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Molecular Detection and Antibiotic Resistance of Acinetobacter Bumannii Isolated from Mastitis Cases

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ABSTRACT: Mastitis in bovine remains as the great economic significant diseases disturbing lactating herds worldwide, with growing anxiety concerning infections caused by displaying resistance to several antibiotics. *Acinetobacter baumannii* has arisen as an opportunistic pathogen with valuable antimicrobial resistance characters, until now its role in bovine mastitis is still under-reported. The present study was conducted to investigate the isolate and identify *A. baumannii* from cases associated with both clinical and subclinical forms of mastitis in milk-producing cattle, to authorize the isolates at the level of molecular detection by real time PCR aiming the *bla*OXA-51 gene, and to assess their patterns of antimicrobial resistance. Overall 150 samples of milk were collected from cows with mastitis in several areas of Baghdad, Iraq. Standard bacteriological approaches, biochemical examinations, API 20E and RapID™ ONE system was used for phenotypic documentation. Molecular verification was carried out utilizing real time PCR. Antibiotics susceptibility analysis exposed high rates of reduce susceptibility to numerous aminoglycosides and β-lactams, while variable susceptibility was detected tocolistin, carbapenems, and tetracycline derivatives. The recognition of the intrinsic *bla*OXA-51 gene established the identity the isolates of *A. baumannii*. The rate of contamination of *A. baumannii* in mastitis cases reach 55 of 150 samples (36.67%). These results highlight the possible role of *A. baumannii* as mastitis related pathogen and underline the necessity for unceasing surveillance of resistance to antimicrobials in dairy farms.

INTRODUCTION

Mastitis in bovine is an inflammatory disorder of the mammary gland that causes considerable financial losses resulting from reduced milk yield, reduce milk quality, rising expenses of treatment, and elimination of infected animals (Kirby, 2019). Even though mastitis is typically related with Gram positive bacteria for example *Streptococcus* spp. and *Staphylococcus aureus*, Gram negative pathogens are progressively documented as important etiological causes (Cobirka *et al.*, 2020). Amongst these, *Acinetobacter baumannii* has increased awareness of its notable capability to adapted to and persist in harsh conditions and obtain resistance to numerous classes of antimicrobials (Mohr *et al.*, 2014). *A. baumannii* is a Gram-negative, non-fermentative, coccobacilli extensively recognized in humans for causing acquired infections in hospital, including wound infections, pneumonia, and septicemia (Mohamed *et al.*, 2022).

The microorganism possesses a wide range of virulence factors and mechanisms of resistance, particularly the release of carbapenem hydrolyzing class D β-lactamases. The *bla*OXA-51 like gene is intrinsic to *A. baumannii* and is considered a dependable molecular indicator for species conformation (Peleg *et al*., 2008). In last year's, information of *A. baumannii* isolation from animals and products of animal have augmented, increasing anxieties about its zoonotic probability and its role as an antimicrobials resistance genes reservoir (Mellace *et al*., 2024). However, records concerning its participation in bovine mastitis, predominantly in Iraq, stay rare. There were several studies established to study the mastitis cases (AH, 2014; Jameel & Hasso, 2018; Al-Rasheed *et al*., 2022; Mahmoud & Yassein, 2024) but none of them refer to importance of *A. baumannii*. Therefore, the herein study was aimed to explore the incidence of *A. baumannii* in samples of mastitis milk, to confirm its identity using molecular approaches, and to evaluate its antimicrobial resistance data.

MATERIALS AND METHODS

Sample Collection

In total, 150 samples of milk were gathered from cows identified with clinical and subclinical mastitis from several local dairy farms in Baghdad, Iraq. Before collection of samples, the teats and udder were carefully cleaned with solution of iodine followed by ethanol 70%. The first milk streams were discarded, and about 10-15 mL of milk was collected aseptically into sterilized screw cap tubs. Samples of milk were submitted to the laboratory for analysis in separated ampules at 4°C and processed within 24 hours, following standard microbiological procedures of Kirby (2019). The clinical and subclinical mastitis that conformed using the clinical examination, then California Mastitis test, and Strip Cup test.

Bacteriological Examination

Samples of milk were primarily cultured onto plates of Nutrient agar and aerobically maintained at 37°C for 24 hours. Putative colonies were further subcultured for subsequent identification on MacConkey agar, Luria–Bertani agar, and Xylose Lysine Deoxycholate agar to assess colony morphology and lactose fermentation characteristics. Gram stain was implemented to definecellular morphology and Gram reaction. Microbiological investigation was done following technique of Angappan (2021).

Biochemical Identification

The biochemical conformations test included indole, catalase, citrate, urease, oxidase, TSI, and DNase. This was done according to the methods of Jinnah and Fatema (2024). The following steps included the conformation using both API 20E and RapID™ one systems.

Molecular Identification by Real-Time PCR

The extracted DNA through using of special kit for Acinetobacter species (QIAamp DNA mini kit, Qiagen, Germany), followed by determine the concentration and purity of the examined DNA using Quantus™ Fluorometer, then the real time PCR was done for detection of *bla*OXA51 gene. This was established according to Turton *et al.* (2006) and Falah *et al.* (2019).

Reagents and Kits Used

Kit/Reagent	Manufacturer	Country
Absolute Ethanol	ROMIL Pure Chemistry	UK
QIAamp DNA Mini Kit	QIAGEN	Germany
GoTaq® qPCR Master Mix & Quantifluords DNA System	Promega	USA
Oligonucleotide Primers	Macrogen	South Korea

Antimicrobial Susceptibility Testing

The antimicrobial sensitivity test was done using disk diffusion test in Mulleller-Hinton agar. This was complete according to Pierce *et al.* (2023). The tupes of antibiotics which used were as follows: levofloxacin, ceftazidime, gentamicin, imipenem,

minocycline, cefepime, eropenem, tigecycline, trimethoprim+sulfamethoxazole, piperacillic+tazobactam, ciprofloxacin, and colisysin.

Ethical Approval: This study was approved by the Animal Utlization Protocol Certification Committee, College of Veterinary Medicine University of Baghdad, Iraq; with the license number P.G./2545 in 7/10/2025. This study did not involve the euthanizing of any animal.

RESULTS AND DISCUSSION

A. bunannii was isolated and identificated from cases of clinical and subclinical mastitis. This was the first time that indicated the presence of *A. bunannii* inside mastitis cases in Iraq. The percentage of identification of the *A. bunannii* reach 55 samples from total of 150 mastitis cases (36.67%) which diagnosed as clinical or subclinical mastitis using clinical examination, then California Mastitis test, and Strip Cup test. The identification was done using MacConkey agar, Gram-stain, biochemical test, API 20E system, RapID® ONE system, molecular analysis using real time PCR (Figure 1). The detection methods similar to the finding of Figueiredo *et al.* (2009), Nowak *et al.* (2012), and Hou and Yong (2015). The percentage of isolation of *A. bunannii* were higher than several previous authors, Dorgham *et al.* (2024) in Egypt whom isolate and identificate the *A. bunannii* to reach 17.14% from 12 positive isolation of 70 subclinical cattle mastitis. Also our result still higher than the percentage that recorded in Brazil which reached 5.5% from 4 positive isolation from total 72 subclinical mastitis cases (de Oliveira *et al.*, 2022). While in China with a huge survey the *A. bunannii* isolated from 3.38% from 1153 clinical mastitis samples (Wang *et al.*, 2026). In a recent study 13 positive isolation of total 335 samples which represent 3.88% of all samples of *A. bunannii* contamination (Hassan *et al.*, 2026). The high recorded rate of *A. bunannii* in the herein study may related to several factors: environmental conditions, herd management, hygienic status, using of antimicrobials, diagnostic techniques. The detection of blaOXA-51 gene will improve the identification methods. The ability of *A. bunannii* to acquire antibiotics resistance and persist in a harsh condition may elevate the public concern of these bacteria in order to limit the transmission of *A. bunannii* between animals, human and the environment.

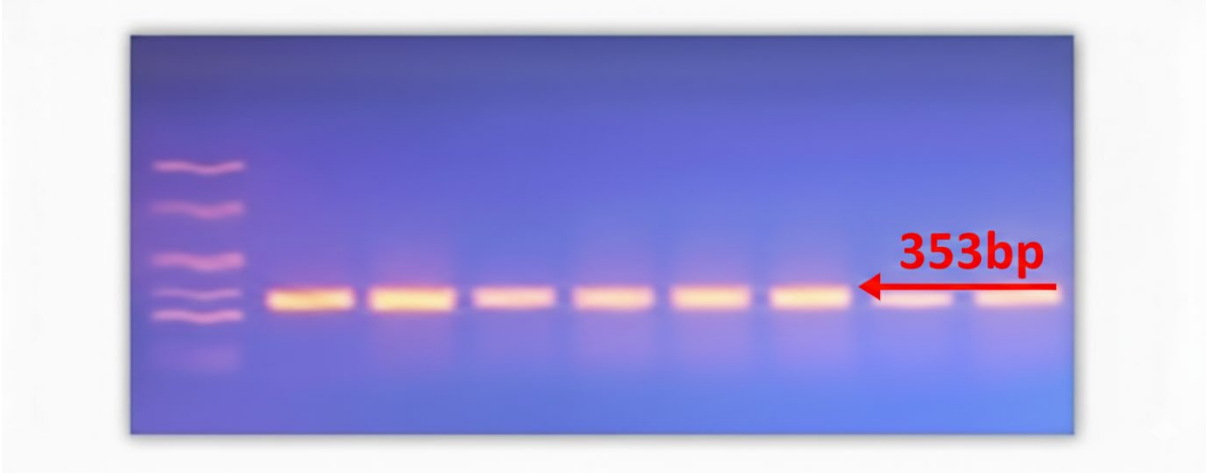


Figure-1: Electrophoresis of PCR products of *A. baumannii* isolated from cases of mastitis utilizing the blaOXA-51 gene with specific primer (353 bp) in 1% agarose gel at 50 V for 1 hour.

Antimicrobial susceptibility analysis established high rates of resistance to piperacillin/tazobactam, cefepime, ceftazidime, ciprofloxacin, and gentamicin. Moderate susceptibility was detected to meropenem and imipenem, whereas minor rates of resistant were noted for tigecycline, colistin, and minocycline (Table 1).

Table 1: Antimicrobial resistance and sensitivity patterns of *A. baumannii* isolates from cases of mastitis

Antibiotic	Resistance	Sensitive	Total no.
Piperacillin/Tazabactam	**38**	**17**	**55**
Ceftazidime	**36**	**19**	**55**

Cefepime	**40**	**15**	**55**
Imipenem	**17**	**38**	**55**
Gentamicin	**42**	**13**	**55**
Tigecycline	**18**	**37**	**55**
Ciproflaxicin	**45**	**10**	**55**
Trimethoprim/sulfamethoxazole	**15**	**40**	**55**
Ceftriaxone	**28**	**27**	**55**
Meropenem	**19**	**26**	**55**
Levofloxacin	**6**	**49**	**55**
Minocycline	**-**	**55**	**55**
Colistin	**16**	**39**	**55**
Ciprofloxacin	**7**	**48**	**55**

Elevated levels of antimicrobial resistance to β-lactam and aminoglycosides antibiotics detected in finding of this study are consistent with former recordation on multidrug resistant strains of *A. baumannii* (Peleg *et al*., 2008; Schuetz *et al*., 2025). The recognition of multidrug resistant *A. baumannii* in mastitis milk is of specific concern, as it may cooperation therapeutic effectiveness and poserisk of the public health through the chain of food. The results highlight the significance of using of prudent antimicrobials and monitoring of routine resistance in dairy farms (Schuetz *et al*., 2025). The distinctly high resistance to fluoroquinolones, mainly levofloxacin and ciprofloxacin, agrees with results stated in both human and animal isolates of *A. baumannii*, where rates of resistance often exceeding 70-80% due to mutations in the target site and over expression of efflux pump (Wong *et al*., 2020).

Moderate resistance to carbapenems, including meropenem and imipenem, detected in the current isolates has been extensively recognized and is frequently related with the production of OXA-type carbapenemases, particularly *bla*OXA-51-like enzymes intrinsic to *A. baumannii* (Turton *et al*., 2006; Higgins *et al*., 2010). The marked antimicrobial resistance to gentamicin described in current study is in agreement with preceding studies representing wide resistance to aminoglycoside among isolates of *A. baumannii*, mainly mediated by enzymes modifying aminoglycoside (Peleg *et al*., 2008).

However, low resistance rate to tigecycline and colistin supports previous outcomes showing that these agents stay among the most effective therapeutic choices against multidrug resistant *A. baumannii* strains, even though developing resistance has been progressively stated (Falagas *et al*., 2010). The variable susceptibility against to trimethoprim/sulfamethoxazole detected in the current study is consistent with former reports proposing restricted but occasionally retained activity of this antimicrobial against isolates of *A. baumannii* (Wong *et al*., 2020).

Conclusions: The herein study recorded a high positive isolation of *Acinetobacter baumannii* reach 36.67% (55 positive isolation from 150 clinical and subclinical mastitis cases). The molecular detection of these bacteria that applied in this study provides the high spread of *A baumannii* among the dairy cattle in Iraq. The antimicrobial resistant of *A baumannii* highlight the importance of prevention and control the spread of this bacteria among mastitis cases, with selection of susceptible antimicrobials antibiotics in order to limit the transmission of resistance strains.

Conflicts of interest

The authors confirm that no conflicts of interest exist regarding the publication of this paper.

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