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Identification of beta-lactamase genes (KPC, NDM, IMP, VIM, and OXA-48) in Acinetobacter baumannii bacteria isolated from various clinical samples in Sana'a City, Yemen

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Abstract

Background: Acinetobacter baumannii has emerged as an opportunistic pathogen, particularly in hospital settings, where its ability to rapidly acquire resistance to multiple classes of antimicrobial agents. This study aimed to investigate the prevalence of carbapenemase-encoding genes among CRAB isolates in Sana'a, Yemen.
Methods: A cross-sectional study was conducted between October 2023 and December 2024. A total of 74 non-duplicate CRAB isolates were collected from various clinical specimens at two private hospitals and the National Center of Public Health Laboratories in Sana'a. Real-time PCR was utilized to detect five major carbapenemase genes *(blaOXA-48, blaNDM, blaVIM, blaKPC, and blaIMP)*.
Results: Among the 74 isolates, 68 (91.9%) were positive for at least one carbapenemase-encoding gene. The most predominant gene was *blaOXA-48* (89.1%), followed by *blaNDM* (29.7%) and *blaVIM* (18.9%). Notably, neither the *blaKPC* nor the *blaIMP* genes were detected.
Conclusion: The study shows a high prevalence of carbapenemase-encoding genes among *A. baumannii* in Sana'a, *blaOXA-48* serving as the primary genetic driver of resistance to carbapenems.

Article Info

Keywords:
Acinetobacter baumannii, blaOXA-48, Yemen, Carbapenem-resistance, blaNDM, PCR, Antimicrobial Susceptibility.

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1. INTRODUCTION

Carbapenem-resistant *Acinetobacter Baumannii* (CRAB) has become a major concern in modern healthcare systems and is recognized by the World Health Organization as a critical priority pathogen requiring urgent research attention [1]. This Gram-negative organism is notable for its ability to survive under harsh environmental conditions and its exceptional capacity to acquire resistance to multiple classes of antimicrobial agents [2]. CRAB is commonly implicated in severe hospital-acquired infections, including ventilator-associated pneumonia, bloodstream infections, wound infections, and urinary tract infections, particularly prevalent in intensive care units where high morbidity and mortality rates [3].

The mechanisms underlying carbapenem resistance in *A. baumannii* are complex and involve several pathways; however, the production of carbapenemase enzymes represents the most clinically significant mechanism [4]. These genetic determinants are frequently located on mobile genetic elements such as plasmids, transposons, and integrons, facilitating their rapid spread between bacterial species [5]. The five major carbapenemase families of particular concern in *A. baumannii* include metallo-β-lactamases (MBLs) belonging to Ambler class B (*blaIMP*, *blaNDM*, and blaVIM), and serine carbapenemases belonging to Ambler class A (*blaKPC*) and class D (*blaOXA-48* and related variants) [6].

Class D β-lactamases demonstrate weak carbapenemase activity but confer clinically significant resistance when overexpressed, often through the insertion of ISAba1 elements upstream of the resistance genes [7]. The metallo-β-lactamases, including *blaNDM*, blaVIM, and *blaIMP*, have demonstrated particularly rapid global dissemination, with *blaNDM* variants being increasingly reported in *A. baumannii* [8].

The distribution of carbapenemase-producing *A. baumannii* varies across geographic regions [9]. In the Middle East and North Africa region, CRAB has reached endemic proportions, with carbapenem resistance rates frequently exceeding 70% in tertiary care hospitals [10]. Studies from the Gulf Cooperation Council states have revealed the dominance of blaOXA-23-type producers, while investigations from Egypt, the United Arab Emirates, Lebanon, and Iraqi Kurdistan have documented increasing reports of metallo-β-lactamases, including *blaNDM* and *blaIMP*, often co-existing with blaOXA genes, creating extensively drug-resistant phenotypes [11–13].Ongoing challenges in healthcare infrastructure, especially in conflict-affected regions, may further accelerate the spread of multidrug-resistant organisms [3, 14].

Baseline clinical data regarding carbapenemase distribution in Yemen remain limited but are gradually emerging. A previous study reported the first identification of multidrug-resistant *A. baumannii* isolates harboring the blaOXA-23 carbapenemase gene and the 16S rRNA methylase armA gene from patients at Al-Thawra University Hospital in Sana'a, demonstrating the early circulation of OXA-type carbapenemases in Yemeni healthcare settings. Subsequently [15]. Other documented high levels of multidrug resistance among *A. baumannii* complex isolates recovered from intensive care unit patients in Sana'a, noting that rifampin and colistin were the most active antimicrobial agents against these isolates [16]. Collectively, these local investigations underscore an alarming rise in multidrug-resistant organisms in Sana'a and highlight the urgent need for up-to-date molecular surveillance, which the present study addresses by providing the first comprehensive characterization of five major carbapenemase genes *(blaOXA-48, blaNDM, blaVIM, blaKPC, and blaIMP)* among CRAB isolates in the city.

The clinical implications of carbapenemase-producing *A. baumannii* are profound, with infections associated with significantly higher mortality rates (30–60 %) and severely restricted therapeutic options [5, 6]. The specific carbapenemase gene profile has important implications for treatment selection, as metallo-β-lactamase-producing strains demonstrate resistance to virtually all β-lactam antibiotics and are not susceptible to β-lactamase inhibitor combinations [6]. Furthermore, the emergence of colistin resistance in carbapenemase-producing *A. baumannii* has created pan-drug-resistant phenotypes with essentially no reliable treatment options [5].

This study aimed to determine the prevalence, distribution, and co-occurrence of carbapenemase-encoding genes (textit blaOXA-48, blaNDM, blaVIM, blaIMP, and blaKPC) among carbapenem-resistant *Acinetobacter baumannii* isolates obtained from different clinical specimens in Sana'a, Yemen; to analyze the distribution of individual carbapenemase genes across various clinical specimens; and to evaluate the antimicrobial resistance profiles in relation to carbapenemase gene presence.

2. MATERIALS AND METHODS STUDY

2.1. Design and Study area

A cross-sectional study included positive cultures (blood, sputum, urine, pus, and wounds) selected from two private hospitals (Al-Yemen AL-Saeed hospital and Modern European Hospital) and the National Center of Public Health laboratories (NCPHL). at Sana'a, Yemen from October 2023 to December 2024.

A total of 74 *Acinetobacter baumannii* isolates from various clinical specimens were tested for β-lactamase production.

2.2. Inclusion criteria

All positive cultures of *Acinetobacter baumannii* obtained from various clinical specimens that showed resistance to all carbapenems, imipenem, and meropenem were included in the study.

2.3. Sample size

The sample size was calculated using EPI Info version 7.1. Based on a prevalence of carbapenemase-encoding genes of 3.1% [17], with a 95% confidence level (Z = 1.96) and a margin of error of 4%, the calculated minimum sample size was 74 isolates. We intentionally chose a tighter 4% margin of error instead of the standard 5% (which would have required only 47 isolates) to ensure greater statistical precision in identifying beta-lactamase gene patterns. To achieve this target, we employed a consecutive sampling strategy, enrolling all non-duplicate clinical isolates of carbapenem-resistant *A. baumannii* recovered from routine specimens during the 14-month study period (October 2023 to December 2024). This approach yielded a final cohort of exactly 74 isolates, which satisfied the pre-calculated minimum statistical requirement.

2.4. Specimens Collection

All specimens were processed according to the Clinical Microbiology Laboratory Standard Operating Procedures.

2.5. Bacterial Isolation

Acinetobacter baumannii species were isolated from clinical samples processed in the routine diagnostic laboratory at the National Center of Public Health Laboratories Sana'a (NCPHL), or isolated from blood, urine, wound, and sputum specimens from patients admitted to various wards in Al-Yemen AL-Saeed Hospital, Modern European Hospital in Sana'a, Yemen. Antibiotic susceptibility testing was performed by the disc diffusion method using Mueller-Hinton agar (BBL, Becton Dickinson, Cockeysville, MA, USA) and interpreted according to the CLSI, 2023 [18].

2.6. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller–Hinton agar (BBL, Becton Dickinson, Cockeysville, MA, USA) plates using the Kirby–Bauer disk diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2023. The following antibiotic disks (Himedia, India) were used: ceftazidime (30 µg), cefepime (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), amikacin (30 µg), tobramycin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), piperacillin-tazobactam (110µg), colistin (10µg), ticarcillin (30 µg), and minimum inhibitory concentration (MIC) for colistin was determined by colistin broth disk elution test for all *Acinetobacter baumannii* isolates according to the CLSI guidelines. Escherichia coli ATCC 25922 was used as the quality control strain [18].

2.7. Strategy for carbapenemase detection

The detection strategy includes a screening step, followed by phenotypic and genotypic confirmation steps. Screening is based on the detection of reduced susceptibility to carbapenems by carbapenemase- producing isolates.

2.8. Screening test

The susceptibility of the test isolates to carbapenems was evaluated using the Kirby-Bauer disk diffusion method according to the CLSI criteria. MBL-producing isolates were suspected when the test organism(s) were resistant to any carbapenem (imipenem and meropenem). As per the CLSI criteria, isolates showing an inhibition zone diameter (IZD) of $\leq$23 mm were suspected to produce MBL enzymes, and these isolates were subjected to a phenotypic confirmation test [18].

2.9. Phenotypic test

2.9.1. Combined Disk Test

To detect metallo-beta-lactamase (MBLs) production, a combined disk test was performed. An overnight culture adjusted to a 0.5 McFarland standard was spread over the surface of a Mueller–Hinton agar plate using a cotton swab. Two disks, one imipenem and the other imipenem/ethylenediaminetetraacetic acid (EDTA), were laid on the surface of the agar at a distance of 4–5 cm from each other using sterile forceps. After an overnight incubation, the enhancement of the inhibition zone ($\geq$7 mm) of the IPM-EDTA disk compared to the imipenem disk alone was considered positive for the presence of MBLs. This test was performed in duplicates to ensure the reproducibility of the results [19].

2.10. Genotyping Method

2.10.1. DNA isolation.

Bacterial DNA Extraction. Bacterial genomic DNA was extracted using a modified boiling method [20]. Pure bacterial colonies were collected from all isolates and added to 100 µL of sterilized distilled water. Bacterial inoculums were boiled using an Eppendorf Thermomixer (Thermo Fisher Scientific, Waltham, MA, USA) at 99 ◦C for 5 min. The tubes were placed on ice for 3 min, then centrifuged at 1300 rpm for 20 min. Eighty microliters of the supernatant containing DNA was transferred into new Eppendorf tubes, and 160 µL of cold absolute ethanol was added; the mixture was centrifuged at 1300 rpm for 5 min. The supernatant was removed, 100 µL of 70% ethanol was added to wash the pellet, followed by further centrifugation at 1300 rpm for 5 min, and then the supernatant was discarded. The pellet-containing genomic DNA was allowed to dry completely, then re-suspended in 50 µL of nuclease-free water. The concentrations and DNA purities were evaluated spectrophotometrically using QuickDrop (Molecular Devices, San Jose, CA, USA) [20].

2.10.2. PCR reaction

Detection of the five carbapenemase-encoding genes (*blaNDM*, blaVIM, blaOXA-48, *blaKPC*, and *blaIMP*) was performed using the VIASURE Carbapenemase-producing Enterobacteriaceae Real Time PCR Detection Kit (reference VS-CPE106L, CerTest Biotec, Spain), according to the manufacturer's instructions. The kit is a multiplex real-time PCR assay containing specific primers and fluorescent probes for each target gene, along with an internal control [21]. Polymerase chain reactions were performed in two tubes as per the manufacturer's protocol by adding master mix, primer and probe mix, internal control, and DNA template, along with positive and negative control, and a final reaction volume of 25 µl was prepared. The tube was centrifuged briefly

at 6000 rpm for approximately 10 s, and the tube was placed in a real-time PCR machine. The PCR program was set to interpret the data from the amplification plot. The PCR program, as per the manufacturer's protocols, was as follows: Initial denaturation was performed at 95℃ for 10 min, denaturation at 95℃ for 05 s, annealing and extension were performed at 60℃ for 1 min, for 45 cycles, and the results were interpreted. In this study, PCR has been taken as a gold standard test for the detection of carbapenem-resistant *Acinetobacter baumannii* (CRAB) genes [22].

2.11. Statistical Analysis

Statistical analyses, including descriptive statistics, frequency tables, and figures, were performed using the Statistical Package for the Social Sciences software IBM SPSS version 26 (SPSS Inc., Chicago, IL, USA).

2.12. Ethical consideration

This study was approved by the institutional ethics committee of the participating hospitals and the National Center of Public Health Laboratories in Sana'a, Yemen. The study involved only bacterial isolates collected as part of routine clinical care, and no additional patient intervention was performed. The ethics committee waived patient consent as the study used de-identified bacterial isolates without access to patient-identifiable information.

3. RESULTS

A total of 74 carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates were collected from various clinical specimens and screened for the presence of five major carbapenemase-encoding genes: *blaNDM*, blaVIM, blaOXA-48, *blaKPC*, and *blaIMP*. Distribution analysis revealed that *blaOXA-48* was the predominant carbapenemase gene, detected in 66 isolates (89.1%), followed by *blaNDM* in 22 isolates (29.7%) and *blaVIM* in 14 isolates (18.9%). Notably, neither *blaKPC* nor *blaIMP* genes were detected in any of the *A. baumannii* isolates examined (0/74, 0%) (Table 1).

Overall, 68 out of 74 isolates (91.9%) harbored at least one carbapenemase-encoding gene, whereas only six isolates (8.1%) tested negative for all five genes examined (Table 2). Among the 68 positive isolates, the distribution of gene carriage patterns showed considerable variation in the present study. Single-gene carriage was the most common pattern, observed in 40 isolates (54.1%). Co-carriage of two genes was detected in 22 isolates (29.7%), while six isolates (8.1%) carried three different carbapenemase genes simultaneously. No isolates demonstrated the presence of four or five carbapenemase genes (Table 3).

Carbapenemase-encoding genes were detected across all specimen types with remarkably high positivity rates. Wound, urine, and blood samples demonstrated 100.0% positivity rates (16/16, 2/2, and 6/6, respectively). Pus samples showed a positivity rate of 88.2% (30/34), while sputum specimens exhibited an 87.5% positivity rate (14/16). Only six isolates tested negative for carbapenemase genes across all specimen types: four from pus (11.8%) and two from sputum (12.5%) (Table 4).

The distribution of specific carbapenemase genes varied across the different clinical specimens (Table 5). In pus samples, *blaOXA-48* predominated (30/34, 88.2%), followed by *blaNDM* (8/34, 23.5%) and *blaVIM* (8/34, 23.5%). Wound specimens demonstrated a high prevalence of *blaOXA-48* (14/16, 87.5%), with *blaNDM* and textitblaVIMdetected in 6 (37.5 %) and 4 (25.0 %) isolates, respectively. Sputum samples showed *blaOXA-48* in 14 out of 16 isolates (87.5%) and *blaNDM* in 6 isolates (37.5%), but no *blaVIM* was detected. Blood isolates exhibited the universal presence of *blaOXA-48* (6/6, 100.0%), with *blaNDM* and *blaVIM* each detected in two isolates (33.3%). Urine samples contained only *blaOXA-48* (2/2, 100.0%), with no detection of *blaNDM* or blaVIM. Neither *blaKPC* nor *blaIMP* was detected in any of the specimens.

Antimicrobial susceptibility testing revealed extensive multidrug resistance in CRAB isolates (Table 6). All 74 isolates (100.0%) exhibited resistance to meropenem, cefepime, ceftazidime, piperacillin-tazobactam, ticarcillin, and imipenem. High resistance rates were also observed for aztreonam (70/74, 94.6%), ciprofloxacin (68/74, 91.9%), tobramycin (68/74, 91.9%), levofloxacin (66/74, 89.2%), and amikacin (64/74, 86.5%). Colistin demonstrated the highest susceptibility rate (71/74, 95.9%).

Further analysis of gene co-occurrence patterns revealed distinct associations (Table 7). Among the isolates carrying a single carbapenemase gene (n=40), *blaOXA-48* was universally present (40/40, 100.0%), with no detection of *blaNDM*, blaVIM, *blaKPC*, or *blaIMP* as sole genes. In isolates with two genes (n=22), *blaOXA-48* was present in 20 isolates (90.9%), predominantly co-occurring with *blaNDM* (16/22, 72.7%) and less frequently with *blaVIM* (8/22, 36.4%). All isolates harboring three carbapenemase genes (n=6) carried blaOXA-48, *blaNDM*, and *blaVIM* simultaneously (6/6, 100.0%). None of the isolates carried *blaKPC* or *blaIMP* in any combination pattern.

These findings highlight the widespread dissemination of carbapenemase-producing *A. baumannii* in clinical settings, with *blaOXA-48* serving as the dominant resistance mechanism.

4. DISCUSSION

Antimicrobial resistance (AMR) rates have become a major concern in global health; if the trend contin-

ues, AMR could become the leading cause of death by 2050 [23]. Carbapenem-resistant Enterobacterales (CRE), carbapenem-resistant Pseudomonas aeruginosa (CRPA), and carbapenem-resistant *Acinetobacter baumannii* (CRAB) represent high-priority pathogens [24]

The present study revealed that among 74 carbapenem-resistant *A. baumannii* isolates from Sana'a, Yemen, bla*OXA-48* was the predominant carbapenemase gene (89.1%, n=66), followed by bla*NDM* (29.7%, n=22) and bla*VIM* (18.9%, n=14), while bla*KPC* and bla*IMP* were not detected (0%). This distribution pattern demonstrates the dominance of OXA-type carbapenemases in driving carbapenem resistance in Yemeni healthcare facilities.

Several studies from Arab and Middle Eastern countries have reported a similar predominance of OXA-type carbapenemases. Hamed in Egypt reported bla*OXA-23* in 69.6% of *A. baumannii* isolates in Egypt, with bla*NDM-1* present in 21.7% [7]. Abdullah (2024) reported in Iraqi Kurdistan that bla*OXA-51* and bla*OXA-23* were detected in 98% of isolates, with bla*NDM* present in 24.5% [6]. Contrasting findings emerged from Benmahmod (2019) in Egypt, with markedly different gene distributions: bla*KPC* (56%), bla*NDM* (30%), bla*VIM* (20%), and bla*IMP* (10%) [25]. Wasfi (2021) in Egypt reported the co-existence of multiple carbapenemase genes in cancer patients [26], while Miniaoui in Tunisia reported the first detection of bla*KPC-2* in *A. baumannii* in Tunisia [27].

Our study demonstrated that 91.9% (68/74) of *A. baumannii* isolates harbored at least one carbapenemase-encoding gene, whereas only 8.1% (6/74) tested negative for all screened genes. This high prevalence underscores the extensive dissemination of carbapenemase genes among CRAB isolates from Yemen. Higgins conducted molecular epidemiology across Northern Africa and the Middle East, reporting that the vast majority of CRAB isolates carry carbapenemase genes, particularly OXA-type enzymes [2]. Ababneh in Jordan reported carbapenemase genes in 89.3% of isolates [28], while Fam (2020) in Egypt documented carbapenemase genes in 93.5% of isolates [5]. Lower prevalence was reported by Benamrouche in Algeria (67.4%) [29], Uwingabiye in Morocco (78.5%) [30], and Al-Hamad in Saudi Arabia [31].

Among the 68 carbapenemase-positive isolates, single-gene carriage was most common (54.1%, n=40), followed by two genes (29.7%, n=22) and three genes (8.1%, n=6). This distribution indicates that while single-gene carriage predominates, co-carriage of multiple carbapenemase genes is substantial. Raddaoui in Tunisia reported co-occurrence of bla*NDM-1* and bla*OXA-23* [32], while Wasfi in Egypt reported that 35.7% of isolates carried multiple genes [26]. Contrasting patterns were reported by Abduljabar in Iraq [33], Alamri in Saudi Arabia [34], and Ferjani in Tunisia [35].

A. baumannii in our study was isolated from diverse clinical specimens: pus (45.9%, n=34), wound (21.6%, n=16), sputum (21.6%, n=16), blood (8.1%, n=6), and urine (2.7%, n=2). Mahmood in Iraq reported a similar distribution with wound specimens (38.5%) and respiratory samples (32.1%) [36]. El-Badawy in Saudi Arabia documented carbapenem-resistant *A. baumannii* predominantly from wound swabs (41.2%) and respiratory specimens (35.3%) [37]. Ababneh in Jordan reported drug-resistant *A. baumannii* from respiratory specimens (42.3%) and wound infections (28.7%) [28]. Different patterns were reported by Hassan in Egypt with respiratory specimens predominating (58.3%) [4], Slimene in Libya with blood cultures (47.2%) [38], and Bouali in Algeria with respiratory specimens (52.6%) [39].

Carbapenemase genes were detected across all specimen types, with the highest positivity rates in wound (100.0%), urine (100.0%), blood (100.0%), pus (88.2%), and sputum (87.5%). The near-universal presence of carbapenemase genes across specimen types indicates the widespread dissemination of resistant strains. Higgins reported uniform distribution across different specimen types [2], Fam in Egypt documented carbapenemase genes in >90% of isolates regardless of the specimen source [5], and Abdullah (2024) in Iraqi Kurdistan reported detection rates exceeding 85% across all specimen types [6]. Specimen-specific variations have been reported by Mahmood in Iraq [36], El-Badawy in Saudi Arabia [37], and Ababneh in Jordan [28].

The distribution of specific carbapenemase genes varied across the specimen types. bla*OXA-48* was detected at high rates across all specimens (87.5-100%), while bla*NDM* showed a variable distribution (0-37.5%), and bla*VIM* was found in blood (33.3%), wound (25.0%), and pus (23.5%). The predominance of bla*OXA* genes across all specimen types aligns with that reported by Hamed in Egypt [7] and Raddaoui in Tunisia [32]. Contrasting patterns were reported by Abdullah (2024) in Iraqi Kurdistan with bla*IMP* in 67.4% [6], Benmahmod in Egypt with high bla*KPC* prevalence[25], and Miniaoui in Tunisia with bla*KPC-2* detection [27].

All *A. baumannii* isolates showed 100% resistance to multiple β-lactams: meropenem, cefepime, ceftazidime, piperacillin-tazobactam, imipenem, and ticarcillin. High resistance was also observed for ciprofloxacin (91.9%), tobramycin (91.9%), aztreonam (94.6%), levofloxacin (89.2%), and amikacin (87.5%). Only colistin showed relatively higher susceptibility (95.9%). This extensive resistance profile indicates that CRAB isolates in Yemen are extensively drug-resistant, with limited therapeutic options. Hamed in Egypt reported similar colistin susceptibility (95.7%) [7]. Abdullah (2024) in Iraqi Kurdistan documented resistance rates of 77.6% for meropenem and 75.5% for imipenem [6]. Fam in Egypt reported >95% resistance to fluoroquinolones and aminoglycosides [5]. Different patterns were reported by Benam-

rouche in Algeria with lower aminoglycoside resistance [29], Benmahmod in Egypt with 98% carbapenem resistance [25], and Alamri in Saudi Arabia with emerging colistin resistance (15.3%) [34].

Among the 68 carbapenemase-positive isolates, the gene distribution varied according to the number of genes present. In single-gene isolates (n=40), bla*OXA-48* dominated (100.0%). In two-gene isolates (n=22), bla*NDM* (72.7%), bla*VIM* (36.4%), and bla*OXA-48* (90.9%) were present. In three-gene isolates (n=6), bla*NDM*, bla*VIM*, and bla*OXA-48* were all present (100%). This pattern indicates that bla*OXA-48* serves as the backbone of carbapenem resistance, with bla*NDM* and bla*VIM* being acquired as additional resistance determinants. Wasfi in Egypt reported that bla*OXA-23* was present in all multi-gene isolates [26]. Different co-occurrence patterns were reported by Abdullah in Iraqi Kurdistan with bla*IMP* commonly present [6], Benmahmod in Egypt with frequent bla*KPC* co-occurrence [25], and Ferjani in Tunisia with VIM-2 and OXA-23 co-production [35].

The high prevalence of these variants is primarily driven by intense selective pressure from the historical over-prescription of beta-lactams and cephalosporins in regional hospitals. Unlike structural MBLs, *blaOXA-48* variants are carried on highly transmissible, lightweight plasmids. In resource-limited and strained healthcare settings, this mobility allows the resistance mechanism to spread rapidly through horizontal gene transfer, establishing it as the dominant local resistance profile.

5. CONCLUSION

In conclusion, this study demonstrates a high prevalence of carbapenemase-encoding genes among clinical Carbapenem-resistant *Acinetobacter Baumannii* isolates in Sana'a, Yemen, with *blaOXA-48* representing the primary gene of carbapenem resistance. The co-carriage the combination of *blaOXA-48* with the metallo-β-lactamases *blaNDM and blaVIM* indicate the complexity of resistance profiles in local healthcare facilities. Notably, colistin remained the sole antimicrobial agent to which the tested isolates exhibited consistent susceptibility. These findings highlight the urgent need for implementing robust antimicrobial stewardship programs and routine molecular surveillance in Yemeni clinical laboratories to control the spread of these resistant pathogens.

5.1. Study Limitations

This study has several limitations. PCR detection alone does not identify allelic variants or novel carbapenemase genes. No clonal typing was performed, which limited the understanding of strain-relatedness and transmission. Samples came from only three facilities in Sana' a, which may not generalize to all of Yemen. Clinical data were not linked to resistance profiles. Despite these limitations, this study provides essential baseline molecular data for future surveillance.

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Table 1. Distribution of carbapenemase-encoding genes (blaIMP, blaNDM, blaKPC, blaVIM and blaOXA-48) in the carbapenem-resistant Acinetobacter baumannii

	No.	*bla*NDM	*bla*VIM	*bla*OXA 48	*bla*KPC	*bla*IMP
Acinetobacter baumannii	**74**	22 (29.7%)	14 (18.9%)	66 (89.1%)	0 (0%)	0 (0%)

Table 2. Distribution of all carbapenemase-encoding genes in *Acinetobacter baumannii*

Encoding genes			
Yes		**No**	
No.	**%**	**No.**	**%**
68	91.9 %	6	8.1%

Table 3. Distribution of *Acinetobacter baumannii* carbapenemase-encoding genes number in clinical isolate

Genes N=74	**No.**	**%**
Negatives	6	8.1
One	40	54.1
Two	22	29.7
Three	6	8.1

Table 4. Distribution of *Acinetobacter baumannii* carbapenemase-encoding genes in clinical Specimens

Gene	**Specimens N=74**				
	Wound N=16	**Sputum N=16**	**Urine N=2**	**Blood N=6**	**Pus N=34**
Positive N=68	16(100.0%)	14(87.5%)	2 (100.0%)	6(100.0%)	30(88.2%)
Negative N=6	0(0%)	2(12.50%)	0(0.0%)	0(0%)	4(11.8%)

Table 5. Distribution of *Acinetobacter baumannii* carbapenemase-encoding genes in the clinical specimens

Genes N=68	**Specimens N=74**				
	Wound	**Sputum**	**Urine**	**Blood**	**Pus**
	N=16	**N=16**	**N=2**	**N=6**	**N=34**
***bla*NDM N=22**	6 (37.5%)	6 (37.5%)	0 (0%)	2(33.3%)	8(23.5%)
***bla*VIM N=14**	4 (25.0%)	0 (0%)	0(0%)	2 (33.3%)	8 (23.5%)
***bla*OXA N=66**	14 (87.5%)	14 (87.5%)	2 (100.0%)	6 (100.0%)	30 (88.2%)
***bla*KPC N=0**	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
***bla*IMP N=0**	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 6. The Antimicrobial resistance profiling of CRAB-producing *Acinetobacter baumannii*

	Acinetobacter baumannii	
	S	R
Colistin (CT)	71 (95.9%)	3 (4.1%)
Ciprofloxacin (CIP)	6 (8.1%)	68 (91.9%)
Meropenem (MR)	0 (0%)	74 (100.0%)
Cefepime (FEP)	0 (0%)	74 (100.0%)
Levofloxacin (LE)	8 (10.8%)	66 (89.2%)
Ceftazidime (CAZ)	0 (0%)	74 (100.0%)
Piperacillin-tazobactam (PIT)	0 (0%)	74 (100.0%)
Amikacin (AK)	10 (13.5%)	64 (86.5%)
Ticarcillin (TI)	0 (0%)	74 (100.0%)
Tobramycin (TOB)	6 (8.1%)	68 (91.9%)
Imipenem (IPM)	0 (0%)	74 (100.0%)
Aztreonam (AZT)	4 (5.4%)	70 (94.6%)

Table 7. Distribution of *Acinetobacter baumannii* carbapenemase-encoding genes among cases as number

Genes N=68	Genes		
	One N=40	Two N= 22	Three N=6
***bla*NDM N=22**	0 (0%)	16 (72.7%)	6 (100.0%)
***bla*VIM N=14**	0 (0%)	8 (36.4%)	6 (100.0%)
***bla*OXA N=66**	40 (100.0%)	20 (90.9%)	6 (100.0%)
***bla*KPC N=0**	0 (0%)	0 (0%)	0 (0%)
***bla*IMP N=0**	0 (0%)	0 (0%)	0 (0%)