance in veterinary clinics, and public education initiatives aimed at mitigating the threat of zoonotic antimicrobial resistance at the animal-human interface.

Conclusion

This study constitutes the first in-depth investigation of the isolation and molecular characterization of *A. baumannii* from conjunctivitis cases in domestic cats in Baghdad, Iraq. The results highlight the emergence of *A. baumannii* as a potential opportunistic ocular pathogen in felines, demonstrating its ability to colonize and persist in the conjunctival niche. The high levels of antimicrobial resistance observed, particularly to antibiotics commonly used in veterinary practice, underscore a significant threat posed by MDR strains in companion animals. These findings reinforce the urgent need for stricter antimicrobial stewardship in veterinary medicine, alongside ongoing surveillance and molecular tracking of resistance mechanisms. Furthermore, this study lays a foundation for future research exploring the zoonotic potential and environmental reservoirs of *A. baumannii* within domestic animal populations.

Ethical Considerations

Compliance with ethical guidelines

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). 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.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Sample collection, diagnosis, experiments and writing the original draft: Ayat A. Hussein; Supervision, review and editing: Sahar M. Hayyawi.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors would like to express their sincere gratitude to the College of Veterinary Medicine, University of Baghdad, and express their special thanks to the Department of Microbiology for their valuable guidance and laboratory assistance throughout the research process.

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