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Carbapenem-resistant *Acinetobacter baumannii* from Jordan: Complicated Carbapenemase Combinations

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Abstract

Acinetobacter baumannii is an opportunistic Gram-negative bacterium that has recently emerged as a clinically important pathogen. It is the most common *Acinetobacter* species associated with hospital-acquired infections worldwide. This study aimed to investigate the resistance characteristics of clinical carbapenem-resistant *A. baumannii* isolates from Jordanian hospitals. Between May 2018 and May 2019, a total of 190 isolates of *Acinetobacter* were collected from patients diagnosed with upper respiratory tract infection (47.5%), sepsis (15.4%), skin infection (14.2%), bronchitis (8.6%), urinary tract infection (4.3%), meningitis (3.7%), diabetes (2.5%), necrotizing fasciitis (1.9%), and cancer, peritonitis, and pneumonia (0.6% each) from seven Jordanian hospitals. Vitek GN ID Cards were employed to identify them, and the identification was validated by the detection of *Acinetobacter* spp. *recA* gene (100%), *A. baumannii* intergenic spacer region (85.3%), and the *A. baumannii rpoB* gene (85.3%). The Vitek AST-N222 and AST-XN05 cards were utilized to determine the minimum inhibitor concentration for a variety of antibiotics including Gentamicin, Tobramycin, Piperacillin-tazobactam, Ticarcillin-clavulanic acid, Ticarcillin, Imipenem, Meropenem, Cefepime, Ceftazidime, Ceftriaxone, Ciprofloxacin, Levofloxacin, Trimethoprim-sulfamethoxazole, Piperacillin, Minocycline, and Tetracycline. The E-test was performed to evaluate the effectiveness of Colistin against all *A. baumannii* isolates. According to the resistance profiles, the isolates had a multidrug resistance profile, with the largest resistance percentage being 98.8% for Tetracycline and the lowest being 23.5% for Minocycline. The Carbapenem-resistant isolates exhibited rates of 98.1% and 87.7% resistance to Meropenem and Imipenem, respectively. On the contrary, the isolates were 98.7% sensitive to Colistin. The most prevalent carbapenem resistance genes among the Jordanian *A. baumannii* isolates were $bla_{OXA\text{-}23\text{-like}}$, $bla_{OXA\text{-}51\text{-like}}$, $bla_{OXA\text{-}69}$, and $_{ISAba1,}$ which were detected in all *A. baumannii* isolates (100%) in this study. Despite the high prevalence of multidrug resistance in the Jordanian isolates, Colistin may be a viable treatment for *A. baumannii* infection.

Keywords: Acinetobacter baumannii, Carbapenemase, Colistin, $bla_{OXA\text{-}23\text{-like}}$, $bla_{OXA\text{-}51\text{-like}}$, $bla_{OXA\text{-}69}$, ISA_{ba1}, Jordan

1. Introduction

Acinetobacter baumannii is an opportunistic Gram-negative bacterium that has recently emerged as a clinically important pathogen due to its association with hospital-acquired infections worldwide. Examples on these infections include wide range of nosocomial infections such as, bloodstream infections, urinary tract infections, wound infections, pneumonia and meningitis, primarily those acquired in the community or from war and natural disasters (Ababneh et al., 2021; El-Khatib et al., 2021; Al-Tamimi et al., 2022). This bacterium lives in the human body and is usually detected in moist environments and on contaminated tools and materials used by patients in hospitals (Shatnawi et al., 2021). Consequently, *A. baumannii* frequently colonize hospitalized patients, resulting in invasive sporadic infection and hospital outbreaks, particularly among critically sick patients and intensive care unit (ICU) patients (Ghaith et al., 2017). Imipenem, a Carbapenem, is the medication of choice for severe infections caused by *A. baumannii*. In recent years, this pathogene has become a major source of concern due to the extensive use of Carbapenems. The emergence of Carbapenem-resistant *A. baumannii* has drastically reduced the number of antibiotics available to individuals infected with multidrug-resistant (MDR) *A. baumannii* (Nguyen and Joshi, 2021). Colistin is another medication that is often used as a first-line therapy for *A. baumannii* infections in Jordan (Samrah et al., 2016). In Jordan, as in many other countries, MDR *A. baumannii* poses a major threat to patients and places an economic burden on healthcare systems from increased healthcare costs, prolonged hospitalization, ICU admissions, higher mortality rates, resource utilization for infection control, challenges in antibiotic development, and the broader impact on public health. Efforts to address antimicrobial resistance and enhance infection control practices are crucial to mitigating these economic challenges.

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Carbapenem resistance in *A. baumannii* is often associated with the production of carbapenemases, enzymes that hydrolyze carbapenem antibiotics. The carbapenemases found in *A. baumannii* belong to different classes. Here are some of the carbapenemases commonly associated with carbapenem-resistant *A. baumannii*: The oxacillinase group, OXA-type carbapenemases, which belong to Class D β-lactamases, such as OXA-23, OXA-24, OXA-58 that are encoded by $bla_{OXA\text{-}23\text{-like}}$, $bla_{OXA\text{-}24\text{-like}}$, and $bla_{OXA\text{-}58\text{-like}}$ genes respectively; OXA-23 is one of the most prevalent carbapenemases in *A. baumannii* and is associated with resistance to carbapenems (Mentasti et al., 2020). Metallo-β-lactamases (MBLs), which belong to Class B β-lactamases, are zinc-dependent enzymes that hydrolyze carbapenems and are resistant to β-lactamase inhibitors such as IMP, and VIM. While MBLs are more commonly associated with other Gram-negative bacteria, they have been found in *A. baumannii* isolates as well (Tooke et al., 2019). New Delhi metallo-β-lactamase (NDM) is a type of MBLs that has been reported in *A. baumannii* strains. The New Delhi metallo-β-lactamase 1 gene (*NDM-1*) carbapenemase has been described in *A. baumannii* worldwide, the highest prevalence was reported in the Indian subcontinent, Middle East, Algeria, Brazil, Pakistan, Balkans, and the United Kingdom (Wu et al., 2019). *Klebsiella pneumoniae* carbapenemase (KPC) has been reported in some *A. baumannii* isolates such as KPC-3 (Caneiras et al., 2018). Furthermore, resistance to carbapenems in *A. baumannii* has been associated with the presence of the *ISAba1* gene, which acts as a promoter for the $bla_{OXA\text{-}51\text{-like}}$ and, most likely, $bla_{OXA\text{-}23\text{-like}}$ carbapenemase genes (Vahhabi et al., 2021).

The current study aimed to identify the carbapenem-resistant genes in Jordanian clinical *A. baumannii* isolates, with a focus on genes encoding carbapenemases class D, oxacillinase *OXA*s, and class B, *NDM-1*.

2. Materials and Methods

2.1. Collection of A. baumannii isolates

From May 2018 to May 2019, a total of 190 bacterial isolates were randomly collected from seven Jordanian hospitals: Al Basheer Hospital, Al Khalidi Medical Center, Islamic Hospital, Jordan University Hospital, Prince Hamza Hospital, Royal Medical Services, and The Specialty Hospital. Information about the patients, including nationality, age, gender, and sample source such as abdominal and pleural fluids, blood culture, bronchoalveolar lavage, cancer tissue, cerebrospinal fluid, pus, specimen collection trap, sputum, swabs, and urine, was collected for each isolate. The isolates were sub-cultured on MacConkey agar (Mast Group, UK) and incubated for 20–24h at 35°C ± 2°C. The pure culture of each isolate was stored at -80°C in 20% glycerol (Cryobank, Mast Group, UK) for further use (Al-Karablieh et al., 2017).

2.2. Identification of the collected isolates

Fresh pure culture of the collected isolates, 1mm in diameter, and the transparent colonies on MacConkey agar were tested for oxidase. The isolates exhibiting negative results in the oxidase test were chosen for further identification by an automated Vitek®2 compact system using GN ID Cards (BioMérieux, Marcy-l'Etoile, France). Furthermore, the genomic DNA was extracted with the Quick–DNA miniprep plus kit (Zymo Research, USA) following the recommendations of the manufacturer. The purity and concentration of DNA were quantified using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, USA). The concentration of the extracted DNA was adjusted to 100ng/µl. Duplex polymerase chain reaction (PCR) was conducted with a programmable thermocycler (S1000 thermal cycler Bio-Rad, USA) targeting the *recA* gene of *Acinetobacter* spp., and an internal 208bp region on the ITS region in *A. baumannii* (Chen et al., 2007). For further validation of the identification, conventional PCR was employed to detect the DNA-directed RNA polymerase subunit beta encoding gene, *rpoB*, in *A. baumannii* (Schleicher et al., 2013). Table 1 lists the all primers used in the present study.

Table 1. List of all primers used in the current study for identification of the collected isolates and detection of carbapenemase-encoding genes.

Primer	Target gene	Sequence (5' - 3')	Size (bp)	Reference
Identification				
P-Ab-ITSF	ITS	AGAGCACTGTGCACTTAAG	208	(Chen et al., 2007)
P-Ab-ITSR		CATTATCACGGTAATTAGTG		
P-rA1-F	*recA*	CCTGAATCTTCTGGTAAAAC	425	(Chen et al., 2007)
P-rA2-R		GTTTCTGGGCTGCCAAACATTAC		
Ac696F	*rpoB*	TAYCGYAAAGAYTTGAAAGAAG	350	(Schleicher et al., 2013)
Ac1093R		CMACACCYTTGTTMCCRTGA		
Carbapenemase-encoding genes				
ISAba1	*ISAba1*	CATTGGCATTAAACTGAGGAGAAA	451	(Ruiz et al., 2007)
ISAba2		TTGGAAATGGGGAAAACGAA		
NDM-F	*blaNDM-1*	GGTGCATGCCCGGTGAAATC	660	(Bonnin et al., 2012)
NDM-R		ATGCTGGCCTTGGGGAACG		
OXA23-F1	$bla_{OXA\text{-}23\text{-like}}$	TGCTCTAAGCCGCGCAAATA	130	(Mesli et al., 2013)
OXA23-R1		TGACCTTTTCTCGCCCTTCC		
OXA24-F	$bla_{OXA\text{-}24\text{-like}}$	CAAATGAGATTTTCAAATGGGATGG	123	(Mesli et al., 2013)
OXA24-R		TCCGTCTTGCAAGCTCTTGAT		
OXA51like-F	$bla_{OXA\text{-}51\text{-like}}$	AACATTAAAGCACTCTTACTTATAAC	171	(Adams-Haduch et al., 2011)
OXA51like-R		TTGTTGGATAACTAAAACACCCGT		
OXA58-F	$bla_{OXA\text{-}58\text{-like}}$	CGCAGAGGGGAGAATCGTCT	102	(Mesli et al., 2013)
OXA58-R		TTGCCCATCTGCCTTTTCAA		
OXA-69A	$bla_{OXA\text{-}69}$	CTAATAATTGATCTACTCAAG	975	(Hamouda et al., 2010)
OXA-69B		CCAGTGGATGGATGGATAGATTATC		

2.3. Antimicrobial susceptibility test of A. baumannii isolates

The minimal inhibition concentration (MIC) was checked for different antibiotics by the Vitek®2 compact instrument, using AST-N222 and AST-XN05 cards to target specific antibiotics (BioMérieux, Marcy-l'Etoile, France) according to the Clinical Laboratory Standards Institute (CLSI, 2019), following the guidelines for antibiotics breakpoints. Twelve antibiotics from eight antibiotics group and three antibiotic combinations were evaluated against the 162 *A. baumannii* isolates, namely, Aminoglycosides (Gentamicin [4–16 μg mL^{-1}], and Tobramycin [4–16 μg mL^{-1}]), β-lactam combination agents (Piperacillin-tazobactam [16/4–128/4 μg mL^{-1}], Ticarcillin-clavulanic acid [16/2–128/2 μg mL^{-1}], and Ticarcillin [16–128 μg mL^{-1}]), Carbapenem (Imipenem [2–8 μg mL^{-1}], and Meropenem [2–8 μg mL^{-1}]), Cephems (Cefepime [8–32 μg mL^{-1}], Ceftazidime [8–32 μg mL^{-1}], and Ceftriaxone [8–64 μg mL^{-1}]), Fluoroquinolones (Ciprofloxacin [1–4 μg mL^{-1}], and Levofloxacin [2–8 μg mL^{-1}]), Folate pathway antagonists (Trimethoprim-sulfamethoxazole [2/38–4/76 μg mL^{-1}]), Penicillins (Piperacillin [16–128 μg mL^{-1}]), Tetracyclines (Minocycline [4–16 μg mL-1], and Tetracycline [4–16 μg mL^{-1}]). Furthermore, the E-test (BioMérieux, Marcy-l'Etoile, France) was conducted to assess the efficacy of Colistin against all *A. baumannii* isolates.

2.4. Molecular detection of carbapenemase-encoding genes

Conventional PCRs were conducted for the detection of intrinsic carbapenemases genes encoding $bla_{OXA\text{-}23\text{-like}}$, $bla_{OXA\text{-}24\text{-like}}$, $bla_{OXA\text{-}51\text{-like}}$, $bla_{OXA\text{-}58\text{-like}}$, $bla_{OXA\text{-}69}$, $bla_{NDM\text{-}1}$, and ISA_{ba1}. The PCR cycles and conditions were performed following the protocol described by the authors of the primers. *A. baumannii* NCTC 13301 was utilized as a positive control for $_{OXA}$ carbapenemases genes and *Klebsiella pneumonia* NCTC 13443 as a positive control for Metallo-β-lactamase ($bla_{NDM\text{-}1}$).

2.5. Statistical Analysis

Statistical analysis was performed using SPSS software, version 21, and analysis of variance (ANOVA) was conducted to determine mean separation at the 0.05 probability level based on the least significant difference.

3. Results

3.1. Identification of the collected isolates

The Vitek®2 GN ID Cards and the detection of the *recA* gene of *Acinetobacter* spp. revealed the presence of *Acinetobacter* spp. in 182 bacterial isolates. The detection of the ITS region of *A. baumannii*, and *rpoB* in 162 bacterial isolates revealed the presence of *Acinetobacter baumannii*. These, 162 isolates, were chosen for further testing as representatives for clinical Jordanian isolates of *A. baumannii*. It is noteworthy that more than half of the *A. baumannii* isolates (51.8%) in this study were collected from military hospitals, in particular Prince Hamza Hospital and Royal Medical Services, followed by public hospitals (29%), particularly Al Basheer Hospital and Jordan University Hospital, and the lowest percentage (19.1%) were collected from private hospitals, particularly Al Khalidi Medical Center, Islamic Hospital, and The Specialty Hospital. (Table 2).

Table 2. The distribution of A. baumannii isolates depends on sampling hospitals.

Hospital	No. of samples	%
AL Basheer Hospital	20	12.3
Al Khalidi Medical Center	12	7.4
Islamic Hospital	14	8.6
Jordan University Hospital	27	16.7
Prince Hamza Hospital	24	14.8
Royal Medical Services	60	37
Specialty Hospital	5	3.1
Total	162	100

3.2. The infections associated with A. baumannii

The distribution of *A. baumannii* isolates was varied according to the origin of the place of the collection, with sputum being the major source of the pathogen (in 47.5% of the samples), followed by blood culture (15.4%), pus (14.2%), specimen collection trap (7.4%), urine (4.3%), swabs (4.3%), cerebrospinal fluid (3.7%), bronchoalveolar lavage (1.2%), abdominal and pleural fluids (0.6%), and cancer (0.6%). The samples were mainly collected from patients diagnosed with upper respiratory tract infection (47.5%), followed by those diagnosed with sepsis (15.4%); skin infection (14.2%); bronchitis (8.6%); urinary tract infection (4.3%); meningitis (3.7%); diabetes (2.5%); necrotizing fasciitis (1.9%); and cancer, peritonitis, and pneumonia (0.6% each) (Table 3).

Table 3. The distribution of *A. baumannii* isolates depends on the infection type.

Infection type	No. of samples	%
Bronchitis	14	8.6
Cancer	1	0.6
Diabetes	4	2.5
Meningitis	6	3.7
Necrotizing fasciitis	3	1.9
Peritonitis	1	0.6
Pneumonia	1	0.6
Sepsis	25	15.4
Skin infection	23	14.2
Upper respiratory tract infection	77	47.5
Urinary tract infection	7	4.3
Total	162	100

A variation was found in the infection with *A. baumannii* isolates according to patient ages, which ranged from 3 to 80 years old. The majority of the infected patients were males (68%). Nearly all patients had Jordanian nationality (97.5%), with few having Iraqi (1.3%) and Libyan (1.2%) nationalities.

3.3. Antimicrobial susceptibility of A. baumannii isolates

The results of the antimicrobial susceptibility test by Vitek AST cards revealed that 98.8% of *A. baumannii* isolates were resistant to Tetracycline; 98.1% were resistant to Ceftazidime, Ceftriaxone, Ciprofloxacin, Meropenem, Piperacillin, Piperacillin-tazobactam, Ticarcillin, and Ticarcillin-clavulanic acid; 97.5% to Cefepime; 95% to Levofloxacin; 87.7% to Imipenem; 73.5% to Gentamicin; 68.5% to Tobramycin; 67.9% to Trimethoprim-sulfamethoxazole; and 23.5% to Minocycline according to (CLSI, 2019) (Table 3).

The results of the E-test of Colistin against *A. baumannii* isolates indicated that 98.7% of *A. baumannii* isolates were sensitive to Colistin (MIC ≤ 0.5), and only 1.3% (two isolates) were resistant to Colistin (Fig. 1).

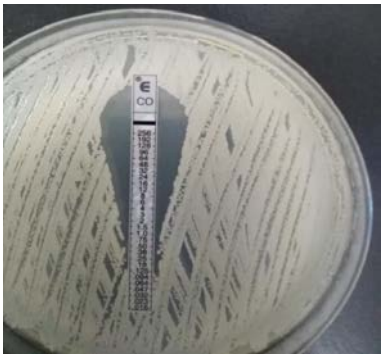

Figure 1. Antimicrobial susceptibility of *A. baumannii* isolates to Colistin by E-test.

All the tested *A. baumannii* isolates (162) resulted in expected amplicon size of the following genes: $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$, $bla_{OXA\text{-}69}$, and *ISAba1*. While 95% of the *A. baumannii* isolates formed the expected amplicon size of the $bla_{OXA\text{-}24\text{-}like}$ gene, 80% formed the expected amplicon size of the *blaNDM-1* gene, and 40% of the isolates formed the expected amplicon size of the $bla_{OXA\text{-}58\text{-}like}$ gene.

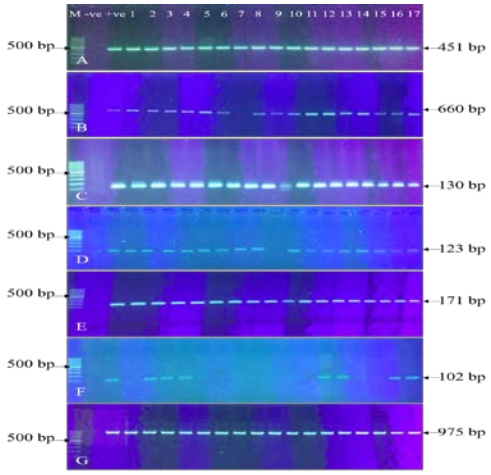

Figure 2. Examples of agarose gel electrophoresis of PCR products: **A**, ISAba1/ISAba2 primers for *ISAba1*, **B**, NDM-F/ NDM-R primers for *blaNDM-1*, **C**, OXA23-F1/ OXA23-R1 primers for $bla_{OXA\text{-}23\text{-}like}$, **D**, OXA24-F/ OXA24-R primers for $bla_{OXA\text{-}24\text{-}like}$, **E**, OXA51like-F/OXA51like-R primers for $bla_{OXA\text{-}51\text{-}like}$, **F**, OXA58-F/ OXA58-R primers for $bla_{OXA\text{-}58\text{-}like}$, **G**, and OXA-69A/ OXA-69B primers for $bla_{OXA\text{-}69}$. The DNA ladder was 1000 bp (M), deionized sterile water was used as negative control (-ve), gDNA of *A. baumannii* NCTC 13301 was used as a positive control for $_{OXA}$ carbapenemases genes and gDNA *Klebsiella pneumonia* NCTC 13443 as a positive control for Metallo-β-lactamase ($bla_{NDM\text{-}1}$) (+ve). Lane 1-17: representative samples of gDNA of Jordanian *A. baumannii* isolates.

4. Discussion

As carbapenems are usually the most practical therapy for many multi-resistant bacterial strains, the emergence of carbapenem-resistant *A. baumannii* has become a global concern (Ababneh et al., 2021; Al-Tamimi et al., 2022). The most common resistance mechanism in *A. baumannii* is the expression of Class D β-lactamases, such as OXA-23, OXA-24, OXA-58 enzymes. These enzymes were initially distinguished by their ability to efficiently

hydrolyze isoxazolyl-type β-lactams such as oxacillin. Due to this substrate preference, these enzymes are also known as oxacillinases or (OXAs) (June et al., 2014). Furthermore, MBLs, which are Class B β-lactamases, can be particularly harmful if found in *A. baumannii*-infected individuals due to their broad spectrum of powerful carbapenemase activity and resistance to β-lactamase inhibitors (Vahhabi et al., 2021). Mobile genetic elements, integrons, and plasmids can help disseminate MBLs across *A. baumannii* (Partridge et al., 2018).

Recent Middle Eastern wars, which have resulted in a significant number of immigrants and refugees, as well as a lack of access to competent medical treatment, are likely contributing factors to multi-drug-resistant organisms, increased resistance patterns, and expanding incidence rates (Nawfal Dagher et al., 2020; Helou et al., 2022). Hence, ongoing monitoring and surveillance are essential for forecasting and mandating suitable steps when necessary (Bessong and Guerrant, 2017). Therefore, Jordanian hospitals must further the invention and adoption of rapid and accurate diagnostic procedures. Collaboration with academic institutions, research centers, and private companies to support innovative projects focused on rapid and accurate diagnostics. Training programs should be provided for healthcare professionals to ensure they are proficient in using new diagnostic technologies. Other strategies include implementing financial incentives for hospitals to adopt and integrate rapid and accurate diagnostic procedures, conducting pilot programs to assess the feasibility and effectiveness of new diagnostic procedures in real-world hospital settings, collaboration with international organizations, research institutions, and healthcare providers to share knowledge and experiences related to the adoption of advanced diagnostics, and educating the public about the benefits of rapid and accurate diagnostics in improving healthcare outcomes. By implementing these strategies, Jordanian hospitals can contribute to the advancement and adoption of rapid and accurate diagnostic procedures, ultimately improving patient care and outcomes.

Using data from the Vitek 2 compact system, 162 *Acinetobacter* isolates were reported with a 99% high accuracy rate in this investigation. Several investigations have indicated that the automated Vitek 2 system is useful, accurate, and rapid in bacterial identification (Al-Karablieh et al., 2022). However, as previously reported, the identification was furthered using the molecular detection of *recA Acinetobacter* spp. at the genus level by 425 bp amplicon detection, and detection of *A. baumannii* ITS and *ropB* genes at the species level by 208 bp and 350 bp amplicons detection, respectively (Chen et al., 2007; Schleicher et al., 2013).

The sensitivity of the *A. baumannii* isolates to Imipenem and Meropenem in the disc diffusion test was lower than that in the test using Vitek AST cards, revealing the accuracy of the Vitek AST cards due to determination of the MIC values. However, the findings regarding the resistance of *A. baumannii* isolates to Imipenem and Meropenem measured by Vitek AST cards were similar to that observed in a previous Jordanian study, 94%, and 88% for Imipenem and Meropenem, respectively (El-Khatib et al., 2021). Moreover, the percentage of MDR *A. baumannii* isolates in this study, 98.8%, was higher than the percentage reported in a previous Jordanian study, 76.8% (Al-Tamimi et al., 2022).

The majority of the isolates in this study were sensitive to Colistin and Minocycline, which is consistent with a previous Jordanian study that found Colistin and Tigecycline to have the lowest resistance rate (Al-Tamimi et al., 2022). A study from the Holy Cities of Makkah and Al-Madinah of Saudi Arabia revealed that all *A. baumannii* isolates were susceptible to Colistin, while 95% of isolates were susceptible to Tigecycline (Al-Sultan, 2021). Another study from Riyadh, Saudi Arabia revealed that 30% of *A. baumannii* isolates were resistant to Colistin, and 56% were resistant to Tigecycline (Al-Agamy et al., 2017).

The tolerance to these antibiotics is crucial due to the global tendency of utilizing Colistin in dealing with of MDR *A. baumannii* infections (Chen et al., 2015). Colistin resistance in *A. baumannii* has previously been reported in Spain (Khoshbakht et al., 2023) and Kingdom of Bahrain (Al-Rashed et al., 2023), and a study from China reported a lower resistance rate to minocycline and tigecycline (Zhu et al., 2022). However, in this study, two Colistin-resistant *A. baumannii* isolates were identified and considered pan-drug resistant bacteria.

According to the findings of this study, Jordan has a higher percentage of carbapenem-resistant *A. baumannii* than surrounding countries, including Lebanon (60%) (Rafei et al., 2015), Turkey (79%) (Kulah et al., 2010), Egypt (70%) (Al-Agamy et al., 2014), Saudi Arabia (85.7%) (Elabd et al., 2015), and Iraq (85%) (Hussein et al., 2013), and this percentage is extremely close to Italy's reported percentage of 90.8% (Mezzatesta et al., 2012). The prevalence of carbapenem-resistant *A. baumannii* is growing in Jordan, with the proportion of carbapenem-resistant *A. baumannii* estimated to be 60% in 2014 (Obeidat et al., 2014). In the current study, carbapenem-resistant *A. baumannii* was found in 98.1% of the tested isolates, which is similar to the previously reported prevalence rates of carbapenem-resistant *A. baumannii* in Jordan of 90.6% and 99.2% (Ababneh et al., 2021; Al-Tamimi et al., 2022), respectively. The reasons for the high prevalence of carbapenem resistance *A. baumannii* might be the result of antibiotic abuse and misuse in healthcare settings, insufficient infection control measures in healthcare facilities, and the movement of individuals across borders, which can contribute to the worldwide spread of antibiotic-resistant strains.

The majority of the carbapenem-resistant *A. baumannii* came from Military hospitals followed by public hospitals, which might be related to challenges facing these hospitals in maintaining optimal hygiene and infection control practices due to the high numbers of patients in these hospitals, which contribute to the persistence of the *A. baumannii* in the environment. The majority of the carbapenem-resistant *A. baumannii* came from Jordanian men, consistent with previous Jordanian studies that found an elevated frequency of *A. baumannii* infections in Jordanian adult males (Ababneh et al., 2021; El-Khatib et al., 2021; Al-Tamimi et al., 2022). Military personnel may sustain injuries during combat, leading to open wounds and increased susceptibility to infections. *A. baumannii*, known for its ability to cause wound infections, can thrive in the environment and infect individuals with compromised immune systems. In conflict zones like

Jordan, injured soldiers may be rapidly evacuated to military hospitals for treatment. The transfer of individuals with infections, including *A. baumannii*, from different locations can contribute to the spread of the bacterium within military healthcare facilities. The use of broad-spectrum antibiotics is common in military settings to treat infections. Prolonged and widespread use of antibiotics can contribute to the development of multidrug-resistant strains of bacteria, including *A. baumannii*.

Similar to a previous Jordanian study (El-Khatib et al., 2021), over half of the carbapenem-resistant *A. baumannii* isolates tested in this study were collected from sputum; however, another Jordanian investigation found that the majority of *A. baumannii* isolates were obtained from wounds (Al-Tamimi et al., 2022).

In dedpth genetic analysis of the studied samples can help in elucidation of many mechanisms that result in *A. baumannii* being both highly infectious and extremely resistant. This study found that the dominant resistant genes among carbapenem-resistant Jordanian *A. baumannii* isolates are $bla_{OXA\text{-}23\text{-like}}$, $bla_{OXA\text{-}51\text{-like}}$, $bla_{OXA\text{-}69}$, ISA_{ba1}, which were found in all tested, 100%, carbapenem-resistant Jordanian *A. baumannii* isolates, followed by 95% for $bla_{OXA\text{-}24\text{-like}}$, 80% for of *blaNDM-1* and 40% for $bla_{OXA\text{-}58\text{-like}}$. The most prevalent encoding genes of MBLs and OXAs in Mediterranean Arab countries are bla_{IMP}, bla_{KPC}, $bla_{NDM\text{-}1}$, $bla_{OXA\text{-}23\text{-like}}$, $bla_{OXA\text{-}24\text{-like}}$, $bla_{OXA\text{-}51\text{-like}}$, $bla_{OXA\text{-}58\text{-like}}$, and $bla_{OXA\text{-}69}$ and bla_{VIM} (Salih and Shafeek, 2019; Shayea and Ali, 2022). The current study is the first in Jordan to show an elevated prevalence of $bla_{NDM\text{-}1}$ of 80% among carbapenem-resistant *A. baumannii* isolates compared to the 10.4% previously reported (Ababneh et al., 2021), indicating the fast expansion of $bla_{NDM\text{-}1}$ in Jordan. Recent investigations from the Arab area have yet to discover bla_{NDM} or $bla_{NDM\text{-}1}$ genes in carbapenem-resistant *A. baumannii*. However, bla_{NDM} was discovered in a few carbapenem-resistant *A. baumannii* isolates collected from Syrian hospitalized patients in Lebanon (Rafei et al., 2014).

5. Conclusion

This study found that *A. baumannii* is widespread in Jordan and can cause major hospital infections. A high frequency of carbapenem resistance was reported among Jordanian *A. baumannii* isolates. The major resistance genes among Jordanian *A. baumannii* isolates were $bla_{OXA\text{-}23\text{-like}}$, $bla_{OXA\text{-}51\text{-like}}$, $bla_{OXA\text{-}69}$, and ISA_{ba1}. This significant resistance will impair the effectiveness of valuable medications that treat *A. baumannii* invasive infections. Despite the high prevalence of multidrug resistance in Jordanian *A. baumannii* isolates, Colistin and Tigecycline may be viable treatment for *A. baumannii* infection. Combining different classes of antibiotics can enhance effectiveness and reduce the risk of resistance development.

Therefore, further studies are warranted to determine the efficiency of combining Colistin and Tigecycline with Jordanian medicinal plants to control *A. baumannii* infection.

6. Ethical approval

The authors have complete confidence that publishing this manuscript will not cause any ethical concerns.

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Conflict of interests

The authors declare that they have no conflicts of interest.

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