Investigation of Biofilm Formation, Virulence Genes and Antibiotic Resistance of *Acinetobacter baumannii* Isolates Obtained from Clinical Samples

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ABSTRACT

The ability of *Acinetobacter baumannii* to form biofilms and its multiple antibiotic resistance may be responsible for the bacterium's survival in various environments. This study was aimed to investigate the biofilm-forming ability, antibiotic resistance phenotypes, frequency of genes associated with biofilm genes (*bap*, *omp*A, *aba*I, *csu*E, *bfm*S), and integron genes (*int*1, *int*2) in *A. baumannii* isolates obtained from human and bovine clinical samples. A total of 30 *A. baumannii* isolates were used, including 25 from human blood samples and 5 from mastitis-infected bovine milk samples. After conventional isolation methods, identification and antibiotic susceptibility tests were performed using an automated microbiology system (BD Phoenix™ 100, USA). Phenotypic biofilm formation was quantitatively assessed using the microplate test, and virulence genes associated with biofilm and integron genes were examined using polymerase chain reaction. Pearson's Chi-square ($\chi 2$) test was used to compare the study data. The highest resistance rate among all isolates was observed against ampicillin, followed by ertapenem and cefepime. Ninety three percent of the isolates exhibited multidrug resistance (MDR), and all could form biofilms (60% strong, 27% moderate, 13% weak). All *A. baumannii* isolates carried at least one gene associated with biofilm formation. The most commonly observed virulence gene associated with biofilm was *bfm*S, followed by *csu*E, *bap*, *omp*A and *aba*I. Integron genes were detected in 90% of the isolates (23 from humans, 4 from bovines). Statistical analysis revealed no significant relationship between the origin of isolates and the severity of biofilm formation. However, significant associations were found between the origin of isolates and the presence of *aba*I and *bfm*S virulence genes, as well as between strong biofilm formation and carrying all virulence genes or integron genes. Analyses of isolates obtained from human and bovine clinical samples indicate that *A. baumannii*'s biofilm formation capacity and resistance to antibiotics pose significant health threats. Particularly, the significant associations between strong biofilm formation and carrying all virulence genes or integron genes highlight the bacterium's complex adaptation strategies. In conclusion, this study demonstrates the comparative analysis of antimicrobial resistance profiles, biofilm formation abilities, and virulence gene carriage of *A. baumannii* isolates, emphasizing its importance as a threat to both human and animal health.

Key words: *Acinetobacter baumannii*; Biofilm; Multiple Antibiotic Resistance.

INTRODUCTION

Acinetobacter baumannii is a member of the *Moraxellaceae* family and is described as a nonmotile, catalase-positive, oxidase-negative, aerobic Gram-negative coccobacil, which is an opportunistic nosocomial pathogen (1). *A. baumannii* can lead to meningitis, urinary tract, skin, and soft tissue

infections in humans (2), with the highest mortality rates observed in ventilator-associated pneumonia and bloodstream infections (3). In the veterinary medicine, there is limited information about *A. baumannii* compared to human medicine (4, 5). *A. baumannii* has been isolated from mastitis, pneumonia, and sepsis in cattle; wound infections, sepsis, bronchopneumonia, neonatal encephalopathy, and eye infections in horses; wound, bloodstream, and urinary tract infections in dogs and cats (4, 5).

In recent years, *A. baumannii* has become an increasingly significant pathogen in both humans and animals due to its escalating antibiotic resistance and ability to form biofilms, making it a common cause of healthcare-associated infections due to its capability to persist and survive on surfaces (6). The virulence factors of *A. baumannii* consist of a series of genes that contribute to its significance as a pathogen in both humans and animals. Some of these genes play crucial roles in functions such as biofilm formation and antimicrobial resistance (7).

Biofilm is a structure where a microbial community attaches to a relevant surface via an extracellular matrix (8). Biofilm formation entails a complex regulatory system that involves bacterial adhesion ability, development of the biofilm, attachment of matrix components, mobility, synthesis, and coordination of relevant gene expression (9). The *bap* (biofilm-associated protein), *ompA* (outer membrane protein A), *csu*E (chaperon-usher pilus), *aba*I (gene for quorum sensing components), and *bfm*RS (two component system) genes have been evaluated as the most significant virulence genes associated with biofilm formation in *Acinetobacter* spp. (9,10). The *bap* protein, associated with biofilm, is a large protein found on the cell surface. Encoded by the *bap* gene, the *bap* protein serves as a surface adhesin involved in intracellular adhesion in mature biofilm and biofilm biomass volume and is homologous to *Staphylococcus* protein. Additionally, it has been found in other bacterial genera typically associated with hospital-acquired infections such as *Enterococcus* spp. and *Pseudomonas* spp. The importance of the bap gene in forming mature biofilm on both biotic and abiotic surfaces has been established (9). *A. baumannii* produces autoinducing signal molecules via the *abaI* gene, which is involved in quorum sensing. These molecules contribute to the bacterium's perception of environmental conditions and particularly to the regulation of biofilm formation. Moreover, they can regulate interactions between *A. baumannii* and other pathogens, potentially increasing the severity of infections (9). *csu*E is part of the CsuA/BABCD chaperone complex critical for *A. baumannii*'s ability to form biofilm. The *csu*E gene encodes part of the csu pilus assembly system and acts as an adhesin attaching to the surface at the onset of biofilm formation. The absence of *csu*E can reduce the bacterium's ability to form biofilm, potentially increasing the risk of infection (9, 11). *omp*A, an outer membrane porin, plays various roles in *A. baumannii*, including antimicrobial resistance, adherence to epithelial cells, and biofilm formation. The *omp*A gene strengthens the bacterial cell membrane, enhancing its resistance to the host and potentially increasing its ability to cause infection (12). The *bfm*S gene aids the bacterium in forming biofilm on inanimate surfaces such as polystyrene. Activation of *bfm*S allows the bacterium to adapt to environmental conditions and enhance its ability to cause infection (9).

In the last decade, *A. baumannii* isolates have become a significant clinical concern due to their ability to develop resistance to all known antibiotics (6). Particularly, the association of isolates showing multidrug resistance (MDR) with nosocomial and community-acquired infections has led the Infectious Diseases Society of America to include *A. baumannii* in the ESKAPE pathogens list alongside *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Enterobacter* spp. (13,14). In recent years, MDR *A. baumannii* has been frequently isolated in infections with high morbidity and mortality rates, often failing to respond to conventional treatments (e.g., penicillins, cephalosporins, carbapenems, and monobactams), primarily due to the acquisition of various resistance genes, especially against β-lactam antibiotics (15).

Integrons are mobile DNA elements that can disseminate multidrug resistance, particularly in Gram-negative pathogens (16). The basic structure of integrons consists of conserved segments containing antibiotic resistance gene cassettes that can be added to or excised from the site-specific recombination catalyzed by integrase (17). To date, five classes of integrons have been identified based on the sequence of the *int* gene; class 1 and class 2 integrons are the most commonly identified integron classes in clinical isolates of *A. baumannii* (16, 17).

Acinetobacter spp. are biofilm producers capable of acquiring and transferring resistance genes, thereby enhancing their antibiotic resistance abilities (18). Studies have shown that

clinical isolates may form more effective biofilms compared to environmental isolates, and there is a significant correlation between biofilm production and multidrug resistance (19,20). To the best of our knowledge, there is currently no study in Türkiye that evaluates both biofilm formation and antibiotic resistance profiles of human and animal-derived *A. baumannii* isolates together. This study aimed to investigate the biofilm-forming ability, antibiotic resistance phenotypes, and the frequency of the most important genes associated with biofilm (*aba*I, *csu*E, *bap*, *omp*A, *bfm*S) and integron genes (*int*1, *int*2) in *A. baumannii* isolates obtained from human (blood culture) and animal (mastitic bovine milk) clinical samples.

MATERIAL AND METHODS

Ethical Approval

The study was conducted with the ethical approval obtained from the Non-Interventional Research Ethics Committee of Aydın Adnan Menderes University, Health Sciences Institute, dated February 12, 2021 (Protocol No: 2021/005).

Material

In the scope of this study, human blood samples were collected from 105 hospitalized patients over a three-month period (May 2021 – July 2021) at Aydın Adnan Menderes University Faculty of Medicine Hospital. Additionally, as animal material, 225 subclinical mastitis milk samples, routinely submitted to the diagnostic laboratory of Aydın Adnan Menderes University Faculty of Veterinary Medicine for various studies, were examined.

Isotion and identification

After homogenizing the samples, one milliliter of Brain Heart Infusion Broth (Merc 110493, Germany) was inoculated. The BHIB cultures were then incubated at 37°C for 18-24 hours. From these cultures, a loopful of broth culture was streaked onto 5% sheep blood agar (Merc 1.10886, Germany) and MacConkey agar (Merck 100205, Germany). The streaked plates were incubated aerobically at 37°C for 18-24 hours. Colonies showing Gram-negative coccobacilli appearance underwent biochemical tests (catalase, citrate, motility, oxidation-fermentation test, hemolysis, gas production, oxidase, H_2S, indole, urea, glucose, and sucrose fermentation) (21). For the identification of suspected *Acinetobacter* spp. colonies, an automated microbiology system (BD Phoenix 100™, USA) was used.

Antibiotic susceptibility test

Antibiotic susceptibility test of the isolates was performed using the NMIC/ID 433 panel on the automated microbiology system (BD Phoenix 100™). A comprehensive panel consisting of 20 different antibiotics from nine antimicrobial classes was utilized for the test, including aminoglycosides such as amikacin, gentamicin; carbapenems such as ertapenem, imipenem, meropenem; cephalosporins such as cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime; beta-lactams such as ampicillin, ceftolozane-tazobactam, amoxicillin-clavulanate; lipopeptides such as colistin; folates such as trimethoprim-sulfamethoxazole; quinolones such as ciprofloxacin, levofloxacin; tetracyclines such as tigecycline. The resistance profiles of the isolates were determined, and interpretation was performed according to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (22). *E. coli* ATCC 25922 was used as a quality control strain. Additionally, *A. baumannii* isolates were classified as MDR when resistant to at least three different antibiotic classes from distinct categories (23).

Biofilm formation

The microplate method was used to quantitatively determine the amount of biofilm production (24). *A. baumannii* isolates were incubated overnight at 37°C in tryptic soy broth (TSB) (Merc 10459, Germany) containing 0.25% glucose. After removal of free cells, the biofilm was washed three times with sterile phosphate-buffered saline (PBS) and then fixed with 99% (v/v) methanol. The biofilm in the wells was stained with 1% (w/v) crystal violet at room temperature for 20 minutes. Subsequently, the crystal violet solutions were dissolved in 33% (v/v) ethanol/acetone (80, 20, v/v), and absorbance values were measured at 595 ηm. The optical density cutoff (ODc) was calculated as three times the sum of the average optical density and standard deviation of the negative control.

Classification of strains was performed according to the following criteria: non-biofilm producer (NB) (OD≤ODc), weak biofilm producer (W) (ODc<OD≤2xODc), moderate biofilm producer (M) (2xODc<OD≤4xODc), and strong biofilm producer (S) (4xODc<OD). The test was repeated three times for each sample, and the average optical density was calculated. *Staphylococcus aureus* 25923 was used as the

Table 1. Primers used in this study.

	Target gene	Primer Sequences (5'-3')	T_m	Amplicon size	Reference
Virulence genes associated with biofilm	*abaI*	CGCTACAGGGTATTTGTTGA TCGTAATGAGTTGTTTTGCG	56.4 54.3	370	11
	csuE	AGACATGAGTAGCTTTACG CTTCCCCATCGGTCATTC	53.0 56.1	516	
	bap	ATAACTCGGCTGTTTACGG ACTGATGGTGTTGGAAGTG	55.2 55.2	358	
	ompA	CTGGTGTTGGTGCTTTCTGG GTGTGACCTTCGATACGTGC	60.5 60.5	352	
	*bfm*S	TTGCTCGAACTTCCAATTTATTATAC TTATGCAGGTGCTTTTTTATTGGTC	60.0 60.9	1428	26
Integron gens	*Int*1	CCTCCCGCACGATGATC TCCACGCATCGTCAGGC	57.2 57.2	280	27
	*Int*2	TTATTGCTGGGATTAGGC ACGGCTACCCTCTGTTATC	51.6 57.3	233	28

T_m: Melting temperature.

positive control, and Müller-Hinton broth (MHB) (Merc 110293, Germany) was used as the negative control.

DNA extraction

Genomic DNA was obtained using the GeneJET™ Genomic DNA Purification Kit (Thermo Scientific™ Biotechnology, Seongnam-Si, Korea, K0721). The purity and quantity of DNA were assessed using nanodrop device (MaestroNano Micro-Volume Spectrophotometer, Malaysia MN-913). DNA purity was determined to be within the range of OD260/280 values of 1.6 to 2.0 (25). For each PCR reaction, 3 µl of template DNA was used.

Polymerase Chain Reaction (PCR)

The presence of genes associated with biofilm (*aba*I, *csu*E, *bap*, *omp*A, *bfm*S) and integron genes (*int*1, *int*2) was examined using PCR as previously described (Table 1). PCR reactions were performed in a volume of 25 µl. The final concentrations for each PCR reaction were adjusted as follows: 1x for 10x Taq enzyme buffer solution, 2 mM for 25 mM $MgCl_2$, 0.2 mM for 10 mM dNTP, 0.4 µmol for 100 ρmol of each primer, 1.5 U for 5 U of Taq DNA polymerase (Fermentas, Massachusetts, USA), and 3 µl of DNA from each sample. Prepared reaction mixtures were loaded into a thermal cycling device (Boeco, Hamburg, Germany).

For DNA amplification, the device used a program consisting of an initial denaturation at 95˚C for 5 minutes; followed by 30 cycles of denaturation at 95˚C for 30 seconds, annealing at 53˚C (*aba*I, *csu*E, *bap*), 56˚C (*int*1, *int*2), and 58˚C (*omp*A, *bfm*S) for 30 seconds, extension at 72˚C for 60 seconds, and a final extension at 72˚C for 10 minutes.

A 2% agarose gel was used for electrophoresis, and the gel was subjected to an electric current of 100 volts for 60 minutes. After electrophoresis, the gel was placed in a transilluminator device (Vilbert Lourmat, Collegien, France) under UV light and photographs were taken. Amplification products were considered to contain the respective gene when they produced a band of the expected size (Table 1).

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 23.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis of the obtained data. Pearson's Chi-square ($\chi 2$) test (Fisher's Exact $\chi 2$ Test) was used to compare frequency data. The following relationships were examined using the $\chi 2$ test: **a.** The relationship between the origin of isolates and the severity of biofilm formation **b.** The relationship between the origin of isolates and the frequency of virulence genes associated with biofilm **c.** The relationship between the strength of biofilm formation and the frequency of virulence genes associated with biofilm **d.** The relationship between the strength of biofilm formation and the frequency of integron genes. The results were evaluated at a 95% confidence

Table 2. Antimicrobial resistance rates of *A. baumannii* isolates.

Antimicrobial Family-Antibiotic Name	Human Blood (n=25), R (%)	Mastitic Bovine Milk (n=5), R (%)	All Isolates (n=30), R (%)
Aminoglycoside			
Amikasin	13 (52)	1 (20)	14 (47)
Gentamicin	17 (68)	2 (40)	19 (63)
Carbapenem			
Ertapenem	25 (100)	0 (0)	25 (83)
Imipenem	16 (64)	0 (0)	16 (53)
Meropenem	17 (68)	0 (0)	17 (57)
Cephem			
Cefazolin	15 (60)	0 (0)	15 (50)
Cefuroxime	14 (56)	0 (0)	14 (47)
Ceftazidime	22 (88)	0 (0)	22 (73)
Ceftriaxone	19 (76)	0 (0)	19 (63)
Cefepim	23 (92)	0 (0)	23 (77)
Penicillin			
Ampicillin	25 (100)	3 (60)	28 (93)
β-Lactam			
CeftolozaneTazobactam	14 (56)	0 (0)	14 (47)
Amoxicillin Clavulanate	19 (76)	4 (80)	23 (77)
Ampicillin Sulbactam	16 (64)	2 (40)	18 (60)
Piperacillin Tazobactam	11 (44)	0 (0)	11 (37)
Lipopeptide			
Colistin	3 (12)	0 (0)	3 (10)
Folate			
Trimethoprim Sulfamethoxazole	16 (64)	3 (60)	19 (63)
Quinolone			
Ciprofloxacin	17 (68)	3 (60)	20 (67)
Levofloxacin	18 (72)	0 (0)	18 (60)
Tetracycline			
Tigecycline	3 (12)	0 (0)	3 (10)

interval, and differences with $p<0.05$ between means were considered statistically significant.

RESULTS

Isolation and identification

In this study, a total of 37 *Acinetobacter* spp. suspected isolates were obtained, including 25 (24%) from 105 human blood samples and 12 (5%) from 225 subclinical mastitis bovine milk samples. On blood agar, the colonies were non-hemolytic, while on MacConkey agar, they typically appeared as light lavender color and displayed a Gram-negative coccobacilli morphology. They were catalase, citrate, glucose fermentation positive; negative for oxidase, indole, urease, hydrogen sulfide production; non-motile, non-hemolytic, and gas non-producing, thus considered as suspected *Acinetobacter* spp. isolates.

Using the automated microbiology system (BD Phoenix 100™), all 25 (100%, 25/25) isolates from human clinical blood samples and 5 (42%, 5/12) out of 12 mastitis bovine milk isolates were identified as *A. baumannii*. Thus, a total of 30 *A. baumannii* identifications were made, consisting of 25 (24%, 25/105) from human clinical samples and 5 (2%, 5/225) from bovine clinical samples.

Antimicrobial susceptibility test

Antimicrobial susceptibility test revealed that among all isolates, the highest resistance rate was observed against ampicillin (93%, 28/30), followed by ertapenem (83%, 25/30), and cefepime (77%, 23/30). All human isolates (100%, 25/25)

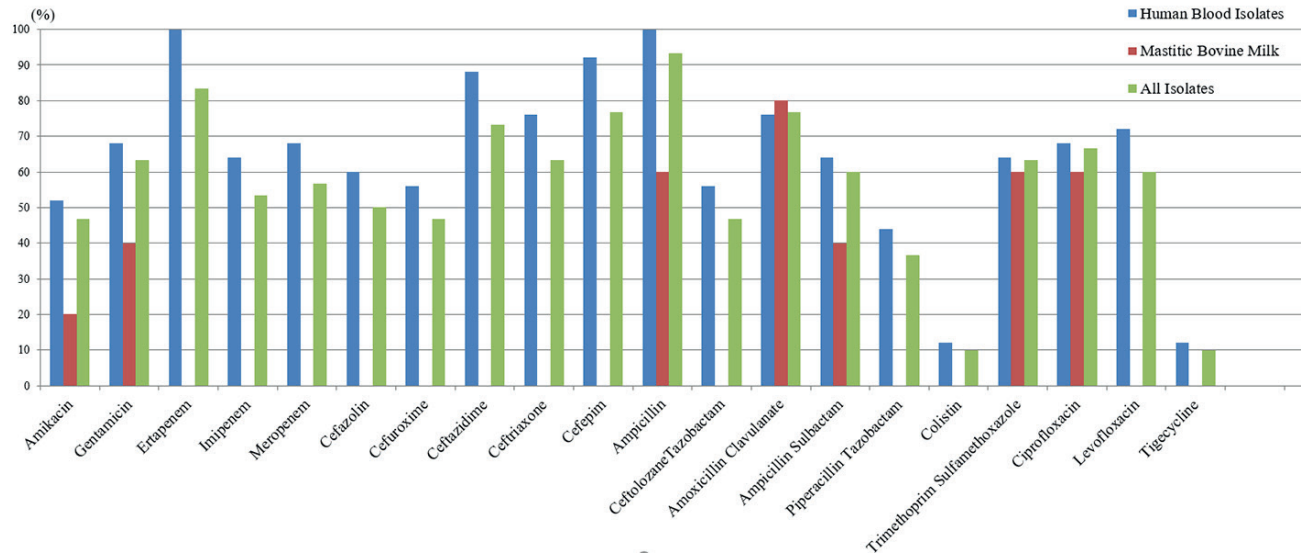

Figure 1. Antimicrobial resistance situations in *A. baumanii*.

Table 3. Antimicrobial resistance phenotypes of the isolates.

Antimicrobial Family	Antibiotic Resistance Fenotypes	Ratio (%), Isolate (n=30)
2*	β-laktam, Folat	3 (1)
2*	β-laktam, Quinolone	3 (1)
3*	Penisilin, β-laktam, Folat	3 (1)
3	Carbapenem, Penisilin, β-laktam	3 (1)
4*	Aminoglikozid, Penisilin, β-laktam, Quinolone	3 (1)
5*	Aminoglikozid, Penisilin, β-laktam, Quinolone, Folat	3 (1)
5	Carbapenem, Cephem, Penisilin, β-laktam, Folat	3 (1)
5	Aminoglikozid, Carbapenem, Cephem, Penisilin, Quinolone	3 (1)
6	Carbapenem, Cephem, Penisilin, β-laktam, Folat, Quinolone	7 (2)
6	Carbapenem, Cephem, Penisilin, β-laktam, Lipopeptid, Quinolone	7 (2)
6	Aminoglikozid, Carbapenem, Cephem, Penisilin, β-laktam, Quinolone	13 (4)
6	Aminoglikozid, Carbapenem, Cephem, Penisilin, β-laktam, Folat	7 (2)
7	Aminoglikozid, Carbapenem, Cephem, Penisilin, β-laktam, Folat, Quinolone	27 (8)
7	Aminoglikozid, Carbapenem, Cephem, Penisilin, β-laktam, Lipopeptid, Folat	3 (1)
7	Carbapenem, Cephem, Penisilin, β-laktam, Folat, Quinolone, Tetrasiklin	3 (1)
8	Aminoglikozid, Carbapenem, Cephem, Penisilin, β-laktam, Folat, Quinolone, Tetrasiklin	3 (1)
8	Aminoglikozid, Carbapenem, Cephem, Penisilin, β-laktam, Lipopeptid, Quinolone, Tetrasiklin	3 (1)

*: Resistance patterns of bovine isolates.

were resistant to ampicillin and ertapenem, while all bovine isolates were susceptible to carbapenems, cephalosporins, lipopeptides, and tetracyclines (Table 2). High (50%-100%) resistance was detected against 70% of the tested antibiotics, moderate (49%-20%) resistance against 20%, and low (19%-1%) resistance against 10% (Table 2, Figure 1).

A total of 17 different antibiotic resistance patterns were identified, with the most common pattern observed in humans being "aminoglycoside, carbapenem, cephalosporin, penicillin, β-lactam, folate, quinolone" resistance pattern, while in bovines, five different resistance patterns were observed for five isolates (β-lactam), Folate), (β-lactam,

Table 4. Number of antimicrobial families to which isolates were resistant.

Isolate origin	Isolate (%)	Number of Antimicrobial Families to Resistance	NMDR/MDR status of isolates
Human (n=25)	1 (4)	3	100% MDR
	2 (8)	5	
	10 (40)	6	
	10 (40)	7	
	2 (8)	8	
Bovine (n=5)	2 (40)	2	40% NMDR
	1 (20)	3	60% MDR
	1 (20)	4	
	1 (20)	5	
Total (n=30)	2 (7)	2	7% NMDR
	2 (7)	3	93% MDR
	1 (3)	4	
	3 (10)	5	
	10 (33)	6	
	10 (33)	7	
	2 (7)	8	

Table 5. Phenotypic capacities of isolates for biofilm formation.

	Weak (%)	Moderate (%)	Strong (%)
Human (n=25)	3 (12)	5 (20)	17 (68)
Bovine (n=5)	1 (20)	3 (60)	1 (20)
Total (n=30)	4 (13)	8 (27)	18 (60)

quinolone), (penicillin, β-lactam), Folate, (aminoglycoside, penicillin, β-lactam, quinolone), (aminoglycoside, penicillin, β-lactam, quinolone, folate)) (Table 3).

Multiple antibiotic resistance

Ninety-three percent (28/30) of all *A. baumannii* isolates were resistant to three or more antimicrobial agents and were considered MDR. Two bovine isolates (40%, 2/5) were resistant to two antimicrobial families (non-MDR, (NMDR)), while all human isolates (100%, 25/25) were MDR (Table 4).

Biofilm formation

All *A. baumannii* isolates have the ability to produce biofilm. Sixty percent of these isolates (18 isolates: 17 human, 1 bovine) demonstrated a strong biofilm-forming ability, while 27% (8 isolates: 5 human, 3 bovine) formed biofilms at a moderate level, and 13% (4 isolates: 3 human, 1 bovine) exhibited a weak biofilm-forming capability (Table 5, Figure 2). These findings indicate that *A. baumannii* possesses varying levels of biofilm-forming ability, observed in both human and bovine isolates. However, there is a higher tendency for strong biofilm formation in human isolates and moderate biofilm formation in bovine isolates.

Biofilm-related virulence genes

All *A. baumannii* isolates harbored at least one virulence gene associated with biofilm formation. The most commonly observed virulence gene related to biofilm formation was *bfm*S (87%), followed by *csu*E (83%), *bap* (73%), *omp*A (70%), and *aba*I (30%). The *aba*I gene was detected at the lowest rate (20%) in human isolates, while its presence in bovine isolates (80%) was higher. Interestingly, while the bap gene was found

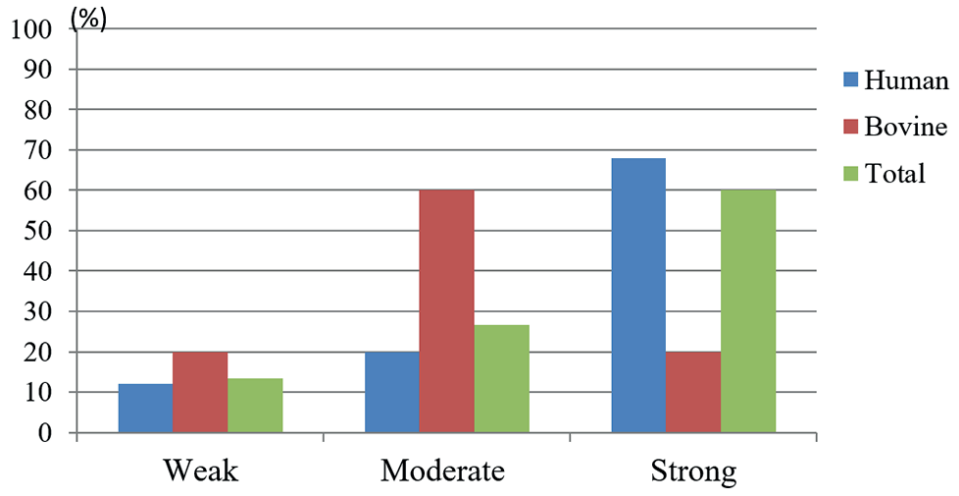

Figure 2. Biofilm formation status of isolates.

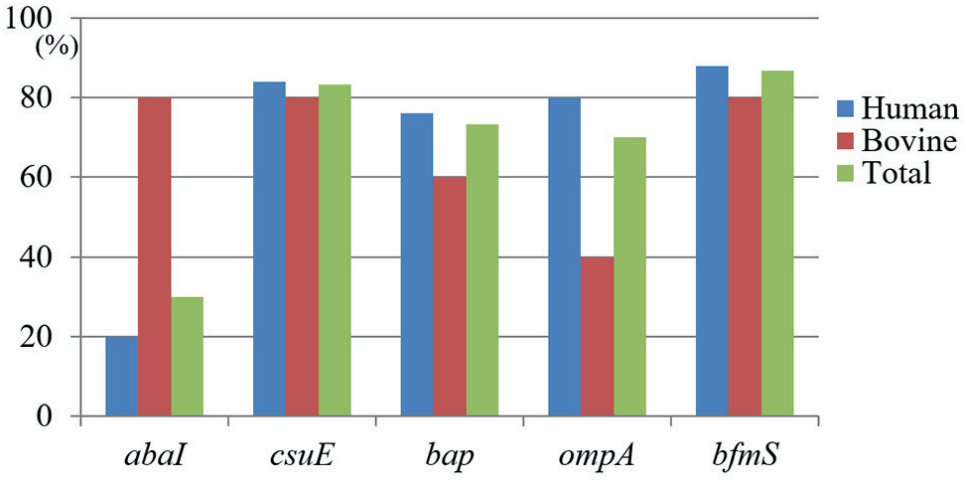

Figure 3. Distribution of biofilm-related virulence genes.

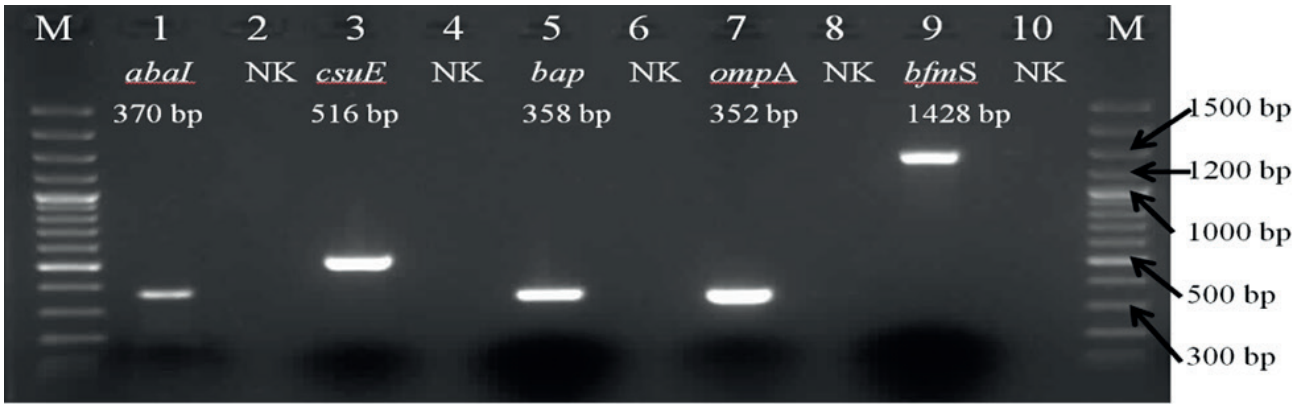

Figure 4. Gel electrophoresis of *A. baumannii* virulence genes **1.** *aba*I (370 bp) **3.** *csu*E (516 bp) **5.** *bap* (358 bp) **7.** *omp*A (352 bp) **9.** *bmf*S (1428 bp **2,4,6,8,10.** Negative Control (NK) (master mix without DNA) **M:** 100 bp DNA ladder (Vivantis).

Table 6. Frequency of biofilm-related virulence genes.

	*aba*I (%)	*csu*E (%)	*bap* (%)	*omp*A (%)	*bfm*S (%)
Human (n=25)	5 (20)	21 (84)	19 (76)	20 (80)	22 (88)
Bovine (n=5)	4 (80)	4 (80)	3 (16)	2 (60)	4 (80)
Total (n=30)	9 (30)	25 (83)	22 (73)	21 (70)	26 (87)

Table 7. Virulence gene phenotypes of isolates.

Number of virulence genes	Virulence gene phenotypes	Ratio (%), Isolate number (n=30)
2	*bfm*S, *omp*A	3 (1)
2	*csu*E, *aba*I	10 (3) (One bovine isolate)
2	*csu*E, *bap*	3 (1)
3	*bfm*S, *csu*E, *omp*A	7 (2)
3	*bfm*S, *bap*, *omp*A	13 (4) (One bovine isolate)
3	*bfm*S, *csu*E, *aba*I	3 (1)
4	*bfm*S, *csu*E, *bap*, *omp*A	43 (13)
4	*bfm*S, *csu*E, *bap*, *aba*I	10 (3) (Two bovine isolates)
4	*bfm*S, *csu*E, *omp*A, *aba*I	3 (1) (One bovine isolate)
5	*bfm*S, *csu*E, *bap*, *omp*A, *aba*I	3 (1)

at a low level in bovine isolates (16%), it was present at a high level (76%) in human isolates (Table 6, Figure 3, Figure 4).

Five isolates carried 2 virulence genes, six isolates (human) carried 2, seven isolates carried 3, fourteen isolates (human) carried 4 genes, seventeen isolates carried 4, and one isolate carried 5 virulence genes. In total, ten different virulence gene patterns were identified, with the most common pattern being the "*bfm*S, *csu*E, *bap*, *omp*A" resistance pattern (Table 7).

Integron genes

In this study, integron genes were detected in 90% of all isolates (23 human isolates and 4 bovine isolates out of a total of 27 isolates). It was determined that the *int*1 gene was carried at a higher rate (92%) compared to the *int*2 gene (52%) in human isolates, while in animal isolates, the presence of both genes was at an equal level (60%). According to the findings, only the class 1 integron gene was present in 37% of the isolates, while only the class 2 integron gene was found in 3% of the isolates. Notably, both classes of integron genes were detected together in 50% of the isolates. However, 10% of the isolates did not contain any class of integron gene (Table 8, Figure 5, Figure 6).

Statistical analysis

In our study, no statistically significant relationship was detected between the origin of the isolates and the severity of biofilm formation (Table 9).

When examining the relationship between the origin of isolates and the frequency of virulence gene presence, a significant association was found between the carriage of *aba*I and *bfm*S virulence genes. However, no significant relationship was detected between the origin of the isolate and the presence of virulence genes for the other three virulence genes (*bap*, *csu*E, *omp*A).

A significant relationship was found between strong biofilm formation and carrying all virulence genes (*aba*I, *csu*E, *bap*, *omp*A, *bfm*S), while similarly, a significant relationship was observed between moderate biofilm formation and carrying *aba*I and *bap* virulence genes, and between weak biofilm formation and carrying *omp*A and *bfm*S virulence genes (Table 11).

A significant relationship was found between strong biofilm formation and carrying integron genes in the isolates, whereas no significant relationship was identified between weak and moderate biofilm formation and carrying integron genes (Table 12).

Table 8. Rates of isolates carrying integron genes.

	*int*1 (%)	Only *int*1 (%)	*int*2 (%)	Only *int*2 (%)	*int*1+*int*2 (%)
Human (n=25)	23 (92)	10 (40)	13 (52)	0 (0)	13 (52)
Bovine (n=5)	3 (60)	1 (20)	3 (60)	1 (20)	2 (40)
Total (n=30)	26 (87)	11 (37)	16 (53)	1 (3)	15 (50)

Table 9. The relationship between the origin of the isolates and the severity of biofilm formation.

Biofilm formation	Origin of isolate		P	χ^2
	Human	Bovine		
Weak (+)	3	1	0.538	0.223
Weak (-)	22	4		
Moderate (+)	5	3	0.102	3.29
Moderate (-)	20	2		
Strong (+)	17	1	0.128	3.87
Strong (-)	8	4		

Table 10. Relationship between the origin of isolates and the frequency of virulence genes related to biofilm formation.

Virulance genes	Origin of isolate		P	χ^2
	Human	Bovine		
***aba*I (+)**	5	4	0.019*	6.91
***aba*I (-)**	20	1		
***csu*E (+)**	21	4	0.046	1
***csu*E (-)**	4	1		
***bap* (+)**	19	3	0.589	0.527
***bap* (-)**	6	2		
***omp*A (+)**	20	2	0.102	3.30
***omp*A (-)**	5	3		
***bfm*S (+)**	22	4	0.017*	6.77
***bfm*S (-)**	3	5		

*: Degree of statistical significance.

DISCUSSION

Acinetobacter species are known for their rapid development of antimicrobial resistance and their ability to persist in the environment for extended periods, which makes them a major concern (9). Moreover, it has been previously reported that *Acinetobacter* spp. can be found in soil, water, and sewage, as well as in the flora of both animal and human skin (29). One of the objectives of this study was to determine the prevalence of *A. baumannii* in clinical samples from humans and animals. In our study, we detected *A. baumannii* in 24% of human clinical samples and 2% of animal clinical samples. These findings indicate that *A. baumannii* is more frequently associated with human-derived isolates, but can also be found in isolates from animals. This highlights the potential significance of infections related to animal health as a source of concern for human health. For example, the prevalence of A. baumannii obtained from milk and dairy products in Egypt was found to be 3% (30). Similarly, a study conducted in Korea reported an incidence rate of 8% for *Acinetobacter*

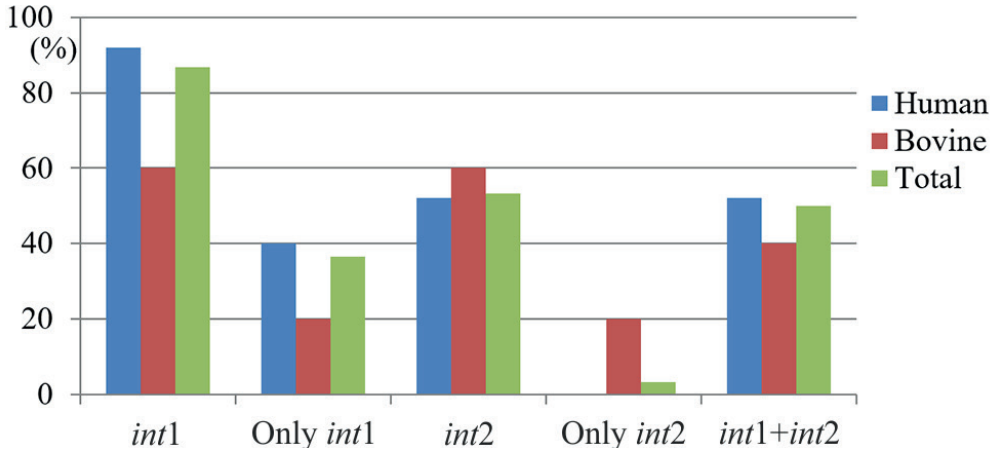

Figure 5. Integron gene carrying status of isolates.

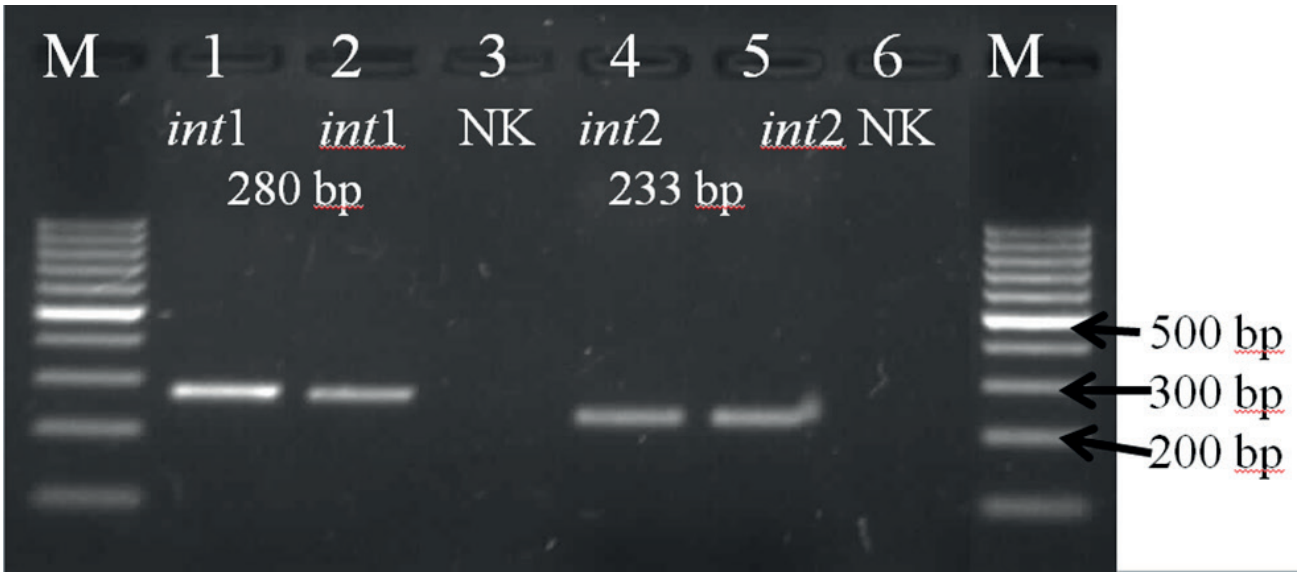

Figure 6. Integron classes of *A. baumannii* isolates. **1,2:** *int*1 gene positive *A. baumannii* isolates **4,5**: *int*2 gene positive *A. baumannii* isolates **3,6:** Negative Control (NK) (DNA-free master mix) **M:** 100 bp molecular marker (Fermentas).

spp. (31). Additionally, *Acinetobacter* spp. were isolated from 23% of raw milk samples from farmers' homes, with *A. baumannii* detected in 20% of these samples. These findings indicate that these microorganisms pose a serious threat to both animal and human health.

Bacterial infections resistant to antibiotics, especially those involving multidrug-resistant bacteria, can pose challenging treatment scenarios, leading to serious health complications and even death due to prolonged hospital stays and unsuccessful treatment attempts (32). In this study, similiar to previous research (33), it was found that all human isolates exhibited multidrug resistance, showing moderate to high levels of resistance to all antimicrobial agents except for tetracycline and lipopeptides. Although resistance rates were lower in animal isolates, resistance to aminoglycosides, penicillin, β-lactams, folate, and quinolone antibiotics were observed. These findings are consistent with prior studies (13). Overall, the high rates of antimicrobial resistance can be associated with the assumption that the samples were largely collected from individuals treated with antimicrobial agents. These results particularly highlight the broad spectrum of antibiotic resistance among human-derived *A. baumannii* isolates. They underscore the need for more effective and personalized treatment strategies to successfully manage *A.*

Table 11. Relationship between the biofilm-forming ability of isolates and the presence of virulence genes associated with biofilm formation.

Genes	Biofilm formation		P	χ2	Biofilm formation		P	χ2	Biofilm formation		P	χ2
	S (+)	S (-)			M (+)	M (-)			W (+)	W (-)		
*aba*I (+)	1	8	0.001***	12.377	5	3	0.016*	6.924	3	6	0.069	4.302
*aba*I (-)	17	4			3	19			1	20		
*csu*E (+)	13	12	0.004**	9.50	8	17	0.287	2.109	4	21	1	0.892
*csu*E (-)	5	0			0	5			0	5		
bap (+)	17	5	0.003**	9.91	3	19	0.016*	6.92	2	20	0.284	1.242
bap (-)	1	7			5	3			2	6		
*omp*A (+)	18	4	0.001***	15.818	4	18	0.158	2.936	0	22	0.003***	12.269
*omp*A (-)	0	8			4	4			4	4		
*bfm*S (+)	18	8	0.018*	6.692	7	19	1	0.006	1	25	0.004***	14.682
*bfm*S (-)	0	4			1	3			3	1		

*: Degree of statistical significance; **???????????; ***p<0.05.

Table 12. Relationship between the biofilm-forming ability of isolates and the presence of integron genes.

Biofilm formation	Integron genes		P	χ2
	Positive	Negetaive		
Weak (+)	3	1	0.360	1.115
Weak (-)	24	2		
Moderate (+)	6	2	0.166	2.636
Moderate (-)	21	1		
Strong (+)	18	0	0.050*	4.833
Strong (-)	9	3		

baumannii infections. Additionally, they demonstrate that *A. baumannii* presents a widespread and serious antimicrobial resistance issue in both humans and animals.

The ability of *A. baumannii* to colonize surfaces and form biofilms is known to be a significant factor contributing to chronic and persistent infections (34). Our findings indicate that all *A. baumannii* isolates possess the capacity to form biofilms; however, notably, there is a significantly higher tendency for strong biofilm formation in human isolates (68%) compared to animal isolates (20%). Our results align with previous reports (10,34), demonstrating that over 58% of *A. baumannii* isolates form strong biofilms. This suggests that human-derived isolates may be better adapted to biofilm formation, potentially increasing their infection potential. Conversely, a moderate tendency for biofilm formation appears to be more common in animal-derived isolates. This indicates that the biofilm-forming ability of *A. baumannii* isolates obtained from animals is milder compared to human isolates and may vary depending on environmental factors.

Studies have shown that *A. baumannii* possesses biofilm-associated genes such as *aba*I, *csu*E, *bap*, *omp*A, and *bfm*S, which influence biofilm formation (10, 11). According to the findings of this study, the presence of the *bfm*S gene was the highest, followed by *csu*E, *bap*, *omp*A, and *aba*I genes, which is consistent with other studies conducted in Iran, Thailand, and Korea (10,11,34,35). The presence of various virulence genes detected in *A. baumannii* isolates indicates various pathogenic mechanisms that enhance the pathogen's disease-causing potential. Specifically, the widespread presence of virulence genes such as *bfm*S, *csu*E, *bap*, *omp*A, and *aba*I is regarded as significant factors that may affect *A. baumannii*'s biofilm formation ability, cell adhesion, antibiotic resistance, and other infection-related properties (10, 11). The presence of different combinations of these virulence genes may reflect the complexity and diversity of the pathogen's infection dynamics.

Differences in the distribution of biofilm-associated virulence genes in *A. baumannii* isolates from human and animal sources highlight the diversity in the pathogen's infection dynamics. Particularly, the higher prevalence of the *aba*I gene in animal-derived isolates suggests the need for a different adaptation strategy, possibly due to the natural microbiome of animals and environmental conditions. During transitions between animals, *A. baumannii* may activate different virulence genes to adapt to envi-

ronmental conditions and initiate infections. However, the higher expression of the *bap* gene in human-derived isolates may suggest a more prominent role of biofilm formation in hospital environments and in the pathogenesis of infections associated with this gene. This finding suggests that the virulence mechanisms of the bacterium may vary depending on environmental factors and infection sources, indicating that different types of infections may exhibit varying pathogenicity characteristics.

The presence of integrons, known as the main reservoirs of antibiotic resistance genes in microbial populations, plays a significant role in the emergence of multidrug-resistant isolates (36). In our study, we found that class I integrons were more prevalent compared to class II integrons, which is consistent with other research findings (10,16). Our findings indicate the widespread presence of integron genes in *A. baumannii* isolates. The notably higher frequency of the *int*1 gene compared to the *int*2 gene in human-derived isolates resembles findings from other studies (16), suggesting a more common occurrence of this gene in human isolates. Conversely, the equal prevalence of both integron genes in animal-derived isolates indicates a different distribution in isolates obtained from animals. Additionally, the low occurrence of isolates carrying only class 1 or class 2 integron genes, and the high occurrence of isolates with both classes together, suggest that combinations of different integron classes may be common in the *A. baumannii* population. These findings suggest that integrons may play a significant role in the spread of antibiotic resistance in *A. baumannii.* However, the concurrent increase in multidrug-resistant isolates and integron resistance genes will lead to the failure of antibiotic treatment strategies. The absence of any class integron gene in some isolates suggests the presence of different resistance mechanisms, which may not be dependent on integrons.

The statistically non-significant relationship between the origin of isolates and the severity of biofilm formation is a noteworthy finding of our study. These results indicate that the biofilm-forming abilities of *A. baumannii* isolates from human and animal origins do not vary depending on their sources. This suggests that the potential for biofilm formation in *A. baumannii* is similarly distributed among isolates from different sources. This finding may imply that the biofilm-forming ability of *A. baumannii* is more dependent on genetic characteristics rather than environmental factors. However, further research with a larger sample size is needed to investigate this aspect more thoroughly.

The investigation of the relationship between the origin of isolates and the frequency of virulence genes reveals an important finding of our study. Specifically, the association of *aba*I and *bfm*S virulence genes with the origin of isolates suggests that certain virulence genes may exhibit different distributions among isolates from human or animal sources. This finding implies that *A. baumannii* isolates from different sources may show variations in virulence gene profiles, indicating that the pathogen employs different genetic strategies to adapt to environmental conditions.

The significant association between strong biofilm formation and the carriage of integron genes highlights the role of integrons in bacterial biology as a critical component in the spread of biofilm formation and antimicrobial resistance. However, the lack of a significant relationship between weak and moderate biofilm formation and the presence of integron genes may suggest that biofilm formation alone may not be sufficient as a determinant factor in resistance development. This finding could contribute to a better understanding of the role of biofilm formation in antimicrobial resistance mechanisms and lay a foundation for future research endeavors.

This study aimed to determine the frequency of *A. baumannii* in human and animal clinical samples, analyze antimicrobial resistance profiles, investigate biofilm formation abilities, and examine the presence of *int*1 and *int*2 genes. Our findings indicate that *A. baumannii* isolates from both human and animal sources have the potential to form biofilms and pose a significant antimicrobial resistance problem. Specifically, human-derived isolates show higher levels of antibiotic resistance compared to those from animals, although isolates from both sources are concerning in terms of antimicrobial resistance. Additionally, our data revealed the more widespread presence of *int*1 compared to *int*2 suggesting the significant role of integrons in the spread of antimicrobial resistance. The diversity of virulence genes and biofilm formation ability are believed to have a decisive impact on the pathogenic potential of *A. baumannii*. In conclusion, the widespread antimicrobial resistance and biofilm formation ability observed in human and animal-derived *A. baumannii* isolates represent a significant public health issue. These findings underscore the need for the development of more effective strategies for the treatment and control of

A. baumannii infections, as well as the necessity for further research in this field.

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