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Original article

Antimicrobial susceptibility and distribution of *traT* and *pld* virulence genes in hospital-acquired *Acinetobacter* spp isolated from intensive care units

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ABSTRACT

Background: *Acinetobacter* is a critical nosocomial pathogen responsible for various infections. It represents global threat due to high antibiotic resistance, including to last-resort options, and possesses multiple virulence factors that lead to significant morbidity and mortality rates. **Objectives:** This study aims to investigate the association between different antibiotic susceptibility patterns and the presence of phospholipase D (*pld*) and serum resistance (*traT*) virulence genes in *Acinetobacter* isolated from clinical samples in ICUs. **Methodology:** Clinical specimens of hospital-acquired infections were collected from ICUs at Tanta University Hospitals. *Acinetobacter* isolates were identified using conventional methods, and their antibiotic susceptibility was assessed through disk diffusion. Colistin susceptibility was tested by broth macrodilution, and virulence genes (*pld* and *traT*) were detected using conventional PCR. **Results:** Out of 135 clinical samples, (20.7%) were identified as *Acinetobacter*, with 96.4% classified as multi-drug resistant (MDR). The isolates showed high resistance to cefotaxime (100%), piperacillin/tazobactam (92.9%), and ceftazidime (92.9%), while low resistance was noted for tetracycline (28.6%) and colistin (10.7%). All isolates (100%) carried the *pld* gene, and (82.1%) had the *traT* gene. Isolates with both virulence genes exhibited significantly higher resistance rates against imipenem (82.6%), ciprofloxacin (87%), and aminoglycosides (73.9%), along with absolute resistance (100%) to cefotaxime and ceftriaxone. MDR levels were notably high in both groups of virulence-associated gene carriage (95.7% for group 1 and 100% for group 2). **Conclusions:** There is high prevalence of antimicrobial resistance of *Acinetobacter* isolates among medical ICU patients with a high proportion of virulence-associated genes.

Introduction

In the last two decades, clinicians worldwide have observed an increasing number of critically ill patients suffering from infections caused by microorganisms of the *Acinetobacter* genus, primarily strains of *Acinetobacter baumannii* [1]. It can cause various infections, including skin and soft tissue infections, ventilator-associated pneumonia, catheter-associated urinary tract infections, meningitis, and bacteremia [2]. *A. baumannii* can be transmitted between patients through various means such as formulas, sinks,

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doors, feeding tubes, and even medical equipment, however, the exact source of infection remains unknown in many cases [3].

Recent reports indicate a rise in *Acinetobacter* infection rates and the ability to resist different antibiotics, which gives this species clinical recognition, especially after the emergence of multidrug-resistant (MDR) strains and pan-resistant strains [4].

Certain virulence factors appear to play a significant role in causing diseases. These factors include biofilm formation, outer membrane porins, surface structures like capsule and lipopolysaccharides, enzymes such as phospholipase D, iron acquisition systems, and regulatory proteins [5]. These virulence factors are thought to be involved in various stages of the infection process, including transmission, binding to host structures, causing cellular damage, and invading the host [6].

The toxicity of *A. baumannii* is significantly influenced by the phospholipase D (PLD) enzyme [7]. This secretory protein contains two active sites and catalyzes phosphatidylcholine to phospholipids, enabling bacteria to penetrate deep into the host tissue to escape host attacks [8].

The *traT* gene encodes a 23 kDa outer membrane protein that is non-covalently associated with peptidoglycan. It functions similarly to several peptidoglycan-associated lipoproteins and exists in the membrane as multimeric aggregates, with a considerable portion exposed on the external surface of the cell's outer membrane. TraT provides bacteria with protection against the lytic action of complement, sharing several characteristics with other major outer membrane proteins, especially the porins. Its role in serum resistance is to inhibit the proper membrane insertion or assembly of the membrane attack complex of complement [9].

The growing clinical significance of *Acinetobacter* species and their heightened antibiotic resistance has driven interest in studying the association between different antibiotic susceptibility patterns and the presence of phospholipase D (*pld*) and serum resistance (*traT*) virulence genes in *Acinetobacter* isolated from clinical samples in ICUs

Methods

Study design

This hospital-based cross-sectional study was carried out in the Medical Microbiology and Immunology Department, and in the Central Research Laboratory Faculty of Medicine, Tanta University, during the period of research from February 2023 to November 2023.

Ethical considerations

Ethical approval for this study was provided by the Ethics and Research Committee, Faculty of Medicine, Tanta University (approval code: 3626MS19/1/23). Written informed consent was obtained from each patient in this research. A code number was given for each sample for adequate provision to maintain the confidentiality of the data.

Patients and sample size determination

Using Openepi, the sample size was calculated and the study was carried out on 135 patients admitted to the ICUs of Tanta University Hospitals with symptoms and signs of infections that appeared at least 48h after admission. Full clinical history was taken from all patients (including age, sex, underlying disease, course of antibiotic treatment, duration of hospital stays, and any predisposing factors).

Specimens' collection and identification of *Acinetobacter*:

Sputum, endotracheal aspirates, bronchoalveolar lavage, bed sore swabs, pus, urine, and blood samples were collected. *Acinetobacter* were identified using microbiological conventional techniques. Samples were cultured on different culture media (**Oxoid, England**) then incubated at 37°C for 24-48 hours, and the isolates in the primary plates were identified in accordance with clinical laboratory guidelines [10] by colonial morphology, microscopic examination, and biochemical reactions (oxidase, catalase, coagulase, citrate utilization, indole, sugar fermentation, triple sugar iron agar, urease and motility tests).

Antibiotic susceptibility test:

Antimicrobial susceptibility testing of *Acinetobacter* isolates was determined by modified Kirby Bauer disc diffusion method (except for colistin) on Muller Hinton agar plates according to Clinical and Laboratory Standard Institute (CLSI) guideline [11] Using the following antibiotics (**Oxoid, England**): imipenem (10 µg), amikacin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), sulfamethoxazole/trimethoprim (1.25/23.75 µg), piperacillin/tazobactam (100/10 µg), cefotaxime (30 µg), meropenem (10 µg), gentamicin (10 µg), ceftriaxone (30 µg), tetracycline (30 µg). The plates

were incubated for 24 hours at 37°C, and then the inhibitory zones were measured and assessed in accordance with the CLSI-recommended protocols.

Minimal inhibitory concentration (MIC) for colistin:

Based on CLSI guidelines, colistin susceptibility among *Acinetobacter* isolates was assessed by detection of colistin MIC using broth macrodilution method. Colistin serial dilutions have been done using Muller Hinton broth and the bacterial isolates were adjusted to 0.5 McFarland standard and were added to each tube, then incubated for 18 hours at 37°C. The highest concentration at which no visible growth was considered the MIC. Susceptibility categories were defined as follows: Intermediate resistance at MIC ≤ 2 µg/ml and full resistance at ≥ 4 µg/ml [11].

Genomic DNA extraction:

ABT bacterial DNA mini extraction Kit (**Applied biotechnology®, Egypt**) was used to extract DNA from *Acinetobacter* isolates in accordance with the kit's instructions. The concentration and purity of DNA were measured by spectrophotometer (**ScanDrop®, analytikjena)** and the extracted DNAs were stored at – 20 °C.

Detection of virulence-associated genes:

All *Acinetobacter* isolates were tested for the presence of virulence genes by amplification of both phospholipase D (*pld*) and serum resistance (*traT*) genes separately using conventional PCR.

Using the primers listed in **Table 1**, the PCR procedure was conducted at a final volume of 25 µl including: 2 µl of template DNA, 1 µl of each of forward primer (10 µM) and reverse primer (10 µM) for the *pld* and *traT* genes**,** 12.5 µl of 2X FastGene® Taq ReadyMix (1.5 mM MgCl2 at 1X)[2] and 8.5 µl of nuclease-free water. *Acinetobacter baumannii* (ATCC® 19606™) was used as a positive control strain for *pld* gene. Amplification at the thermocycler (**Conventional PCR-Biometra, AnalytikJena AG, Germany**) was programmed as follows: Initial denaturation at 95 ºC for 3 minutes, followed by 35 cycles of denaturation at 95 ºC for 30 seconds, annealing at 87°C for *pld* gene and 68°C for *traT* gene, and extension at 72 °C. Finally, the final extension step at 72 °C. The amplicons were run on a 1.5% agarose gel, stained with ethidium bromide, and visualized under an ultraviolet transilluminator before being photographed as illustrated in F**igure 1**.

Statistical Analysis

In this study, statistical data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 20 (Armonk, NY: IBM Corp). A p-value < 0.05 was considered significant, a p-value < 0.001 was deemed highly significant, while a p-value > 0.05 was regarded as statistically not significant. The chi-square test was performed for categorical variables to compare different groups. The Z test of proportions was used to determine the true difference in proportions between two independent groups within a given confidence interval.

Results

Clinical characteristics and distribution of *Acinetobacter* isolates among clinical samples

Twenty-eight (20.7%) *Acinetobacter* isolates were recovered from 135 clinical samples from patients admitted to the ICUs of Tanta University Hospitals with different types of hospital-acquired infections. Out of 28 *Acinetobacter*- infected patients, (64.3%) were males and (35.7%) were females. The patients' ages ranged from (20 to 80) years, with the majority of patients (67.9%) being between (60 and 80) years old. Regarding the predisposing factors, *Acinetobacter* infection was notably high in patients with diabetes mellitus and hypertension, with a p-value of < 0.05 (21.4% and 14.3%, respectively). *Acinetobacter* with the highest isolation rate recovered from endotracheal aspirate samples (32.1%), sputum (21.4%), and bronchoalveolar lavage (14.3%) as illustrated in **Table 2.**

Antimicrobial susceptibility test of *Acinetobacter* isolates

Based on the results obtained from the disc diffusion method, all isolates (100%) were resistant to cefotaxime. High resistance rates were observed against piperacillin-tazobactam and ceftazidime (96.36% for each). Furthermore, (89.3%) were resistant to sulfamethoxazole-trimethoprim, (82.1%) were resistant to each of ciprofloxacin and ceftriaxone, and (78.6%) were resistant to each of amikacin, imipenem, and gentamicin. Moreover, (75%) of isolates showed resistance to meropenem, while the least resistance rate (28.6%) was observed for tetracycline as shown in **Table 3.**

The majority of *Acinetobacter* isolates were categorized as MDR (96.4%), as they were non-susceptible to at least one agent in three or more antimicrobial categories, including penicillins,

cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems.

Colistin susceptibility

Regarding colistin susceptibility, the results revealed that (89.3%) of isolates showed intermediate sensitivity to colistin with MIC values ≤ 2 µg/ml, while (10.7%) were resistant with MIC values ≥ 4 µg/ml **(Table 4).**

Detection and analysis of *pld* and *traT* virulence genes

The PCR results showed that the *pld* gene was detected in all *Acinetobacter* isolates (100%), while the *traT* gene was detected in (82.1%) isolates only. Analysis of the cross-resistance profile revealed a statistically significant association between *traT* genes and MDR *Acinetobacter* isolates **(Table 5).**

Categorization of virulent *Acinetobacter* isolates:

Acinetobacter isolates were further categorized into two groups based on the presence of virulence-associated genes (*pld* and *traT* genes):

• **Group (1):** Isolates carried both investigated virulence genes (*pld* and *traT* genes) = 23 isolates

• **Group (2):** Isolates carried only one virulence gene (*pld* gene only) = 5 isolates

The relationship between virulence-associated genes in both groups of *Acinetobacter* isolates and their antibiotic susceptibility profile is presented in **Table 6.** The results demonstrated that group (1) isolates showed higher resistance rates against imipenem (82.6%), ciprofloxacin (87%), sulfamethoxazole/trimethoprim (91.3%), and piperacillin/tazobactam (91.3%), with statistically significant higher resistance rates against aminoglycosides; amikacin (73.9%) and gentamicin (73.9%). Furthermore, these virulent isolates showed absolute resistance (100%) against each of cefotaxime and ceftriaxone. Interestingly, none of the group (2) isolates were resistant to colistin and none of them were sensitive to tetracycline. Moreover, results indicated that MDR was considerably high in both groups of virulence-associated genes; however, it was slightly higher in group (2).

Table 1. The sequence of primers used and their product length [33]*:*

Target gene		**Sequence (5′→ 3′)**	**Product size (bp)**
Pld	**F**	CTGCAGATTATGGCACAATCCTTTCATTCCA	1743
	R	CTGCAGGTAGAAGGCCATGATGTAAAAAGTT	
traT	**F**	GGTGTGGTGCGATGAGCACAG	290
	R	CACGGTTCAGCCATCCCTGAG	

Table 2: Demographic characteristics and clinical samples of studied patients:

	***Acinetobacter*- infected patients (n = 28)**	**Other patients (n = 107)**	**Test of sig.**	**p**
Gender				
Male	18 (64.3%)	58 (54.2%)	χ^2=1.398	0.237
Female	10 (35.7%)	49 (45.8%)		
Age (years)				
20 – 40 years	1 (0.9%)	1 (0.9%)	χ^2= 1.541	^{MC}p= 0.532
40 – 60 years	8 (28.6%)	34 (31.8%)		
60 – 80 years	19 (67.9%)	72 (67.3%)		
Mean ± SD	60.64 ± 9.51	62.69 ± 6.35	U=1303.50	0.290
Median (Min. – Max.)	62 (20 – 70)	64 (40 – 70)		
Associated comorbidity				
Trauma	2 (7.1%)	3 (2.8%)	χ2=1.172	0.279
Diabetes mellitus	6 (21.4%)	45 (42.1%)	χ2=4.017*	0.045*
Chronic heart disease	0 (0%)	2 (1.9%)	χ2=0.531	^{FE}p=1.000
Hypertension	4 (14.3%)	1 (0.9%)	χ2=11.0922*	^{FE}p=0.007*
Chest disease	2 (7.1%)	4 (3.7%)	χ2=0.606	^{FE}p=0.604
Chronic kidney disease	2 (7.1%)	11 (10.3%)	χ2=0.251	^{FE}p=1.000
Cancer	4 (14.3%)	4 (3.7%)	χ2=4.429	^{FE}p=0.058
Length hospital stay (Days)				
Mean ± SD.	12 ± 4.8	13.16 ± 4.09	U=1266.50	0.207
Median (Min. – Max.)	13 (5 - 22)	14 (5 - 20)		

Clinical samples	
Sample	***Acinetobacter* isolates (n = 28)**
Endotracheal aspirate	9 (32.1%)
Sputum	6 (21.4%)
Bronchoalveolar lavage	4 (14.3%)
Urine	3 (10.7%)
Blood	3 (10.7%)
Bedsore swab	2 (7.1%)
Pus	1 (3.6%)

Table 3: Antibiotic resistance profile of *Acinetobacter* isolates to different antimicrobial agents:

	Antibiotic	***Acinetobacter* isolates (n = 28)**
Carbapenems	**Imipenem**	22 (78.6%)
	Meropenem	21 (75%)
Aminoglycosides	**Amikacin**	22 (78.6%)
	Gentamicin	22 (78.6%)
Cephalosporins	**Ceftazidime**	26 (92.9%)
	Cefotaxime	28 (100%)
	Ceftriaxone	23 (82.1%)
Fluoroquinolones	**Ciprofloxacin**	23 (82.1%)
Folate pathway antagonists	**Sulfamethoxazole trimethoprim**	25 (89.3%)

B-lactam combinations	**Piperacillin/tazobactam**	26 (92.9%)
Tetracyclines	**Tetracycline**	8 (28.6%)

Table 4: Colistin MIC values for *Acinetobacter* isolates:

MIC values (µg/ml)		**No. of isolates Total (n=28)**
Intermediate	**0.25**	0
	0.5	15
	1	6
	2	4
	Total	**25 (89.3%)**
Resistant	**4**	0
	8	2
	16	0
	32	1
	64	0
	128	0
	Total	**3 (10.7%)**

Table 5: Distribution of virulence-associated genes among *Acinetobacter* resistance categories:

	Non-MDR (n = 1)		**MDR (n = 27)**		**P**
***pld* gene**					
Negative	0	0.0	0	0.0	–
Positive	1	3.6	27	96.4	
***traT* gene**					
Negative	0	0.0	5	17.9	0.013*
Positive	1	3.6	22	78.6	

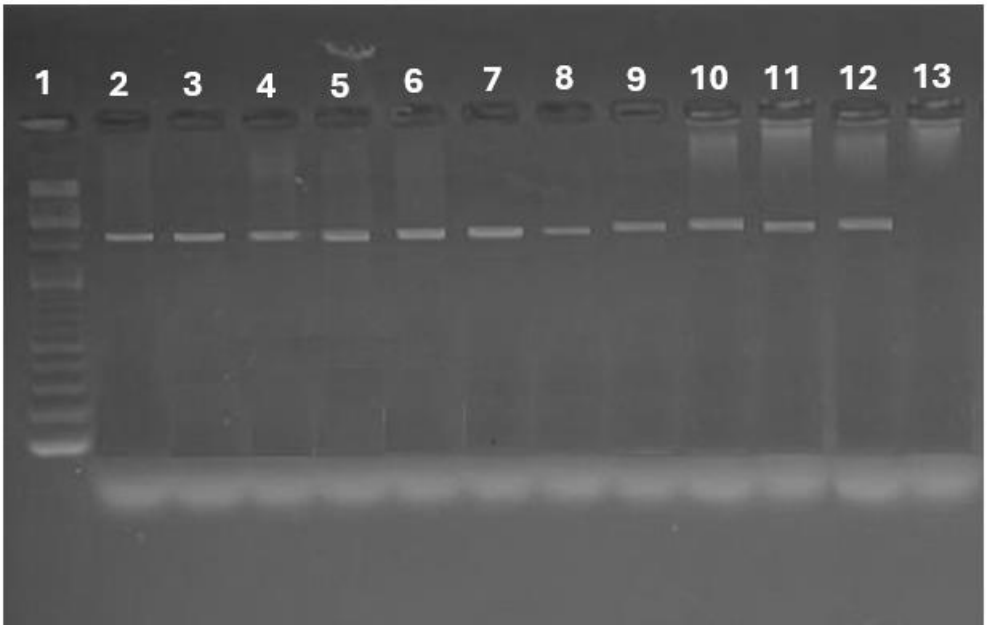
Table 6: Relationship between virulence-associated genes in both groups of *Acinetobacter* isolates and antibiotic susceptibility, and distribution of resistant *Acinetobacter* categories among both groups of virulence-associated genes:

Antibiotic	**Result**	**Group (1) (*pld* and *traT* genes) (n = 23)**	**Group (2) (*pld* gene only) (n = 5)**	**P**
Imipenem	Resistant	19 (82.6%)	3 (60%)	0.332
	Intermediate	1 (4.3%)	2 (40%)	0.110
	Sensitive	3 (13%)	0 (0%	0.063
Amikacin	Resistant	17 (73.9%)	5 (100%)	**0.004***
	Intermediate	3 (13%)	0 (0%	0.063
	Sensitive	3 (13%)	0 (0%	0.063
Ceftazidime	Resistant	21 (91.3%)	5 (100%)	0.139
	Intermediate	1 (4.3%)	0 (0%)	0.307
	Sensitive	1 (4.3%)	0 (0%)	0.307

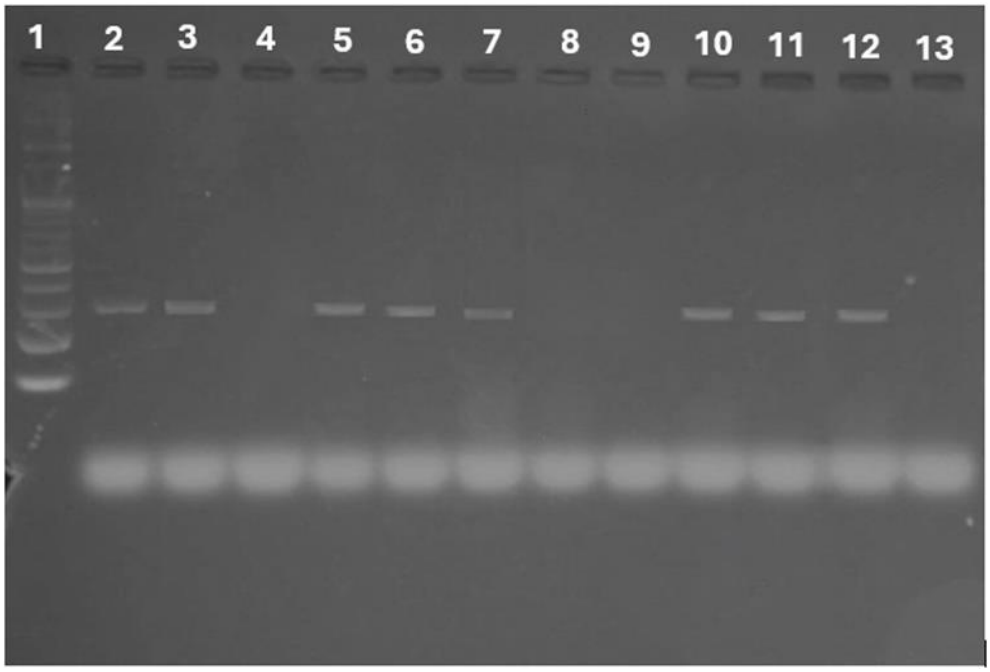
Ciprofloxacin	Resistant	20 (87%)	3 (60%)	0.241
	Intermediate	2 (8.7%)	2 (40%)	0.168
	Sensitive	1 (4.3%)	0 (0%)	0.307
Sulfamethoxazole/ trimethoprim	Resistant	21 (91.3%)	4 (80%)	0.548
	Intermediate	1 (4.3%)	1 (20%)	0.395
	Sensitive	1 (4.3%)	0 (0%)	0.307
Piperacillin/tazobactam	Resistant	21 (91.3%)	5 (100%)	0.139
	Intermediate	1 (4.3%)	0 (0%)	0.307
	Sensitive	1 (4.3%)	0 (0%)	0.307
Cefotaxime	Resistant	23 (100%)	5 (100%)	-
	Intermediate	0 (0%)	0 (0%)	-
	Sensitive	0 (0%)	0 (0%)	-
Meropenem	Resistant	17 (73.9%)	4 (80%)	0.762
	Intermediate	3 (13%)	0 (0%)	0.063
	Sensitive	3 (13%)	1 (20%)	0.717
Gentamicin	Resistant	17 (73.9%)	5 (100%)	**0.004***
	Intermediate	4 (17.4%)	0 (0%)	**0.028***
	Sensitive	2 (8.7%)	0 (0%)	0.139
Ceftriaxone	Resistant	23 (100%)	5 (100%)	-
	Intermediate	0 (0%)	0 (0%)	-
	Sensitive	0 (0%)	0 (0%)	-
Tetracycline	Resistant	5 (21.7%)	3 (60%)	0.104
	Intermediate	5 (21.7%)	2 (40%)	0.438
	Sensitive	13 (56.5%)	0 (0%)	**0.000***
Colistin	Resistant	3 (13%)	0 (0%)	0.063
	Intermediate	20 (87%)	5 (100%)	0.063
	Sensitive	0 (0%)	0 (0%)	-
Acinetobacter resistance categories				
	Group (1) (both genes) (n = 23)	**Group (2) (*pld* gene only) (n = 5)**	**χ^2**	**^{FE}p**
Non- MDR	1 (4.3%)	0 (0%)	0.225	1.000
MDR	22 (95.7%)	5 (100%)		

χ^2: **Chi square test** **FE: Fisher Exact test,** p: p value for comparing between the studied groups.,
Multidrug-resistant **(MDR), *:** statistically significant

Figure 1: A**):** Agarose gel electrophoresis of amplified pld gene.
B): Agarose gel electrophoresis of amplified traT gene



A



B

A**):** Agarose gel electrophoresis of amplified pld gene. "Lane 1": The molecular weight size marker (100 to 3000 bp). "Lane 2-11": Detected pld gene at 1743 bp. "Lane 12": Positive control. "Lane 13": Negative control.
B): Agarose gel electrophoresis of amplified traT gene. "Lane 1": The molecular weight size marker (100 to 3000 bp). "Lane 2, 3, 5, 6, 7, 10, 11, and 12": Detected traT gene at 290 bp. "Lanes 4, 8, and 9": Non detected traT gene. "Lane 13": Negative control.

Discussion

Acinetobacter species can cause serious illnesses, particularly in patients with risk factors such as advanced age, immunocompromised state, invasive procedures, and prolonged hospitalization [12].

During the ten months of this study, 28 (20.7%) cases of positive *Acinetobacter* culture were identified among the 135 patients enrolled.

The current study found a male predominance (64.3%) among *Acinetobacter*-infected patients, consistent with previous studies by *Modi et al.* [13], *Kipsang et al.* [14], and *Singh et al.* [15] which reported that males were more affected by *Acinetobacter* than females. On the contrary, *Moulana et al.* [16] reported that (60%) of *Acinetobacter* isolates were collected from female patients. This variation may result from differences in immunological responses between males and females. Generally, adult females demonstrate stronger innate and adaptive immune responses compared to males [17].

Among *Acinetobacter*-infected patients, most of them (67.9%) were aged 60-80 years. *Mukhtar et al.* [18] found (75.3%) of isolates came from those aged 51-70 years, also *Liu et al.* [5] noted most patients were 69-79 years old. In contrast, *Kipsang et al.* [14] in Kenya found the majority were aged 45-60 years. Advanced age is an independent risk factor for *Acinetobacter* infection [18].

In this study, we found that among *Acinetobacter*-infected patients, (21.4%) had diabetes mellitus and (14.3%) had hypertension, both of which are significant risk factors for infection (P-value $\leq$ 0.05). This aligns with *Ceparano et al.* [19], who noted hypertension and diabetes as the most common comorbidities. In contrast, *Prata-Rocha et al.* [20] identified renal disease (30.1%) and malignancy (13.7%) as the most common comorbidities, while *Despotovic et al.* [21] found cardiovascular disease to be the most prevalent (54.2%).

In the current study, the majority of *Acinetobacter* isolates were obtained from respiratory samples; endotracheal aspirate (32.1%), sputum (21.4%), and bronchoalveolar lavage (14.3%), followed by blood and urine (10.7% each), bedsore swab (7.1%), and pus (3.6%). Different studies, for instance, *Mukhtar et al.* [18] and *Rajkumari et al.* [22] have highlighted the prevalence of *Acinetobacter* strains in broncho-pulmonary samples. The upper respiratory tract has been reported as the preferred site for *Acinetobacter* colonization therefore, this pathogen frequently causes mechanical ventilation-associated infections [23]. In contrast, *Makled et al.* [24] in Egypt found the highest isolation rate of *Acinetobacter* in urine samples (33.3%), followed by burn swabs (23.3%) and blood samples (16.7%). *Singla et al.* [25] noted that blood samples (30%) were the most common source, followed by respiratory samples (25.6%). The variation in isolation rates among different studies could be attributed to the difference in the hospital environment, the patients' clinical conditions, and the number of samples investigated [26].

The antimicrobial susceptibility testing of *Acinetobacter* isolates in this study using the disk diffusion method for various antibiotics revealed a high resistance rate to piperacillin-tazobactam was observed at (92.9%), aligning with *Singh et al.* [15], who reported rates of (93%). In contrast, *Singla et al.* [25] reported a lower rate of (36.7%). Resistance rates against cephalosporins were also high in the present study: cefotaxime (100%), ceftriaxone (82.1%), and ceftazidime (96.36%), consistent with findings by *Mohammed et al.* [17] and higher than those reported by *Dessie et al.* [27]. Aminoglycosides appear to retain activity against many *Acinetobacter* isolates but as with all antimicrobial agents and MDR pathogens, resistance to aminoglycosides is increasing [15]. In the present study, both amikacin and gentamicin exhibited the same resistance rate (78.6%). *Singh et al.* [15] also reported similarly high resistance rates for aminoglycosides (71.9%) for amikacin and (82.2%) for gentamicin. On the other hand, *Mirzaei et al.* [28] reported higher resistance rates than the current study (88.9%) for amikacin and (100%) for gentamicin.

A high resistance rate was observed for fluoroquinolones, with ciprofloxacin showing a resistance rate of (82.1%). This result aligns with *Singh et al.* [15], who reported a resistance rate of (83%). Conversely, the study by *AL-Kadmy et al.* [29] found that *A. baumannii* exhibited a (100%) resistance to ciprofloxacin. Tetracyclines are not typically used for *Acinetobacter* infections, but recent practices include doxycycline and minocycline with other antibiotics to improve treatment efficacy [13]. The current study reports a resistance rate of (28.6%) for tetracycline, consistent with *Beheshti et al.* [30] (21.53%). However, *Singh et al.* [15] reported a higher rate of (72.4%).

The high resistance pattern of trimethoprim/sulfamethoxazole has been observed in different studies, reaching as high as (92%) as reported by *Said et al.* [31]. These findings are consistent with the current study, which found that (89.3%) of isolates were resistant to sulfamethoxazole/trimethoprim.

In the present study, resistance rates for imipenem and meropenem were (78.6%) and (75%),

aligning with *Castilho et al.* [32], who reported resistance rates of (76.7%) for both antibiotics. However, a study by *Singh et al.* [15] reported higher resistance rates of imipenem and meropenem (96% and 82%) respectively.

From the results of this study regarding colistin sensitivity; (89.3%) were intermediate, while (10.7%) of *Acinetobacter* isolates demonstrated resistance to colistin using the tube macrodilution method. The resistance rate result closely aligns with those reported by *Makled et al.* [24] (10%) and *Moulana et al.* [16] (6%). On the other hand, *Sadr et al.* [34] reported (100%) sensitivity to colistin with no resistant strains.

The present study revealed high rates of drug resistance among *Acinetobacter* isolates, with (96.4%) classified as MDR. This is similar to *Castilho et al.* [32] who reported (91.1%) and *Tolba et al.* [34] in Egypt, showing (95.1%) as MDR. However, this study's MDR rate exceeds *Singh et al.* [15], where (79%) were MDR. The high incidence of MDR strains in ICUs may be due to excessive antimicrobial use [32].

Antibiotic resistance rates vary across geographical regions and even within different hospital units over time. Factors influencing these rates include the characteristics of the studied population, general health conditions, sample types, adherence to infection control, antibiotic stewardship programs, and definitions of MDR in different countries [22, 35].

The genotypic detection of virulence-associated genes in this study showed that all isolates expressed the *pld* gene (100%), consistent with *Bahador et al.* [36] and *Depka et al.* [37], who reported rates of (100% and 99%) in Iran and Poland, respectively. In contrast, *Sadr et al.* [34] found a lower rate of (76.67%) in 198 *Acinetobacter* isolates in Iran. The *traT* gene rate in this study was (82.1%), aligning with *Mohajeri et al.* [38], who reported (80%). However, findings differ from *Sadr et al.* [34], and *Liu et al.* [5], who reported *traT* gene presence in (66.67%, and 0%) respectively. The discrepancy in percentages of the same virulence genes among different studies could be attributed to their endemicity, and prevalence variation worldwide. Also, this difference could be due to phenotypic and genotypic detection techniques for these virulence factors [39, 40].

The carriage rate of *pld* and *traT* virulence genes in MDR *Acinetobacter* isolates was significantly higher than that of the non-MDR isolate. This finding was in agreement with a study conducted by *Liu et al.* [5] who stated that the *pld* gene was also highly associated with MDR isolates compared to non-MDR isolates.

To the best of our knowledge, the current study is the first of its kind that aims to compare between single and multiple virulence genes in association with antibiotic resistance. The isolates were further divided into two groups based on the presence of virulence-associated genes (*pld* and *traT* genes). The results showed significantly higher resistance rates against aminoglycosides such as amikacin (73.9%) and gentamicin (73.9%).

There are a few limitations to discuss this finding. Firstly, the number of isolates carrying only the *pld* gene is considered relatively small. Secondly, the generalizability of results is limited because the effect of whole virulence genes of *Acinetobacter* was not analyzed in relation to each other. Consequently, further studies involving larger sample numbers and analyzing the whole virulence genes are necessary to clarify the relationship between antibiotic resistance and virulence genes in *Acinetobacter*.

Conclusion

Acinetobacter isolates are frequently found in ICUs, particularly in respiratory samples. These isolates exhibit high levels of resistance to many antibiotics, including cephalosporins, aminoglycosides, carbapenems, and fluoroquinolones. However, they remain susceptible to tetracycline and colistin. A statistically significant association was found between isolates carrying both virulence-associated genes (*pld* and *traT*) and antibiotic resistance, particularly to aminoglycosides such as amikacin and gentamicin. This suggests a correlation between the presence of these genes and increased drug resistance. The relationship between virulence and antimicrobial resistance is quite complex, highlighting the urgent need for the discovery of new therapeutic targets and the development of innovative diagnostics for this pathogen.

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