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Evaluation of Antibiotic Resistance and Susceptibility Patterns in Multidrug-Resistant Acinetobacter spp

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ABSTRACT

Antimicrobial-resistant Acinetobacter species (spp.) have emerged as significant healthcare-associated pathogens because they can develop resistance to many antibiotics, making infections difficult to treat. This study aims to evaluate antibiotic resistance and susceptibility patterns among Acinetobacter (A.) species. Six hundred twenty-four (624) clinical samples were collected from both inpatients and outpatients. Forty-four (44) Acinetobacter spp. isolates were identified by culture and biochemical characteristics and then confirmed with the VITEK 2 system. The antibiotic susceptibility profile was determined using the VITEK 2 AST-419 card. Acinetobacter spp. represented 7.1% of all isolates. The Acinetobacter baumannii complex was the most common, with 35 (79.6%), followed by A. haemolyticus 7 (15.9%) and A. lwoffii 2 (4.5%) isolates. The antibiotic susceptibility test showed the highest resistance rates to ampicillin/sulbactam 38 (86.4%), piperacillin 34 (77.3%), cefotaxime 29 (65.9%), levofloxacin 29 (65.9%), ciprofloxacin 28 (63.6%), ceftazidime 28 (63.6%), ticarcillin/clavulinate 27 (61.4%), and imipenem 27 (61.4%). Most isolates were sensitive to minocycline 34 (77.3%) and colistin 2 (4.5%), respectively. The data showed increased resistance to common antibiotics. Colistin, minocycline, and amikacin were effective antibiotic for managing infections induced by Acinetobacter species. Extensive drug resistance (XDR) and multidrug resistance (MDR) were the most prevalent among the Acinetobacter spp. isolates.

Introduction

Opportunistic infections are often associated with Acinetobacter spp., which are Gram-negative, nonfermentative bacteria that are present ubiquitously [1]. Human infections attributed to these bacteria involve both hospital-acquired and community-acquired infections, notably in critically ill patients with weakened host defenses. These infections include pneumonia, meningitis, and endocarditis, cutaneous and wound infections, peritonitis in patients on peritoneal dialysis, urinary tract infections (UTIs), and bacteremia [2]. The evolutionary timeline, phylogenetic diversity, and ecological prevalence are evident in the abundance of Acinetobacter species. Until September 2025, the genus had 87 species with validly published names, alongside several other taxonomically distinct provisional taxa and strains awaiting taxonomic clarification [3].

A multi-year hospital study revealed that A. baumannii was the most prevalent causative agent of Acinetobacter infections, followed by the non-baumannii species: A. pittii and A. nosocomialis; infections with A. haemolyticus, A. johnsonii, A. junii, A. calcoaceticus, A. lwoffii, A. schindleri, A. ursingii, and A. courvalinii have also been identified [4].

The emergence of Antimicrobial resistance has generated great concern, especially inside hospitals, as those receiving medical care or having compromised immune defenses are generally more susceptible to infections. Additionally, bacteria resistant to antibiotics can transmit within and across healthcare facilities, thereby resulting in diseases in patients termed healthcare-associated infections (HAIs), or they can spread to the community or environment (like soil and water) [5]. The significant frequency of resistant isolates among many antibiotic classes complicates initiation of successful empirical therapy. Owing to the variable multidrug resistance patterns exhibited by Acinetobacter spp., it is crucial to determine the susceptibility profiles common within the institution [6]. Numerous types

of acquired resistance mechanisms, including the production of extended-spectrum beta-lactamase enzymes (ESBLs), modification enzymes for aminoglycosides, modified binding sites for quinolones, and diverse efflux mechanisms, lead to significant challenges for clinicians when selecting an appropriate empirical antimicrobial agent. [7]. Consequently, in 2017, the World Health Organization (WHO) identified carbapenem-resistant Acinetobacter baumannii (CRAB) as a critical priority for the discovery and formulation of newly developed antibiotics [8]. This study aims to isolate and identify Acinetobacter spp. from various clinical samples, evaluate the antibiotic resistance and susceptibility patterns of Acinetobacter spp. (Multidrug-resistant, extensively drug-resistant, and pan-drug-resistant).

Materials and Methods

Study design, setting and patients

This cross-sectional study was conducted between February 2026 and June 2026 at Azadi Teaching Hospital in Kirkuk City, Iraq. Six hundred twenty-four (624) different clinical samples were obtained from both inpatients and outpatients, including urine, blood, sputum, burn swabs, wound swabs, high vaginal swabs, cerebrospinal fluid (C.S.F.), and bronchial wash. The criteria for inclusion in the study were suspected bacterial infections, whereas duplicate cultures and contaminated samples were excluded.

Sample collection, isolation, and bacteriological identification of Acinetobacter spp.

The samples collected from patients were classified as infected based on the clinical manifestations exhibited by each patient. All samples were collected by following strict aseptic protocols and were conveyed to the microbiology laboratory for processing.

The specimens were subsequently inoculated onto MacConkey agar, blood agar, and a medium, then incubated at 37°C for 24 hours. Bacterial isolates were identified by conventional microbiological methods based on colony morphology and preliminary testing, such as Gram staining, catalase, and oxidase assays. Traditional biochemical tests used, including Kligler iron agar (KIA), indole, citrate, urease, and motility assays (semi-solid manitol; SSM), to identify Acinetobacter species. Finally, all Acinetobacter species were confirmed using VITEK2 Gram-negative (GN) identification cards following the manufacturer's guidelines.

Antimicrobial susceptibility testing of Acinetobacter spp.

Testing for susceptibility to antimicrobial agents was carried out on all isolated Acinetobacter spp. using the VITEK 2 GN AST-N419 cards. The system-determined MIC values were measured according to CLSI 2026 standards [9], and the susceptibility level of each isolate was assessed as sensitive (S), intermediate (I), or resistant (R). According to the Centers for Disease Control and Prevention (CDC), isolates that are resistant to at least one antibiotic from three or more classes are defined as MDR. Isolates that demonstrate resistance to at least one antibiotic from each group of antibiotics are identified as XDR. Pan-drug-resistant (PDR) isolates are defined as strains that are resistant to all antibiotics evaluated in all groups of antimicrobial agents [10]. The identification of drug-resistant patterns (MDR, XDR, PDR, and Carbapenemase Acinetobacter spp. isolates) was obtained using VITEK 2 results, and percentages were determined in relation to the total isolates.

Statistical analysis

The data were analysed utilising SPSS software (version 27.0), and the chi-square (X^2) test was applied to assess the significance of percentages at probability levels of 0.05 and 0.01.

Results and Discussion

Patients and Acinetobacter spp. isolates characteristics

Six hundred twenty-four (624) clinical samples were obtained from patients at Azadi Teaching Hospital in Kirkuk City. Based on culture and biochemical testing, 190 (30.4%) of the 624 samples showed bacterial growth, including 44 (7.1%) Acinetobacter spp. The remaining 146 (23.3%) involved other bacteria, whereas 434 (69.6%) samples exhibited no bacterial growth, as listed in **Table (1)**.

Table (1): Culture results for different clinical samples.

Sample Culture		Number (No.)		Percentage (%)	
Bacterial Growth	**Acinetobacter spp.**	190	44	30.4%	7.1%
	Other bacteria		146		23.3%

No Bacterial Growth	434	69.6%
Total	624	100%

Our results were similar to those of the previous study [11], which reported that bacterial growth accounted for 28% in Thiruvallur City, India, and that the frequency of Acinetobacter spp. isolates was 9.9% among different clinical samples.

Furthermore, a previous study [12] reported a 4.9% frequency of Acinetobacter spp., agreeing with our findings. By contrast, our findings disagreed with a study carried out in Babylon Province [13], which found 20 (3.33%) Acinetobacter spp. isolates among 600 isolates from different clinical samples. The observed differences in frequency might be resulting from differences in study design, methodology, and timing [14].

The frequency of Acinetobacter spp. isolates in clinical samples was highest in burn swabs (29.6%), followed by urine (25%), blood (18.2%), sputum (13.6%), wound swabs (4.5%), high vaginal swabs (4.5%), cerebrospinal fluid (2.3%), and bronchial wash (2.3%), as shown in **Table (2)**.

Table (2): Isolation source and percentage of Acinetobacter species.

Isolation Source	Number of Acinetobacter spp.	Percentage of Acinetobacter spp.
Burn swabs	13	29.6%
Urine	11	25%
Blood	8	18.2%
Sputum	6	13.6%
Wound swabs	2	4.5%
Higher vaginal swabs	2	4.5%
Bronchial wash	1	2.3%
C.S.F.	1	2.3%
Total	44	100%

A comparable study carried out by [15] reported that the frequency of Acinetobacter spp. isolates was high in burn swabs (41%). Additionally, another study [16] conducted in Duhok Province reported that the frequency of Acinetobacter baumannii isolates was high in burn swabs (36.5%), consistent with our findings regarding the burn swabs prevalence rate. An increased number of isolates were observed in burn swabs due to the increased vulnerability of burns and wounds to contamination from the hospital environment, thus facilitating the spread of resistant strains. Additionally, most antibiotics demonstrate limited ability to penetrate the sites of burns and wounds [17].

The second most prevalent Acinetobacter spp. frequency in our study was observed in urine samples at a rate of 25%, which aligns with study [18], which reported a frequency of Acinetobacter spp. in urine of 25.43%. The high recovery rate from urine samples may reflect healthcare-associated urinary tract infections. A. baumannii is capable of causing infections at various clinical sites in individuals receiving hospital care. This pattern might reflect the frequent use of invasive medical devices, specifically urinary catheters [19].

Out of 624 patients, 352 were inpatients and 272 outpatients. The frequency of Acinetobacter spp. was higher in inpatients, 31 (70.5%), relative to outpatients, 13 (29.5%). Our findings revealed an increased prevalence of this infection among inpatients, which clearly reflects the nosocomial origin of this opportunistic pathogen. A similar occurrence was observed in other studies [20, 21]. It may last for extended periods in hostile environments (walls, surfaces, and medical equipment) within healthcare settings, and it is chromosomally resistant to many antibiotic classes [22].

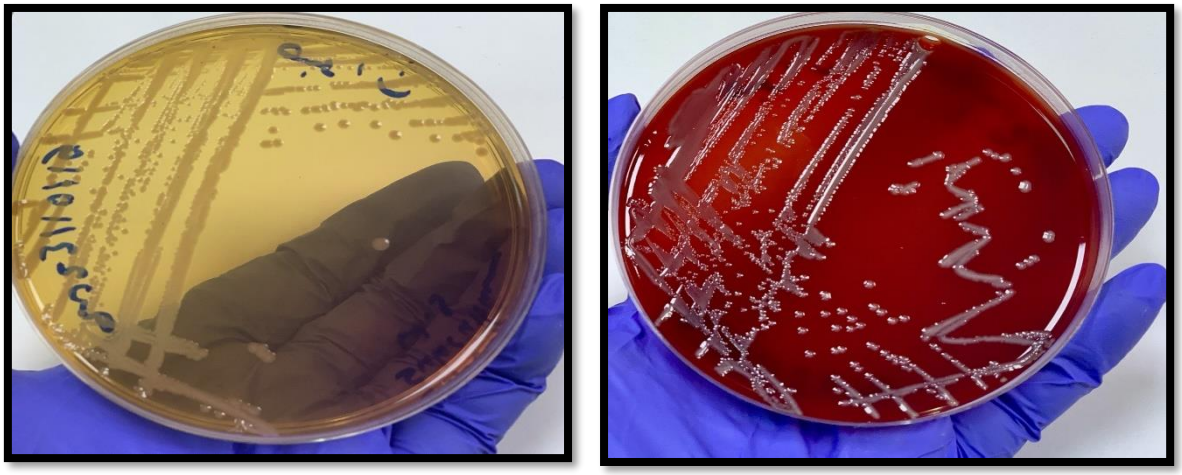
There were 367 females and 257 males aged from less than one to more than 80 years. The females 30 (68.2%) appeared to be more infected than males 14 (31.8%). This finding agreed with study [23] conducted in Wasit, Iraq, which found that the infection rate in females was 38 (65.5%) more than in 20 (34.5%) males. This might be attributed to the higher frequency of hospital visits reported by females in comparison to males [20].

Regarding patient ages, the distribution of Acinetobacter spp. was highest in 16 patients (36.4%) aged 21-40 years whereas 10 patients (22.7%) were aged 61-80 years, 7 patients (15.9%) were aged 41-60 years, 5 patients (11.4%) were aged less than one year, 4 patients (9.1%) aged 1-20 years and 2 patient (4.5%) belonged to the age group more than 80 year.

Most infections occurred in age group 21-40 years (36.4%), and the lowest percentage was observed in the age group older than 80 years (4.5%); these results are in line with the prevalence reported by a recent local study [24] carried out in Babylon and Al Najaf City. The increasing frequency in the 21–40 age range could be linked to increased exposure to traumatic injuries, surgical interventions, and healthcare facilities, particularly in contexts where young and middle-aged individuals constitute a substantial portion of patients. A study [25] revealed a high distribution of Acinetobacter spp. among those aged over 50 years; our results align with this and showed a second high rate in the 61-80 age range, followed by the 41-60 group. This suggests that elderly individuals, particularly those with underlying comorbidities, are at greater risk for A. baumannii infections.

Isolation and bacteriological identification of Acinetobacter spp. isolates

The colonies morphology on MacConkey and blood agar were used for the preliminary identification of Acinetobacter spp. isolates (No. 44). The colonies of Acinetobacter spp. seen on MacConkey agar were round, pale-colored, and non-lactose fermenting. The colonies were observed to be grey or white with a convex morphology on blood agar, as shown in **Figure (1)**.



A. MacConkey agar. B. Blood agar.

Figure (1): Culture characteristics of Acinetobacter spp. on MacConkey and blood agar.

Biochemical tests, including oxidase (negative), catalase (positive), urease (variable), citrate utilization (positive), KIA (alkaline/alkaline), motility (non-motile), and indole (negative), were performed on the bacterial isolates as shown in **Figure (2)**.

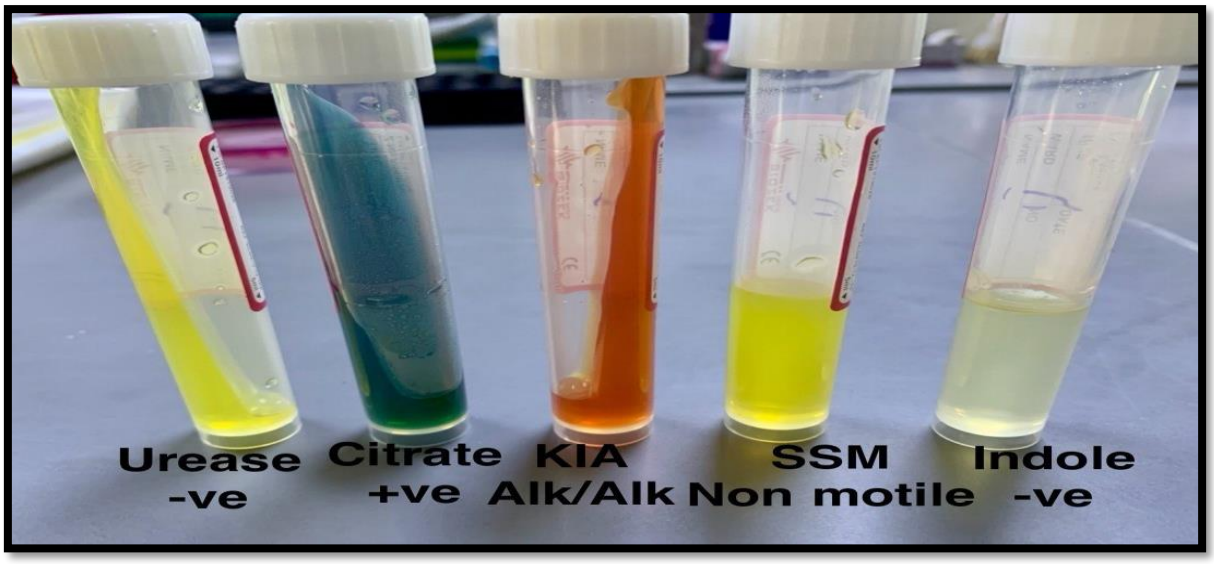


Figure (2): Biochemical characterization of Acinetobacter spp. isolates.

In addition, the identification of 44 isolates of Acinetobacter species was confirmed by the VITEK 2 system, which differentiated them into three different species: Acinetobacter baumannii complex, Acinetobacter lwoffii, and Acinetobacter haemolyticus. The A. baumannii complex was found to be the most common, with 35 (79.6%), followed by A. haemolyticus 7 (15.9%) and A. lwoffii 2 (4.5%) isolates, which agreed with the findings of other studies [26] but disagreed in that they also isolated A. radioresistens and A. junii.

Antimicrobial Susceptibility Testing (AST)

The highest resistance pattern was detected against ampicillin/sulbactam 38 (86.4%), piperacillin 34 (77.3%), cefotaxime 29 (65.9%), levofloxacin 29 (65.9%), and ciprofloxacin 28 (63.6%). Additionally, resistance levels toward ceftazidime were 28 (63.6%), ticarcillin/clavulinate 27 (61.4%), and imipenem 27 (61.4%). An intermediate resistance pattern was observed toward cefepime 26 (59.1%), trimethoprim/ sulfamethoxazole 25 (56.8%), gentamicin 25 (56.8%), tobramycin 24 (54.5%), piperacillin/ tazobactam 24 (54.5%), and meropenem 23 (52.3%). The lowest resistance was observed against colistin 2 (4.5%) and minocycline 9 (20.4%), respectively, as shown in **Table (3).**

Table (3): Antibiotic susceptibility tests of Acinetobacter species.

Class of antibiotics	Antibiotics	R	%	I	%	S	%	T
Penicillins	Piperacillin	34	77.3	2	4.5	8	18.2	44
B-lactam combination agents	Ampicillin/ Sulbactam	38	86.4	1	2.3	5	11.3	44
	Ticarcillin/Clavulinate	27	61.4	3	6.8	14	31.8	44
	Piperacillin/ Tazobactam	24	54.5	1	2.3	19	43.2	44
Cephalosporins	Ceftazidime	28	63.6	0	0	16	36.4	44
	Cefepime	26	59.1	6	13.6	12	27.3	44
	Cefotaxime	29	65.9	3	6.8	12	27.3	44
Carbapeneams	Imipenem	27	61.4	1	2.3	16	36.4	44
	Meropenem	23	52.3	2	4.5	19	43.2	44
Lipopeptide	Colistin	2	4.5	0	0	42	95.5	44
Aminoglycosides	Amikacin	16	36.4	8	18.2	20	45.4	44
	Gentamicin	25	56.8	1	2.3	18	40.9	44
	Tobramycin	24	54.5	0	0	20	45.5	44
Tetracycline	Minoycycline	9	20.4	1	2.3	34	77.3	44
Fluoroquinolones	Ciprofloxacin	28	63.6	3	6.8	13	29.6	44
	levofloxacin	29	65.9	3	6.8	12	27.3	44
Folate pathway antagonists	Trimethoprim/ Sulfamethoxazole	25	56.8	0	0	19	43.2	44

* Total: T.

The resistance patterns to the selected antibiotics revealed a significant variation among Acinetobacter spp. isolates. The high resistance to piperacillin shown in our results aligns with study results [27], which reported a resistance rate of 88.8%.

A recent study [19] carried out in Baghdad City in 2026 reported high resistance to cefotaxime, with 30 (76.9%) showing resistance, followed by ceftazidime with 23 (59.0%) resistant. The resistance rates to piperacillin/tazobactam were 21 (53.8%), aligning with the findings of the current study. A variety of β-lactamases present in Acinetobacter species hydrolyze β-lactams and provide resistance to cephalosporins, carbapenems, and penicillins. Chromosome-encoded AmpC cephalosporinases confer resistance to broad-spectrum cephalosporins [27].

Our findings were in agreement with the study [28] in which the resistance rate toward Ciprofloxacin exhibited 77.7% resistance, followed by levofloxacin 66.6% and gentamicin 63.9%. Another study [17] showed that the tobramycin resistance level was 60.9%, which was similar to our findings. Lower sensitivity to fluoroquinolones further limits the accessibility of efficacious oral therapeutic alternatives for multidrug-resistant infections [29].

A study [30] carried out in Europe reported that, among the carbapenem class, imipenem had a resistance rate of 66%, comparable to our results, but disagreed in that they reported higher meropenem resistance (74%) than our findings. This increasing resistance is alarming, particularly as many patients previously had received treatment at primary or secondary care facilities with combinations of β-lactams, aminoglycosides, or fluoroquinolones, which contributed to selective pressure and resistance [31].

The low resistance patterns of amikacin demonstrate that they are effective therapeutically; a similar result was obtained from a retrospective study [32] carried out in India, in which the resistance level toward amikacin was 46%, indicating high aminoglycoside activity.

This study's results closely aligned with the study [33] carried out in Jordan that observed the level of resistance to ampicillin-sulbactam of 80% and trimethoprim/sulfamethoxazole of 56%, respectively.

Regarding minocycline, our result was similar to the study [21], which reported resistance to minocycline was 14.28%.

A local study [17] reported that the sensitivity rate of A. baumannii isolates to colistin was 97.5%, aligning with the results of our study. Colistin was the most effective antibiotic for managing infections induced by Acinetobacter species.

In this study 44 isolates of Acinetobacter spp., of these isolates, 9 (20.5%) were found to be MDR strains, 25 (56.8%) isolates XDR, 1 (2.2%) isolates PDR and 9 (20.5%) were non-MDR isolates, which was statistically highly significant (p-value < 0.001*) as mentioned in **Table (4)**. Antibiotic susceptibility testing performed by the VITEK-2 system indicated that 28 (63.6 %) of the Acinetobacter spp. isolated strains were carbapenemase producers phenotypically, as mentioned in **Table (5)**.

Table (4): Drug resistance patterns for Acinetobacter species.

Type of resistance	Number	Percentage	P-value
MDR	9	20.5	p<0.001*
XDR	25	56.8	
PDR	1	2.2	
Non-MDR isolates	9	20.5	
Total	44	100	

***** $X^2 = 27.64$.

Table (5): Detection of carbapenemase-producing Acinetobacter species.

Acinetobacter species	Number	Percentage
CR-Acinetobacter spp.	28	63.6
Non -CR-Acinetobacter spp.	16	36.4
Total	44	100

Such resistance patterns have been observed in a local study [34] carried out in Diyala City, and [24] conducted in Babylon and Al-Najaf Cities, where A. baumannii isolates (38.0%) MDR and (62.0%) XDR strains reported were nearly comparable with the results of this study but disagreed in that there were also PDR and non-MDR isolates in our isolates. The significant proportion of MDR and XDR isolates identified in this study can be attributed to the exceptional adaptability of A. baumannii. Bacteria can develop many strategies for acquiring resistance to antimicrobial drugs, including the production of β-lactamases and carbapenemases, reducing outer membrane permeability, modifying targets, and enhancing the expression of multidrug efflux systems [19].

In a hospital-based prospective study [18] found that among carbapenem-resistant isolates screened (83.72%) confirmed the production of carbapenemase by the isolates which was higher percentage than our results. The observed variation may be attributed to elevated local antibiotic pressure, variations in methodology, the prevalence of resistance clones, and variations in geographical or institutional strategies for managing nosocomial infections [12]. Therefore, local monitoring studies of this nature play an important role in determining the most effective treatments for Acinetobacter infections.

CONCLUSION

Among Acinetobacter species, the A. baumannii complex appears to be isolated most frequently, followed by A. haemolyticus; the highest prevalence was found in burn swabs, followed by urine samples. Most Acinetobacter species were extensively drug-resistant, multidrug-resistant, and carbapenemase producers. This demonstrates the significance of alternative treatment approaches, stringent infection control protocols, and appropriate antibiotic use, especially in burn and medical wards.

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