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Molecular Detection and Antibiotic Sensitivity of *Acinetobacter baumannii* isolated from Clinical Samples in Thi-Qar- Southern Iraq

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Abstract

Acinetobacter baumannii is a gram-negative coccobacillus and a critical global health threat due to its high antibiotic resistance, particularly in hospital settings and intensive care units (ICUs). As an ESKAPE pathogen, it causes severe infections such as pneumonia, wound infections, and bloodstream infections, especially in immunocompromised patients.This study aimed to characterize A. baumannii clinical isolates in Southern Iraq, determine their antibiotic resistance profiles, and identify key resistance genes. A total of 50 clinical isolates were collected from Nasiriyah and Al-Hussein Hospitals, including sputum (28%), burns (48.3%), and blood samples (24%). Identification and antimicrobial susceptibility testing were performed using the VITEK-Compact system, while molecular characterization was carried out using PCR to detect resistance genes. Among the isolates, high resistance rates were observed in MDR strains, particularly to ampicillin-sulbactam (95%), cefazoline (95%), piperacillin (90%), doxycycline (90%), ceftazidime (80%), cefotaxime (80%), ciprofloxacin (80%), levofloxacin (75%), ceftriaxone (75%), and imipenem (50%). Molecular analysis confirmed the presence of resistance genes including blaOXA-51, BAP, CsuE, and OmpA. The findings reveal an alarming prevalence of multidrug-resistant A. baumannii in clinical settings in Southern Iraq, highlighting the urgent need for effective antimicrobial stewardship programs, strict infection control measures, and the development of innovative therapeutic strategies to combat these resistant pathogens.

1.1. Introduction

Acinetobacter involve a group of bacteria that are Gram-negative aerobic bacilli or coccobacilli (1) ; catalase-positive, oxidase-negative; Including its one types is Acinetobacter has emerged as one of the most pathogens . A. baumannii has been remarked by its ability to up regulate and obtain the determinants of antibiotic resistance (2). There are of antibiotic resistance mechanisms of A. baumannii including: Opposition can be developed by reducing membrane permeability and rising efflux of antibiotics, thus restricting access to the aim. Or, microbe can through genetic mutation or post-translational modification a protect the antibiotic target and thus, antimicrobial agents can be suppressed directly by variety of modification process (3). which is called This rise of multidrug –resistant (MDR) pathogens have become an increasingly serious concern in both nosocomial and community-acquired infections. The extensive misuse of antibiotic and inefficient management have contributed to the emergence of MDR isolates. These isolates are commonly linked to a medical history of prolonged hospital stays, catheter use and mechanical ventilation. Additionally, immunocompromised and critically ill patients are more susceptible to invasive infections (4). MultiDrug Resistant pathogens (MDR) that are most common and serious which is called acronym “ESKAPE,” these are, S. aureus, , K. pneumoniae, E. faecium, A. baumannii, P. aeruginosa and Enterobacter spp. (5). Series of infections caused by A. baumannii a, such as meningitis, respiratory and urinary tract infections, endocarditis, wound infections and bacteremia, especially in the patients admitted in the intensive care units (6). A.

baumannii has an ability potential to form biofilm this interpret resistance and survival in the hospital environment, this is an essential pathogenic mechanism in such infections..(7) Consequently, the clinical isolates of A. baumannii can survive for longer periods under the highly desiccated conditions on abiotic surfaces(8). In this study was conducted for identify the spread of *A. baumannii* in clinical infections in Southern Iraq. Evaluate its resistance patterns to commonly used antibiotics. Characterize its molecular properties and genetic determinants of resistance. By combining phenotypic and molecular methods, this research aims to detection of *A. baumannii* in clinical samples from infected patients, molecular diagnosis, and assessment of its antibiotic resistance levels against different types of antibiotics to provide actionable data to inform treatment protocols and infection control measures.

1.2. Methodology and Approaches

1.2.1. Collection of Sample

In this study, 200 samples were collected from different wards of the hospital; which was 76(36%) were isoleted from blood (Septicemia, infective endocarditis and systemic infections), 51(25.5%) from swab burn(burn wound infections, necrotizing infections and gangrene) and 73(36.5%) from sputum(hospital-acquired pneumonia, chronic bronchitis and tuberculosis). The swabs were taken during the period from November, 2023 to May, 2024.Collected swabs of different age groups (male 110(55%) and female 90(45%)) aged between <1 and 60 years.

1.2.2. Isolation of A. baumannii from clinical samples

The elaboration of all culture media including MacConkey and blood agar, according to as specified by the guidelines of the manufacturer; the autoclave was used to sterilize media at 121Co (15 Ib/In2) for 15 minutes, and at (35–37Co)for (24–48) hours, was incubated. Colony morphology, such as shape, color, and size, was recorded.

1.2.3. Identification of *A. baumannii*

The VITEK-2 system, which involved multiple procedures in accordance with Maha et al. (2022)(9), was employed in this investigation to ensure the diagnosis of *A. baumannii* isolates. Biochemical assays, such as oxidase, catalase, indole, and urease tests, were performed. Bacterial suspension preparation: *A. baumannii* colonies from a pure culture were transferred using a sterile swab and individually suspended in 3 ml of sterile saline in a clear plastic test tube. The objective was to use BioMerieux/Densi CHEK plus (France) to bring the turbidity of the bacteria down to 0.5 MacFarland, or 1.5*108 C F U/ml.

1.2.4. Antibiotic Susceptibility Test

The VITEK-2 system (bioMérieux, France) was used to determine the antibiotic susceptibility of *A. baumannii* isolates. The following antibiotics were tested: ciprofloxacin, ceftazidime, imipenem, and colistin, among others. Were determined Minimum Inhibitory Concentrations (MICs) according to Clinical and Laboratory Standards Institute (CLSI- 2023) guidelines.

1.2.5. Molecular Techniques

1.2.5.1. DNA Extraction

Genomic DNA was extracted from isolates using Proteinase K and column-based purification methods (standard protocol), DNA purity was assessed using a Nanodrop spectrophotometer at 260/280 nm.

1.2.5.2. PCR Amplification

Specific primers targeting 16S rDNA and resistance genes (blaOXA-51, BAP, CsuE, OmpA) were used. PCR reactions were carried out under optimized conditions, as per manufacturer guidelines. Thermal cycling conditions included initial denaturation (94°C for 5 min), followed by 35 cycles of denaturation, annealing, and extension..

Table (1) Preparation of PCR reaction mixture

PCR	Volume
Master mix	**10 µL**
Nuclease free water	**10.5**
Forward primer (10pmol)	**1**
Reverse primer (10pmol)	**1**
DNA template	**2**
$Mgcl_2$	**0.5**
Total	**25**

Table (2) Primers Using in RAPD PCR

Primer name		Sequence (5….3)
Bap	F	TGCTGACAGTGACGTAGAACCACA
Bap	R	TGCAACTAGTGGAATAGCAGCCCA (184bp)
CsuE	F	CATCTTCTATTTCGGTCCC
CsuE	R	CGGTCTGAGCATTGGTAA(168)
OmpA	F	GTTAAAGGCGACGTAGACG
OmpA	R	CCAGTGTTATCTGTGTGACC(578)
$bla_{\text{OXA-51}}$	F	TAATGCTTTGATCGGCCTTG
$bla_{\text{OXA-51}}$	R	TGGATTGCACTTCATCTTGG (353bp)

1.2.5.3. Gel Electrophoresis

PCR products were resolved on a 1% agarose gel, stained with ethidium bromide, and visualized under UV light. A DNA ladder was used to assure the sizes of amplicons.

1.3. Results and Conclusions

1.3.1. Distribution of samples

Out of 200 samples, 50 isolates were identified as *A. baumannii*. There were isolates from sputum14(28%), from burns 24(48.3%), and from blood 12(24%).Fig. 1. describe the percentage of *A. baumannii* Allocation according to samples .Tables

1.3.2. Antibiotic Resistance

The isolates exhibited high resistance rates to ceftazidime , ciprofloxacin , and imipenem etc.. with MIC values exceeding standard thresholds.

Table(3): Antibiotic Sensitivity Patterns of *A. baumannii*, Using the VITEK 2 System

No	Antimicrobial	R.	(MIC)	S.	(MIC)	I.	(MIC)
1	Piperacillin (PI)(100ug)	46 92%	≥ 128	2 4%	≤ 16	2 4%	(32-64)
2	Ampicillin-sulbactam(AMS) (10\10ug)	47 94%	≥ 64/32	1 2%	≤ 8/4	2 4%	(16/8-32/16)
3	Ceftazidime(Caz) (30ug)	40 80%	≥ 64	3 6%	≤ 8	7 14%	(16-32)
4	Cefepime (Fep)(30ug)	35 70%	≥ 64	5 10%	≤ 8	10 20%	(16-32)
5	Cefazoline (30 ug)	47 94%	≥ 128	1 2%	≤ 16	2 4%	(32-64)
6	Cefotaxime(CTX) (30 ug)	40 80%	≥ 64	3 6%	≤ 8	7 14%	(16-32)

7	Ceftriaxone (Cro) (30 ug)	37 74%	≥ 64	3 6%	≤ 8	10 20%	(16-32)
8	Imipenem (IPM) (10 ug)	25 50%	≥ 16	10 20%	≤ 2	15 30%	(4-8)
9	Meropenem(MEM)(10 ug)	22 44%	≥ 16	13 26%	≤ 2	15 30%	(4-8)
10	Gentamicin (GEN) (10 ug)	30 60%	≥ 32	10 20%	≤ 4	10 20%	(8-16)
11	Tobramycin(TOB) (10 ug)	32 64%	≥ 32	5 10%	≤ 4	13 26%	(8-16)
12	Amikacin(AK)(30 ug)	15 30%	≥ 128	15 30%	≤ 16	20 40%	(32-64)
13	Netilmicin (NET)	20 40%	≥ 128	12 24%	≤ 16	18 36%	(32-64)
14	Doxycyline (DY) (30 ug)	45 90%	≥ 16	2 4%	≤ 4	3 6%	(8)
15	Tigecycline ()	10 20%	≥ 8	25 50%	≤ 2	15 30%	(4)
16	Ciprofloxacin(CIP)(5ug)	40 80%	≥ 4	2 4%	≤ 1	8 16%	(2)
17	Levoflaxacin(LEV) (5ug)	37 74%	≥ 8	3 6%	≤ 2	10 20%	(4)
18	Trimethioprim-Sulfamethoxazole(SXT) (1.25\23.75ug)	42 84%	≥ 8/152	3 6%	≤ 2/38	5 10%	(4/76)

R(resistant), S (susceptibl), I (intermediate) No.(number).

According to Table 3 above, the results of the antibiotic sensitivity test for *Acinetobacter baumannii* isolated from clinical samples show that there were 20 (40%) that showed MDR pattern against antimicrobial agents these isolates distributed as follows: 15(30%) of them were resistant to piperacillin and ampicillin-sulbactam within the group of penicillins and ceftazidime and cefazoline within fourth generations cephalosporin group and tobramycin and amikacin within aminoglucacosides group. The other 5(10%) MDR isolates were resistant to meropenem, doxycyline and levofloxacin.

1.3.3. Molecular Findings

PCR amplification revealed the presence of resistance genes, including bla_{OXA-51}, BAP, CsuE, OmpA) Sequencing and phylogenetic analysis highlighted genetic variability among isolates, suggesting multiple sources of infection. As shown in Figures.

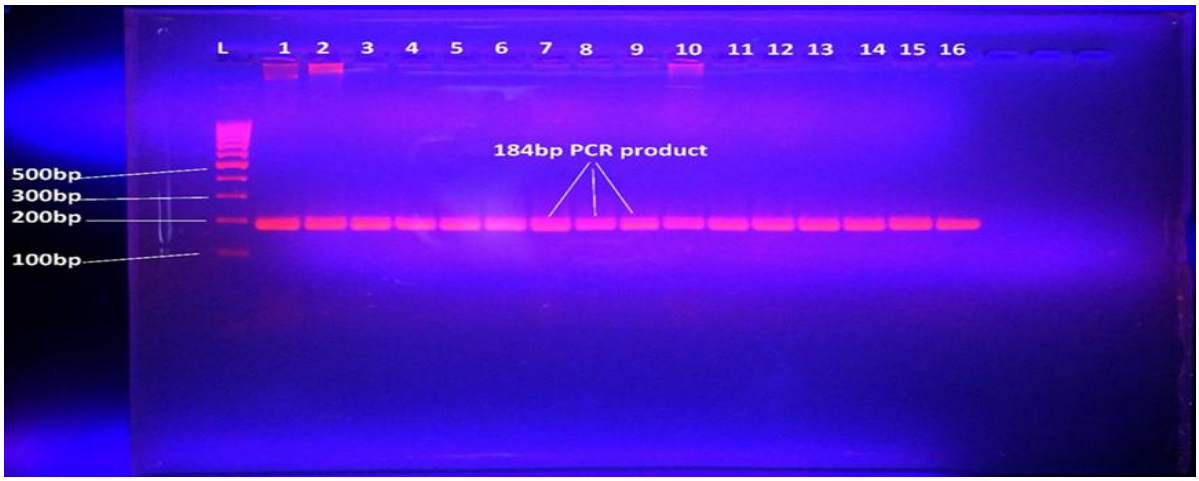

Fig.(1) Detection of Bap gene by PCR , the specific amplicon size is 184bp, lane L 100 bp DNA ladder , other lanes show positive for all isolate.

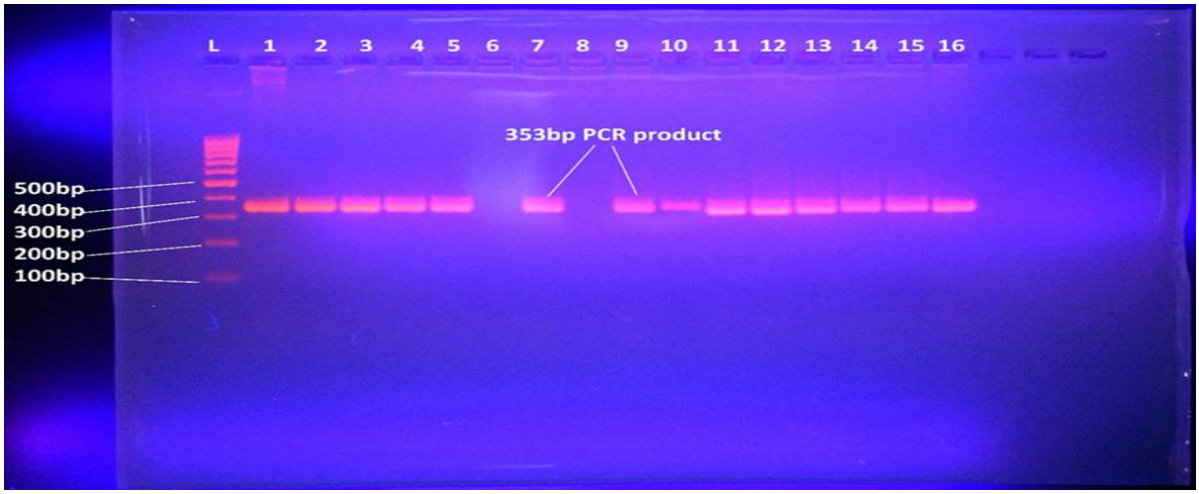

Fig.(2) Detection of blaOXA-51 gene by PCR , the specific amplicon size is 253bp, lane L 100 bp DNA ladder , lane 6 and 8 negative isolate, other lanes are positive.

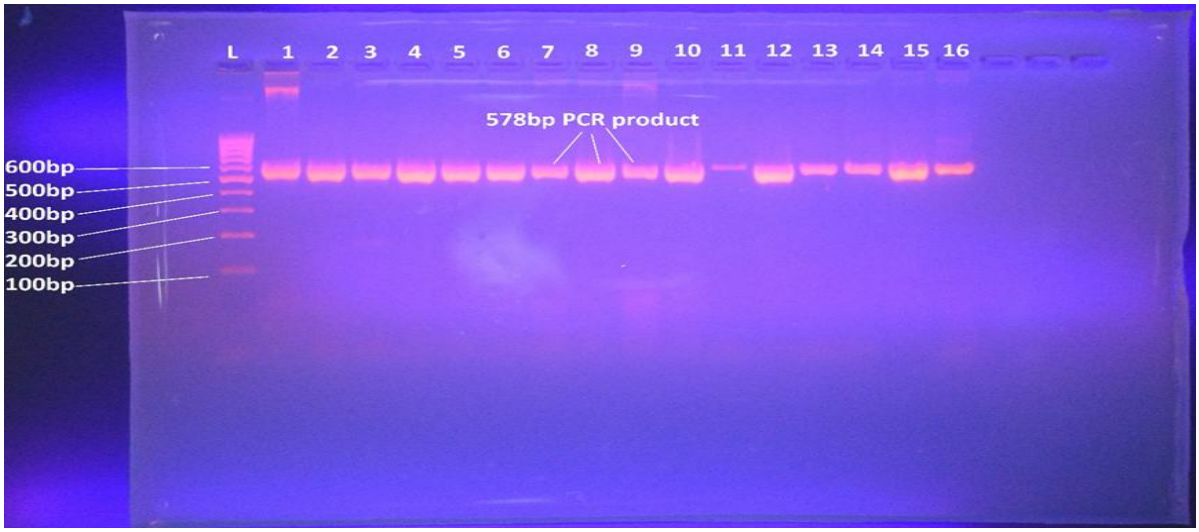

Fig.(3) Detection of OmpA gene by PCR , the specific amplicon size is 578bp, lane L 100 bp DNA ladder , other lanes are positive isolate.

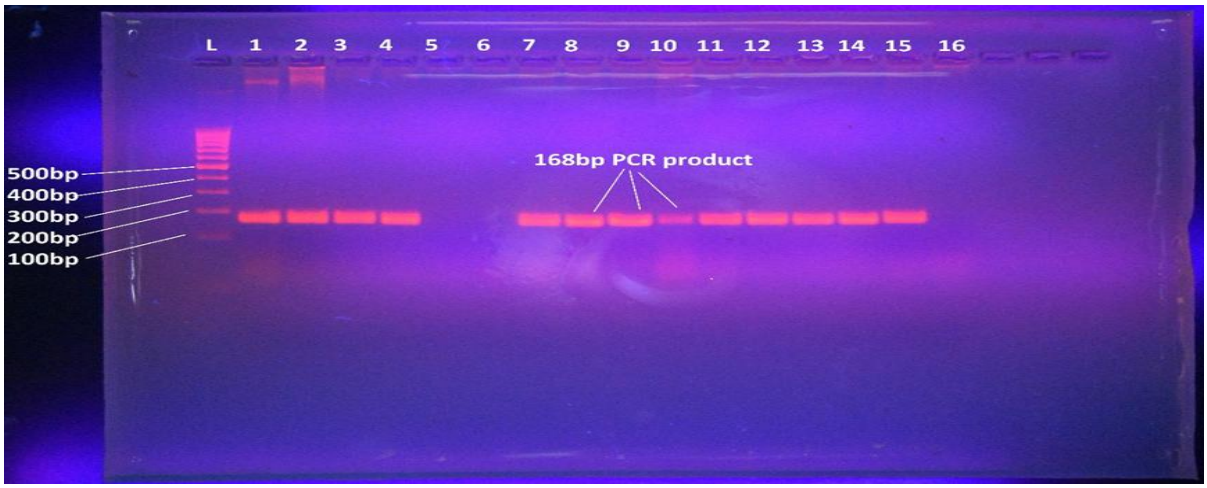

Fig.(4) Detection of CsuE gene by PCR , the specific amplicon size is 168bp, lane L 100 bp DNA ladder , lane 5,6 and 16 negative isolate, other lanes are positive.

Discussion

The study highlights the alarming resistance levels of *Acinetobacter baumannii* to commonly used antibiotics in Southern Iraq, aligning with global trends. Molecular findings suggest a high resistance to cefepime, cefazolin, trimethoprim, gentamicin, piperacillin, imipenem, ciprofloxacin, ampicillin, ceftriaxone, and ceftazidime. Previous reports have shown that between 30% and 83.9% of *A. baumannii* strains are multidrug-resistant (MDR) (10). Additionally, studies have demonstrated increased resistance to cephalosporins, extended-spectrum penicillins, carbapenems, aminoglycosides, and fluoroquinolones (11). A study by Maha E. Zidan et al. (2022)(9) on Acinetobacter strains showed a significant prevalence of resistance to tetracycline (92%) and amikacin (72%), as well as ciprofloxacin (76%) and meropenem (72%). The 86 *A. baumannii* isolates had a multidrug resistance (MDR) profile, including DR (47.6%), XDR (37.6%), and MDR (15.1%). The susceptibility profiles of these isolates showed considerable variation. The increasing multidrug resistance in *A. baumannii* has led to significant medical challenges in treating infected patients. Several factors contribute to this resistance, including environmental conditions and antibacterial application patterns, which vary by country and healthcare setting. *A. baumannii* has been isolated from blood, sputum, and burn samples, demonstrating its ability to proliferate in hospital environments and contaminate exposed tissues. Moreover, it has been shown to acquire and disseminate antibiotic resistance while contaminating respiratory system insertion devices. According to Silvia D'Arezzo et al. (2011)(12), differences in antibiotic usage patterns and the lack of resources to combat hospital infections influence the transmission and development of resistant microorganisms across hospital departments. These findings emphasize the urgent need for innovative treatment strategies and strict antibiotic stewardship programs to control the spread of drug-resistant *A. baumannii*. Strengthening infection control measures and developing alternative therapeutic approaches are essential to mitigating the clinical impact of this highly resistant pathogen.

Conclusion

In this study confirms the high prevalence of MDR *A. baumannii* in clinical samples from Nasiriyah Hospital. The integration of phenotypic and molecular methods provided comprehensive insights into resistance mechanisms. Future efforts should focus on implementing infection control measures and monitoring resistance trends. Regular monitoring of antibiotic resistance patterns in healthcare settings, Rational use of antibiotics to minimize resistance and xploring alternative therapies and novel antimicrobial agents.

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