Microbes and Infectious Diseases 2025; 6(1): 319-328

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Phenotypic and molecular characterization of carbapenem-resistant *Acinetobacter baumannii* isolates from ventilator associated pneumonia patients

Rehab A. Rabie[1*], Nagwan A. Ismail[2] and Hanaa M. El Maghraby[1]

1- Department of Medical Microbiology and Immunology, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

2- Department of Chest, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

ARTICLE INFO

Article history:
Received 13 August 2024
Received in revised form 15 October 2024
Accepted 19 October 2024

Keywords:
A. baumannii
Carbapenems
Carbapenemases
Ventilator associated pneumonia

ABSTRACT

Background: Multi- drug resistant *Acinetobacter baumannii* (*A. baumannii)* is considered a major cause of ventilator associated pneumonia (VAP) among intensive care unit (ICU) admitted patients. The objectives of this study were to determine the prevalence of carbapenem - resistant *A. baumannii* causing VAP in ICU and to investigate the presence of carbapenem hydrolyzing class D β-lactamase genes among the studied isolates. **Methods:** In a cross-sectional study, *A. baumannii* was isolated from VAP patients and its antibiotic susceptibility was detected. Carbapenem resistance was determined phenotypically by modified carbapenem inactivation method (mCIM) and genotypically by polymerase chain reactions for detection of class D β-lactamases including; bla_{OXA}-51-like gene, bla_{OXA}-23-like, bla_{OXA}-24-like, and bla_{OXA}-58- like genes. **Results:** Forty-six *A. baumanii* were isolated from 140 respiratory specimens collected from VAP patients. Using disc diffusion methods, all *A. baumanii* isolates were multidrug resistant (100%) and 39 (84.7%) of them were carbapenem resistant. Carbapenemases genes were positive in 36 (92.3%) of carbapenem resistant *A. baumanii*. Using mCIM test, carbapenemases were detected in 34 (87.2%) carbapenem resistant *A. baumanii.* Non-significant agreement existed between phenotypic and genotypic detection methods of carbapenemases (Kappa= 0.1 and *p* value 0.4). **Conclusion:** Carbapenem-resistant *A. baumannii* isolates were responsible for a significant number of VAP in intensive care unit admitted patients. Carbapenemases genes especially *OXA* 23 were frequent in *A. baumannii*.

Introduction

About 50% of cases of hospital-acquired pneumonia are caused by ventilator-associated pneumonia (VAP), a lung infection that appears 48 hours after endotracheal intubation. Fever, purulent tracheal discharge, and respiratory troubles when bacteria are present are its characteristic symptoms [1]. With an incidence range of 5 to 40%, it has been found to be among the most common infections acquired in critical care units (ICUs) [2].

Still, VAP continues to be identified as a significant cause of morbidity and death in ICUs [3]. Pathogenic bacteria may invade artificial airways; the most common pathogens are *Enterobacter*, *Klebsiellae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *especially methicillin resistant staphylococcus aureus (MRSA*) and

DOI: 10.21608/MID.2024.312046.2156

* *Corresponding author:* Rehab Ahmed Rabie
E-mail address: *dr.rehabrabie@yahoo.com*

© 2020 The author (s). Published by Zagazig University. This is an open access article under the CC BY 4.0 license **https://creativecommons.org/licenses/by/4.0/.**

Acinetobacter species. Prior research has indicated that a significant number of coronavirus disease of 2019 (COVID-19) patients admitted to intensive care units have carbapenem-resistant *A. baumannii* (CRAB) which has been identified as one of the most prevalent bacteria in the intensive care unit (ICU) that causes serious infections [4].

A. baumannii is known to be resistant to a variety of antibiotics. Since carbapenems are powerful antibiotics typically used as a last resort to treat *A. baumannii* infections, resistance to them is the most alarming [5].

Several mechanisms, such as efflux, and impermeability resulting from the loss of major porins, can mediate carbapenem resistance in *A. baumannii* [6]. Most often, it is mediated by the drug's enzymatic hydrolysis, namely by class D β-lactamases (oxacillinases) that hydrolyze carbapenem. They are poor carbapenem hydrolysers, but when they are overexpressed, they provide resistance because they bind to mobile elements like ISA_{ba1}, which has a powerful promoter [7].

Despite the fact that oxacillinases -related resistance to carbapenem has been documented in a variety of geographic locations. Regarding their distribution and prevalence in our area, not much is known. We thus set out to determine the prevalence of carbapenem - resistant *A. baumannii* causing VAP in ICU and to investigate the presence of carbapenem hydrolyzing class D β-lactamase genes among the studied isolates.

Methods

Study design and settings

This cross-sectional study was carried out at the Medical Microbiology and Immunology and ICU of Chest Departments, Faculty of Medicine, Zagazig University throughout 10 months from July 2023 to April 2024

The estimated sample size was 46 isolates of *A. baumannii* calculated using Epi software version 6 assuming that total number of cases admitted to ICU was 130 and estimated prevalence of *A. baumannii* was 32.6 % [8] at 80% power and 95% CI.

Ethical approval

The study protocol was reviewed and approved by the ethics Institutional Review Board of the Faculty of Medicine, Zagazig University (ZU-IRB; #10970 -25-7-2023). This study was done in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). For each participant, signed informed consent was obtained.

Patients whose age ≥18 years; on invasive mechanical ventilation > 48 h and who met the diagnostic criteria of VAP according to National Healthcare Safety Network (NHSN) [9,10], were eligible for inclusion.

Clinical samples collection and identification

Twenty pleural fluid specimens, thirty-six bronchoalveolar lavage (BAL) fluid specimens, and eighty-four endotracheal aspirate (ETA) specimens were among the 140 respiratory samples taken from VAP patients admitted to the pulmonary ICU. They were collected aseptically and transported immediately to the laboratory. The ETA smear was analyzed in a low power field, and the quality of the samples was evaluated. Less than 10 squamous epithelial cells or more than 25 pus cells/low power field were approved for cultivation. For specimen cultivation, 5% blood agar and MacConkey agar were utilized. The specimens were then incubated at 37°C for 16 to 18 hours [11]. Conventional microbiological methods were used for isolates identification [12]. Confirmation of isolated *A. baumanii* was done by detection of the bla_{OXA}-51-like gene which is an intrinsic gene for this species identification [13, 14].

Antimicrobial susceptibility testing

Isolates' susceptibility to various antibiotics was investigated on Mueller–Hinton agar **(Oxoid, UK)** by using the disc diffusion agar method according to the Clinical and Laboratory Standards Institute [15]. Antibiotic disks **(Oxoid, UK)** used in the study were "ceftazidime (CAZ, 30μg), cefotaxime (CTX, 30ug), Ampicillin/ sulbactam (SAM, 10/10μg), piperacillin (PRL, 100 μg), piperacillin/tazobactam (PTZ, 100 μg/10μg), ciprofloxacin (CIP, 5μg), levofloxacin (LEV, 5 μg), gentamicin (GM, 10μg), amikacin (AK, 30 μg), imipenem (IMI, 10μg), meropenem (MEM, 10 μg), sulphamethoxazole-trimethoprim (SXT, 1.25 + 23.75 μg) and tigacycline (TGC, 15 μg). Broth microdilution was used to demonstrate colistin susceptibility with colistin concentrations ranging from 0.25 to 32 μg/ml were applied and resistant means MIC ≥4 μg/mL. Quality control strains including; *Pseudomonas aeruginosa*ATCC®27853 and *Escherichia coli* ATCC 25922 were used

(American Type Culture Collection [ATCC], Manassas, VA, USA). Multidrug resistant (MDR) isolates were those resistant to more than three classes of antibiotics [16].

Phenotypic tests for screening carbapenemase production; Modified Carbapenem inactivation method (mCIM)

It was done according to the CLSI guidelines; a 1 µL of CRAB was inoculated on 2 mL tube of tryptic soy broth. A 10 µg meropenem disk was placed in the tube and it was incubated at 35 °C for 4 h ± 15 min. Then, the disk was picked from the tube and placed on Mueller-Hinton agar plate recently loaded with a 0.5 McFarland suspension of a carbapenem-susceptible *E. coli* ATCC 25922. Subsequently, the plate was incubated at 35 °C for 18 to 24 h. A zone size ≥19 mm was considered negative, 6 to 15 mm was positive, or intermediate (defined as positive) if pinpoint colonies are present within a 16 to 18 mm zone [17,18].

PCR for *OXA* genes

Genomic DNA extraction from isolates was done as per the manufacturer's guidelines of the QIAamp DNA Mini Kit (Qiagen, GmbH, Germany).

Veriti ®96-Well thermal cycler (Applied Biosystems, Singapore) was used for the multiplex PCR for bla_{OXA}-51-like gene, bla_{OXA}-23-like, bla_{OXA}-24-like, and bla_{OXA}-58- like genes using 50 µl PCR bead of Maxime PCR PreMix Beads (iNtron, Certified Company, Germany).

Each bead reaction contained; 30 ng of the DNA, 20 pM of each of the primers and completed with sterile deionized water.

PCR for ISA_{b1} gene

Single reaction mixture using 20 µl PCR bead of Maxime PCR PreMix Beads (iNtron, Certified Company, Germany). Each reaction contained; 30 ng of DNA, 10 pM of each primer and completed with sterile deionized water.

Primers sequences and cycling conditions listed in **Tables 1 & 2**. Amplified products were visualized on 1.5% agarose gel under UV light.

Statistical analysis

Quantitative variables were described using their means, median and standard deviations, while categorical values were represented as percentage. Cohen's kappa coefficient was used to measure reliability for categorical items. SPSS version 27 inc. Chicago, USA was used to analyze data. Level of significance was set at p value ≤ 0.05.

Results

Forty-six isolates of *A. baumanii* were found in 140 respiratory specimens (32.8%) obtained from VAP patients in this cross-sectional research. **Table 3** demonstrates demographic and clinical criteria of patients. The highest isolation was from ETA (N=30) with 65.2% followed by BAL (N=14) with 30.4% and the least from pleural fluid (N=2) with 4.4% **(Figure 1)**.

Antibiotic susceptibility testing result is demonstrated in **(Figure 2)**. All *A. baumanii* isolates were MDR (100%). Phenotypically, 39 (84.7%) *A. baumanii* isolates were CRAB. Using mCIM, carbapenemases were positive in 34 (87.2%) out of 39 *A. baumanii* isolates.

Oxa genes were detected in 36 (92.3%) *A. baumanii* isolates; *oxa* 23 was detected in all of them, *oxa* 58 co -existed with *oxa* 23 in 2.6% of *A. baumanii* isolates, isa_{b1} was detected in 25 (64.1%) of isolates upstream to *oxa* 23. However, *oxa* 24 was not detected **(Table 4)**.

The identification of carbapenemases by genotypic and phenotypic methods showed statistically non-significant agreement (Kappa= 0.1 and p value 0.4) **(Table 5)**.

Table 1. Primers sequences used in *OXA* and ISA_{b1} genes PCR

Primer	Sequence 5′-3′	Length (bp)	References
0XA-51-like	F TAA TGC TTT GAT CGG CCT TG R TGG ATT GCA CTT CAT CTT GG	353 bp	[19]
OXA 23	F GAT CGG ATT GGA GAA CCA GA R ATT TCT GAC CGC ATT TCC AT	501	
OXA 24	F GGT TAG TTG GCC CCC TTA AA R AGT TGA GCG AAA AGG GGA TT	246	
OXA 58	F AAG TAT TGG GGC TTG TGC TG R CCC CTC TGC GCT CTA CAT AC	599	
ISA_{b1}	F CAC GAA TGC AGA AGT TG R CGA CGA ATA CTA TGA CAC	549	[7]

Table 2. Cycling conditions for *OXA* and ISA_{b1} PCR [20]

Steps	*Oxa* genes cycling conditions	ISA_{b1} gene cycling conditions
Initial denaturation	One cycle 94◦C for 3 min	One cycle 95◦C for 5 min
Denaturation	30 cycles 94◦C for 25 s	35 cycles 95◦C for 45 s
Annealing	30 cycles 52◦Cfor 40 s	35 cycles 56◦C for 45 s
Extension	30 cycles 72◦C for 50 s	35 cycles 72◦C for 3 min
Final extension	One cycle 72◦C for 5 min	One cycle 72◦C for 5 min

Table 3. Patient demographics and clinical features

Variable	Value
Age (mean ± SD)	45.6 ±15.5
Gender N (%)	
-Male	90 (64%)
-Female	(50) (36%)
Symptoms N (%)	
-Fever	30 (21.4%)
-Chest pain	14 (10%)
-Excessive amount of purulent secretions	75 (53.5%)
Routine Lab investigations	
-CRP (mg/dl) median (range)	45 (22-58)
-Procalcitonin (ng/dl) median (range)	3.5 (2.1-4.7)
Radiological	
Chest x-ray and /or Bedside Ultrasonography N (%)	
-Lung infiltrates only	120 (85.7%)
-Lung infiltrates with pleural effusion	20 (14.28%)

CRP: C reactive protein, SD: Standard deviation

Table 4. Frequency of oxa genes among CRAB isolates

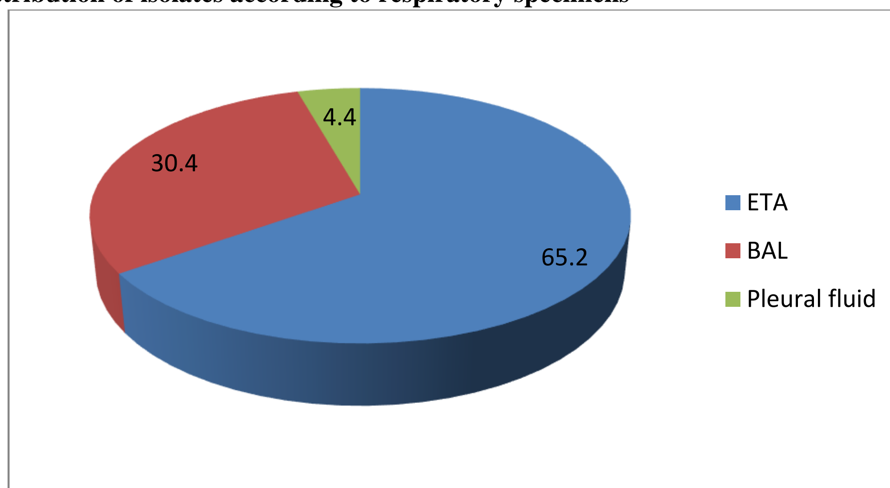
Gene	**No (%)**
***Oxa* genes positive isolates**	**36(92.3%)**
Oxa 23 only	10 (25.6%)
Isa_{bA1}/*oxa* 23	25(64.1%)
Oxa 23/*Oxa* 58	1(2.6%)
Oxa 24	0(0.0%)
***Oxa* genes negative isolates**	**3(7.7%)**
Total	39(100%)

Table 5. Comparison of isolates' carpabenemase genotypic and phenotypic testing results

Variables		Genotypic methods		Total	Kappa test	*P* value
		positive	Negative			
Phenotypic Methods (mCIM)	Carpabenemase positive	31(79.5%)	3(7.7%)	34(87.2%)	0.1	0.4
	Carpabenemase negative	5(12.8%)	0(0.0%)	5(12.8%)		
Total		36(92.3%)	3(7.7%)	39(100%)		

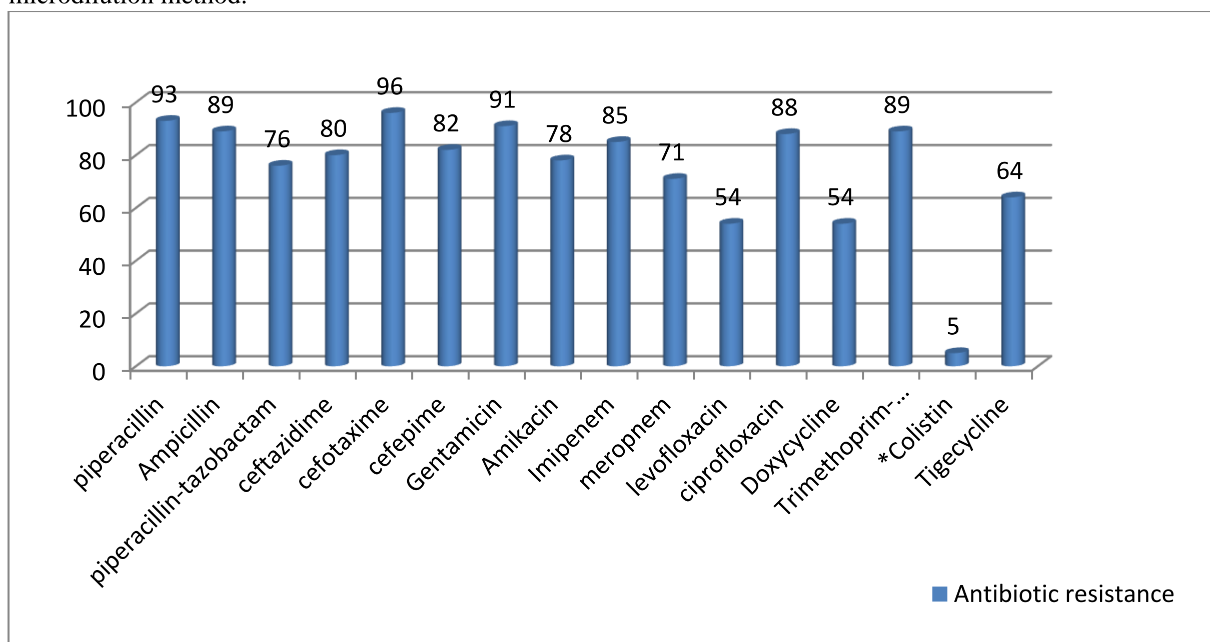
Non-significant Kappa test, 95% confidence interval: from-0.19 to -0.022. mCIM: modified carbapenem inactivation method.

Figure 1. Distribution of isolates according to respiratory specimens



ETA: Endotracheal aspirates, BAL: broncho-alveolar lavage.

Figure 2. Antibiotic susceptibility by disc diffusion method. *Colistin susceptibility was detected by broth microdilution method.



Discussion

In critically ill patients, VAP is a common nosocomial infection. Over the past 20 years, *Acinetobacter* species have emerged in intensive care unit and have been linked to serious infections including VAP. The fact that this bacterium can develop resistance to practically all of the existing antibiotics used in clinical practice is one of the biggest worries associated with it. Furthermore, it has a strong propensity to rapidly disseminate this resistance, affecting healthcare settings [21].

There is little information available on VAP brought on by *A. baumannii* in Egypt. In this work, we assessed antibiotic resistance and some genetic features of carbapenem resistance among *A. baumannii* in VAP patients.

In our research, out of 140 respiratory specimens obtained from individuals with VAP, 46 isolates (32.8%) of *A. baumanii* were identified. This was consistent with previous results; Saleem et al. [11] reported 30.7% prevelance rate in Saudi Arabia, Huang et al. [21] found the incidence around 36% in all VAP patients in China, and in research carried out in India, *Acinetobacter* spp. (36.76%) were determined by Sharma et al. [22] to be the most prevalent causal agent linked to VAP. On the other hand, during the second wave of COVID-19, Elwakil et al. [23] reported that the isolation rate of *A. baumannii* among patients on mechanical ventilation was 27.4% in Egypt.

Effective treatment still requires the identification of resistance mechanisms, especially for transferable resistance mechanisms on plasmids or transposons. Additionally, early and precise identification of the carbapenemase-producing *A. baumannii* is necessary for treatment and control [21].

Every *A. baumanii* isolate in the current investigation was MDR (100%) and concerning carbapenems which are the drug of choice due to their effectiveness and low toxicity, unfortunately, 84.7% of our isolates showed carbapenem resistance which reflected the overuse and abuse of theses antibiotics. Also, a high resistance to almost all tested antibiotics was observed and only colistin appeared to be the most active against theses isolates (5% resistance rate). In Egypt, several previous studies gave comparable results; In their isolates from Assiut, Abdel-Rahim et al. [24] discovered a strong pattern of resistance to all classes of antibiotics, Said et al. [25] observed high levels of quinolones, aminoglycosides and carbapenem resistance in Mansoura, and in an investigation carried out by Sánchez-Urtaza et al. [26] on isolates from Alexandria, 85 % of carbapenem, 100% of fluoroquinolone and 86% of aminoglycoside resistance were noted however the isolates were still sensitive to colistin. Colistin resistance, on the other hand, was found in as many as 55% of *A. baumannii* Egyptian isolates according to investigations conducted by Makharita et al. [27] and Fam et al. [28] who attributed this to the increase in its use as an active agent and a last resort against multidrug-resistant *A. baumannii*.

Moreover, In Nebal, research by Joshi et al. [29] described that all *A. baumannii* isolates were MDR with 97.7% carbapenem resistance and were fully susceptible to colistin. In KSA, another study by El-Badawy et al. [30] found 71 % of their isolates were CRAB and all of them were MDR, about 91% were resistant to all β–lactams that were investigated. Although the isolates' resistance rates to aminoglycosides, quinolones and tetracycline ranged from 51% to 100%, all isolates remain sensitive to colistin.

polymyxins are currently used either alone or in conjunction with other medications as first-line antimicrobials against CRAB. They exhibit strong in vitro efficacy against strains of *A. baumannii*; nevertheless, their narrow therapeutic spectrum, lack of therapeutically meaningful susceptibility breakpoints, and severe nephrotoxicity and neurotoxicity side effects are their main drawbacks. Additional significant problems that could lead to poor clinical results include resistance developing during therapy as a result of heteroresistant bacteria and challenges in identifying heteroresistance [31].

The current study's findings also demonstrated that carbapenem resistance was caused by the *oxa* 23 gene (92.3 %) indicating the horizontal dissemination of harboring plasmids, and *oxa* 58 co-existed with *oxa* 23 in only one isolate (2.6%). Similar findings had been reported by Chang et al. [32] who observed the main carbapenemase causing resistance in their isolates was the *oxa* 23 gene and Alhaddad et al. [33] supported the same finding in Saudi Arabia. Also, Joshi et al. [29] found *oxa* 23 in all their isolates explaining that the *oxa* 23 gene is positioned on plasmid and is passed between *A. baumannii* through conjugation increasing antibiotic resistance rapidly worldwide, and added

that their analyzed isolates did not contain any of the other class D β-lactamase genes, which were common in Europe, such as *oxa* 24 and *oxa* 58.

In *A. baumannii*, the presence of the ISA_{ba1} promoter sequence linked to *OXA* genes significantly increases carbapenem resistance [34]. In our study, ISA_{ba1} was detected upstream to *oxa* 23 in 64.1% of isolates suggesting overexpression of carbapenemase hydrolyzing activity. A recent investigation in Taiwan by Su et al. [35] that characterized the majority of isolates (77.4%) had an ISA_{ba1} sequence which encodes a promoter leading to increased expression of *OXA* genes in addition to a transposase, and ultimately causes carbapenem resistance. Joshi et al. [29] observed ISA_{ba1} in all of their *oxa* 23 positive isolates and described a link between higher minimal inhibitory doses of carbapenems and *A. baumannii* expressing the isa_{b1}/ *oxa* 23 gene.

Among our study isolates, 79.5% were positive by both mCIM and genotyping. Similarly, Abouelfetouh et al. [36] reported that 68.9% of their isolates were also carbapenemase positive phenotypically and genotypically. However, in this study, we observed 3 isolates (7.7%) with carbapenemase activity only phenotypically and this finding could be explained by the involvement of alternative carbapenem resistance mechanisms, such as altered permeability, efflux pump, lower drug affinity due to PBP downregulation, and decreased membrane porin density or extra carbapenemase enzymes not investigated in this work [37].

On the other hand, regarding the agreement between phenotypic and genotypic detection of carbapenemases, our results were non-significant (*p value*=0.4). Currently, there is no exclusive recommendations for phenotypic detection of carbapenemases among *A. baumannii* according to CLSI guidelines [15]. Hence, several studies have been trying to find the most suitable and affordable method for early detection of carbapenemase producing *A. baumannii* especially when dealing with high-resistant burden and resource-limited settings [38,39]. Khuntayaporn et al. [40] recommended the combination of 2 phenotypic tests helping to identify carbapenemase producing *A. baumannii.*

In conclusion, in this work we highlighted the predominance of CRAB isolates among VAP patients which is a concerning issue. The probable prevalent *OXA* carbapenem resistant gene among these isolates was *oxa* 23. Although inexpensive and readily available, phenotypic screening assays are not thought to be ideal for the early detection of carbapenemase producing *A. baumannii.*

We recommend further studies for more investigations, continuous monitoring and surveillance for antimicrobial susceptibility which are crucial for formulating strategies of control and treatment.

Author contributions

HME, RAR contributed to the study conception and design. All authors contributed to methodology, analysis, interpretation of data, writing the manuscript draft reviewing and editing. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest.

Funding

This work was not funded by any organization for the research, authorship, and/or publication of this article.

References

1- Kalanuria AA, Ziai W, Mirski M. Ventilator-associated pneumonia in the ICU. Crit Care 2014; 18(2): 208. doi: 10.1186/cc13775.

2- Papazian L, Klompas M, Luyt CE. Ventilator-associated pneumonia in adults: a narrative review. Intensive Care Med 2020; 46(5): 888-906. doi: 10.1007/s00134-020-05980-0. Epub 2020.

3- Cihan Semet. The ongoing challenge of ventilator-associated pneumonia: epidemiology, prevention, and risk factors for mortality in a secondary care hospital intensive care unit. Infection Prevention in Practice 2023; 5(4): 100320. https://doi.org/10.1016/j.infpip.2023.100320.

4- Ceparano M, Baccolini V, Migliara G, Isonne C, Renzi E, Tufi D, et al. *Acinetobacter baumannii* Isolates from COVID-19 Patients in a Hospital Intensive Care Unit: Molecular Typing and Risk Factors. Microorganisms

2022; 10(4): 722. doi: 10.3390/microorganisms10040722.

5- Kuo HY, Chang KC, Kuo JW, Yueh HW, Liou ML. Imipenem: a potent inducer of multidrug resistance in *Acinetobacter baumannii.* Int J Antimicrob Agents 2012; 39(1): 33-8. doi: 10.1016/j.ijantimicag.2011.08.016.

6- Słoczyńska A, Wand ME, Bock LJ, Tyski S, Laudy AE. Efflux-Related Carbapenem Resistance in *Acinetobacter baumannii* Is Associated with Two-Component Regulatory Efflux Systems' Alteration and Insertion of ΔAbaR25-Type Island Fragment. Int J Mol Sci 2023; 24(11): 9525. doi: 10.3390/ijms24119525.

7- Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, et al. The role of ISAba1 in expression of OXA carbapenemase genes in *A. baumannii*. FEMS Microbiol Lett 2006; 258(1): 72-7. doi: 10.1111/j.1574-6968.2006.00195.x.

8- Amr GE & Abdel Razek GM. Characterization of Carbapenem Resistant *Acinetobacter baumannii* causing Ventilator associated Pneumonia in ICUs of Zagazig University Hospitals, Egypt. Int.J.Curr.Microbiol.App.Sci 2016; 5(12): 660-671. doi: http://dx.doi.org/10.20546/ijcmas.2016.512.074

9- National Healthcare Safety Network. Pneumonia (Ventilator-associated [VAP]and non-ventilator-associated Pneumonia [PNEU]) event. NHSN. Retrieved February16, 2023, from https://www.cdc.gov/nhsn/pdfs/pscmanual/ 6pscvapcurrent.pdf.

10- Zhang T, Xu X, Xu CF, Bilya SR, Xu W. Mechanical ventilation-associated pneumonia caused by *Acinetobacter baumannii* in Northeast China region: analysis of genotype and drug resistance of bacteria and patients' clinical features over 7 years. Antimicrob Resist Infect Control 2021; 10(1): 135. doi: 10.1186/s13756-021-01005-7.

11- Saleem M, Khaja AS, Hossain A, Alenazi F, Said KB, Moursi SA, et al. Molecular Characterization and Antibiogram of *Acinetobacter baumannii* Clinical Isolates Recovered from the Patients with Ventilator-Associated Pneumonia. Healthcare 2022; 10(11): 2210. https://doi.org/10.3390/healthcare10112210

12- Forbes BA, Sahm DF, Weissfeld AS. 12th ed. Missouri: Elsevier; Bailey and Scott's Diagnostic Microbiology.2007; P 811.

13- Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of *Acinetobacter baumannii* by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. J Clin Microbiol 2006; 44(8): 2974-6. doi: 10.1128/JCM.01021-06.

14- Thirapanmethee K, Srisiri-A-Nun T, Houngsaitong J, Montakantikul P, Khuntayaporn P, Chomnawang MT. Prevalence of OXA-Type β-Lactamase Genes among Carbapenem-Resistant *Acinetobacter baumannii* Clinical Isolates in Thailand. Antibiotics (Basel) 2020; 9(12): 864. doi: 10.3390/antibiotics9120864.

15- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. CLSI, 2023, M100, 33rd ed., Wayne, PA.

16- Dent LL, Marshall DR, Pratap S & Robert BH. Multidrug resistant *Acinetobacter baumannii*: a descriptive study in a city hospital. BMC Infect Dis 2010; 10: 196. https://doi.org/10.1186/1471-2334-10-196

17- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial

susceptibility testing. CLSI, 2018, M100, 28th ed., Wayne, PA.

18- Pierce VM, Simner PJ, Lonsway DR, Roe-Carpenter DE, Johnson JK, Brasso WB, et al. Modified Carbapenem inactivation method for phenotypic detection of Carbapenemase production among *Enterobacteriaceae*. J Clin Microbiol 2017; 55(8): 2321–33.

19- Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter spp*. Int J Antimicrob Agents 2006; 27(4): 351-3. doi: 10.1016/j.ijantimicag.2006.01.004.

20- Elabd FM, Al-Ayed MS, Asaad AM, Alsareii SA, Qureshi MA, Musa HA. Molecular characterization of oxacillinases among carbapenem-resistant *Acinetobacter baumannii* nosocomial isolates in a Saudi hospital. J Infect Public Health 2015; 8(3): 242-7. doi: 10.1016/j.jiph.2014.10.002.

21- Huang Y, Zhou Q, Wang W, Huang Q, Liao J, Li J, et al. *Acinetobacter baumannii* Ventilator-Associated Pneumonia: Clinical Efficacy of Combined Antimicrobial Therapy and in vitro Drug Sensitivity Test Results. Front Pharmacol 2019; 10: 92. doi: 10.3389/fphar.2019.00092.

22- Sharma V, Sharma R & Vyas A. Phenotypic Characterization of *Acinetobacter spp.* Isolated from Ventilator-Associated Pneumonia in an Intensive Care Unit of Tertiary Care Hospital. International Journal of Medical Research & Health Sciences 2021; 10(4): 156-163. ISSN No: 2319-5886

23- Elwakil WH, Rizk SS, El-Halawany A, Rateb ME, Attia AS. Multidrug-resistant *Acinetobacter baumannii* infections in the United Kingdom versus Egypt: trends and potential natural products solutions. Antibiotics 2023; 12(1): 77. doi: 10.3390/antibiotics12010077.

24- Abdel-Rahim MH, Abd–ElNasser RA, Hassnein KM, Mahmoud MA, Elkhawaga AA. Outcome of *Acinetobacter baumannii* Ventilator Associated Pneumonia in Upper Egypt: does the resistance profile play a role? Egypt J Chest Dis Tuberc 2024; 73(2): 146-153. doi: 10.4103/ecdt.ecdt_39_23

25- Said HS, Benmahmod AB, Ibrahim RH. Co-Production of AmpC and extended spectrum beta-lactamases in cephalosporin-resistant *Acinetobacter baumannii* in Egypt. World J. Microbiol. Biotechnol 2018; 34 (12): 189. doi: 10.1007/s11274-018-2571-z

26- Sánchez-Urtaza S, Ocampo-Sosa A, Molins-Bengoetxea A, El-Kholy MA, Hernandez M, Abad D, et al. Molecular characterization of multidrug resistant *Acinetobacter baumannii* clinical isolates from Alexandria, Egypt. Front Cell Infect Microbiol 2023; 13: 1208046. doi: 10.3389/fcimb.2023.1208046.

27- Makharita RR, El-Kholy I, Hetta HF, Abdelaziz MH, Hagagy FI, Ahmed AA, et al. Antibiogram and genetic characterization of carbapenem-resistant Gram-negative pathogens incriminated in healthcare-associated infections. Infection Drug resistance 2020; 13: 3991–4002. doi: 10.2147/IDR.S276975

28- Fam N, Gamal D., Mohamed SH, Wasfy RM, Soliman MS, El-Kholy AA, et al. Molecular characterization of Carbapenem/Colistin-resistant *Acinetobacter baumannii* clinical isolates from Egypt by whole-genome sequencing. Infection Drug resistance 2020; 13: 4487–4493. doi: 10.2147/IDR.S288865

29- Joshi PR, Acharya M, Kakshapati T, Leungtongkam U, Thummeepak R, Sitthisak S. Co-existence of blaOXA-23 and blaNDM-1

genes of *Acinetobacter baumannii* isolated from Nepal: antimicrobial resistance and clinical significance. Antimicrob Resist Infect Control 2017; 6: 21. doi: 10.1186/s13756-017-0180-5.

30- El-Badawy MF, Abdelwahab SF, Alghamdi SA, Shohayeb MM. Characterization of phenotypic and genotypic traits of carbapenem-resistant *Acinetobacter baumannii* clinical isolates recovered from a tertiary care hospital in Taif, Saudi Arabia. Infect Drug Resist 2019; 12: 3113-3124. doi: 10.2147/IDR.S206691.

31- Shields RK, Paterson DL, Tamma PD. Navigating Available Treatment Options for Carbapenem-Resistant *Acinetobacter baumannii-calcoaceticus* Complex Infections. Clin Infect Dis 2023; 76(Suppl 2): S179-S193. doi: 10.1093/cid/ciad094.

32- Chang Y, Luan G, Xu Y, Wang Y, Shen M, Zhang C, et al. Characterization of carbapenem-resistant *Acinetobacter baumannii* isolates in a Chinese teaching hospital. Front Microbiol 2015; 6: 910.

33- Alhaddad MS, AlBarjas AK, Alhammar LE, Al Rashed AS, Badger-Emeka LI. Molecular Characterization and Antibiotic Susceptibility Pattern of *Acinetobacter baumannii* Isolated in Intensive Care Unit Patients in Al-Hassa, Kingdom of Saudi Arabia. Int J Appl Basic Med Res 2018 Jan-Mar; 8(1): 19-23. doi: 10.4103/ijabmr.IJABMR_91_17.

34- Sajjad Y, Ehsan N, Ezzat Allah G, Naemeh J, Ali H, Abdollah A. Carbapenem-resistant *A. baumannii* isolates carrying bla OXA genes with upstream ISAba1: First report of a novel OXA subclass from Iran. J Glob Antimicrob Resist 2019; 18: 95-99.

35- Su PW, Yang EC, Moi SH, Yang CH, Chuang LY. Prevalence of Carbapenem Resistance Genes among *Acinetobacter baumannii* Isolated from a Teaching Hospital in Taiwan. Antibiotics 2023; 12: 1357. https://doi.org/10.3390/antibiotics12091357

36- Abouelfetouh A, Torky AS, Aboulmagd E. Phenotypic and genotypic characterization of carbapenem-resistant *Acinetobacter baumannii* isolates from Egypt. Antimicrob Resist Infect Control 2019; (8): 1-9. doi: 10.1186/s13756-019-0611-6.

37- Santajit S, Bhoopong P, Kong-Ngoen T, Tunyong W, Horpet D, Paehoh-ele W, et al. Phenotypic and Genotypic Investigation of Carbapenem-Resistant *A. baumannii* in Maharaj Nakhon Si Thammarat Hospital, Thailand. Antibiotics 2023; 12: 580. https://doi.org/10.3390/antibiotics12030580

38- Vu TN, Byun JH, D'Souza R, Pinto NA, Nguyen LP, Yong D, et al. Adjustment of modified carbapenem inactivation method conditions for rapid detection of carbapenemase-producing *Acinetobacter baumannii.* Ann Lab Med 2020; 40(1): 21–26. doi: 10.3343/alm.2020.40.1.21

39- Zhang S, Mi P, Wang J, Li P, Luo K, Liu S, et al. The optimized carbapenem inactivation method for objective and accurate detection of carbapenemase producing *Acinetobacter baumannii.* Front. Microbiol 2023; 14: 1185450. doi: 10.3389/fmicb.2023.1185450

40- Khuntayaporn P, Thirapanmethee K, Kanathum P, Chitsombat K, Chomnawang MT. Comparative study of phenotypic-based detection assays for carbapenemase-producing *Acinetobacter baumannii* with a proposed algorithm in resource-limited settings. PLoS One 2021; 16(11): e0259686. doi: 10.1371/journal.pone.0259686.

Rabie RA, Ismail NA, El Maghraby HM. Phenotypic and molecular characterization of carbapenem-resistant *Acinetobacter baumannii* isolates from ventilator associated pneumonia patients. Microbes Infect Dis 2025; 6(1): 319-328.