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Epidemiology and antibiotic resistance assessment of *Acinetobacter baumannii* isolates from respiratory specimens collected at Can Tho General Hospital

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ABSTRACT

Acinetobacter baumannii is a significant nosocomial pathogen causing hospital-acquired pneumonia (HAP) in many health-care settings. This study aimed to determine the prevalence and antibiotic resistance of *A. baumannii* isolates causing HAP in Can Tho General Hospital, Vietnam. Respiratory specimens were collected from patients diagnosed with HAP, and bacterial strains were isolated and identified using microbiological techniques. The minimal inhibitory concentration of testing antibiotics against *A. baumannii* isolates was evaluated using the Vitek-2 Compact automated microbial identification system. The presence of carbapenemase-encoding genes (*bla*OXA-23, *bla*OXA-51, and *bla*IPM) was determined in carbapenem-resistant *A. baumannii* (CRAB) isolates using polymerase chain reaction. Of the 434 patient samples analyzed, *A. baumannii* was the most prevalent infection, accounting for 31.1% of positive cases. The study showed a high prevalence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) *A. baumannii* strains, with 80.7%, 15.6%, and 3.7% of isolates categorized as MDR, XDR, and PDR. CRAB isolates accounted for 89.6% of *A. baumannii* strains, with the *bla*OXA-23 and *bla*OXA-51 gene being the most prevalent. The study emphasizes the high prevalence of *A. baumannii* causing HAP in the hospital setting and the urgent need to implement infection control measures to reduce the spread of antibiotic-resistant strains.

1. INTRODUCTION

Healthcare-associated infections are a significant public health issue, leading to increased morbidity, mortality, and imposing a large economic burden on society [1,2]. *Acinetobacter baumannii* is one of the common causes of healthcare-associated infections [2] and is considered an opportunistic pathogen responsible for serious nosocomial infections, such as pneumonia, sepsis, and meningitis [3,4]. In addition, *A. baumannii* has the ability to form biofilms and can grow in a wide temperature range of 15–44°C with simple nutritional requirements and high resistance to disinfectants and antiseptics [5-7]. These traits allow for the prolonged survival and spread of *A. baumannii* in the environment.

Carbapenems are the first-line treatment for infections caused by *A. baumannii* due to their high efficacy and low toxicity [5,8]. However, the emergence and spread of carbapenem-resistant *A. baumannii* (CRAB) has become a major concern globally and has been classified as a critical pathogen by the World Health Organization (WHO) [2]. CRAB is resistant to many antibiotics, including carbapenems and third-generation cephalosporins [2]. In cases of CRAB infections, last-line antibiotics such as colistin, polymyxin B, or tigecycline are effective, but their high toxicity limits their usage [5,9]. Several studies have reported the effectiveness of combination therapy using anti-virulence agents (inhibitors active against virulence factors of pathogens) and available antibiotics against *A. baumannii* [3,10]. Further, information on the resistance of clinical isolates of *A. baumannii* could contribute to the long-term effectiveness of these treatments.

The susceptibility pattern of *A. baumannii* to antibiotics varies among hospitals [11]. Can Tho General Hospital is a large medical facility that treats patients from various provinces and cities in the Mekong Delta. This study aimed to reports the clinical factors and the antibiotic resistance profile of *A. baumannii* strains from Can Tho General Hospital. Thereby, we intended to contribute information to improve the effectiveness of the treatment of *A. baumannii* infections.

2. MATERIALS AND METHODS

2.1. Bacterial Isolation and Species Identification

This study aimed to comprehensively analyze respiratory specimens collected from patients diagnosed with hospital-acquired pneumonia (HAP), including ventilator-associated pneumonia, at Can Tho General

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Hospital (Vietnam) from April 2021 to April 2022. The specimens were obtained from various departments within the hospital, including the intensive care unit (ICU), the department of internal medicine (DIM), the department of endocrinology (DOE), and other departments. Only one bacterial strain for one patient was selected for the study despite the possibility of obtaining multiple specimens from each patient. The bacterial isolates were identified using a combination of standard microbiological techniques, including Gram staining, catalase and oxidase tests, motility testing, and automated identification using the Vitek 2 GN ID card with the Vitek-2 Compact system (Biomerieux, France) [12-14]. *A. baumannii* strains are Gram-negative, twitching motility, oxidase negative, and catalase positive [6].

Our study was conducted after obtaining approval from the Scientific Council and the Medical Council of Can Tho University of Medicine with approval number 21.128-DHYDCT.

2.2. The Minimal Inhibitory Concentration of Testing Antibiotics against *A. baumannii* Isolates

The antimicrobial susceptibility of the bacterial isolates was assessed using the AST-GN67 card with the Vitek-2 Compact automated microbial identification system. The susceptibility interpretation was based on the Clinical Laboratory Standards Institute guidelines 2014 [15]. The antibiotics tested in this study included penicillin agents (ampicillin/sulbactam – SAM and piperacillin/tazobactam – TXP), cephalosporin agents (cefazolin – CZO, ceftazidime – CAZ, ceftriaxone – CRO, and cefepime – FEP), carbapenem agents (imipenem – IPM, ertapenem – ETP, and meropenem – MEM), aminoglycoside agents (gentamicin – GEN and tobramycin – TOB), fluoroquinolone agents (ciprofloxacin – CIP and levofloxacin – LVX), folate pathway inhibition agent (trimethoprim/sulfamethoxazole – SXT), and polymyxin agent (colistin – COL). The bacterial isolates were classified into three categories based on their resistance patterns: Multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR). MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories, and PDR was defined as non-susceptibility to all agents in all antimicrobial categories [16]. The *A. baumannii* strains were designated as carbapenem-resistant *A. baumannii* (CRAB) when they were non-susceptible to both imipenem and meropenem [8,15].

2.3. Polymerase Chain Reaction (PCR) Detection Carbapenemase-encoding Genes of CRAB Isolates

The presence of the *bla*OXA-23, *bla*OXA-51, and *bla*IMP genes was determined in all CRAB isolates. Based on the previous research results in Vietnam (data not show), the *bla*OXA-51 and *bla*OXA-23 genes represent class D beta-lactamase biosynthetic genes. The *bla*IPM gene represents the class B beta-lactamase biosynthetic gene. Genomic DNA was extracted from the cells using the Invisorb® Spin Universal kit (Invitek Molecular, Germany). The PCR amplification was carried out using the MyTaq™ Red Mix Kit (Meridian Bioscience, USA) and the C1000 Touch Thermal Cycler system (Bio-Rad, USA), and the reaction mixture included specific primers [Table 1] for each gene described by Woodford *et al.* [17]. The PCR amplification conditions included an initial denaturation step at 95°C for 3 min, followed by 40 cycles of amplification at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. The final extension step was maintained at 72°C for 5 min [18]. The PCR products were analyzed through a 2% agarose gel and visualized using the gel documentation device (Bio-Rad, USA).

Table 1: Primers used to identify carbapenemase-encoding genes in this study [17].

Gene	Primers sequence (5'→3')	Tm (°C)	Product (bp)
*bla*OXA-23			
Forward primer	GATCGGATTGGAGAACCAGA	56.72	501
Reverse primer	ATTTCTGACCGCATTTCCAT	55.69	
*bla*OXA-51			
Forward primer	TAATGCTTTGATCGGCCTTG	58.93	353
Reverse primer	TGGATTGCACTTCATCTTGG	56.50	
*bla*IMP			
Forward primer	GGAATAGAGTGGCTTAAYTCT	50.37	189
Reverse primer	CCAAACYACTASGTTATCTC	44.39	

F: Forward primer, R: Reverse primer

2.4. Statistical Analysis

The difference in detection rates of bacterial strains in categorical variables was analyzed using the Chi-square test, which was implemented using the R programming statistical package.

3. RESULTS

3.1. The Epidemiological Characteristics of Isolated *A. baumannii* Strains

The study aimed to investigate the prevalence of bacterial infections and the specific role of *A. baumannii* in such infections. The study analyzed 434 patient samples, with a majority of positive cases caused by *A. baumannii* (31.1%, 135/434 case), followed by *Klebsiella pneumoniae* (23.2%) and *Escherichia coli* (20.5%). The study highlights the need for greater attention toward *A. baumannii* as a potential source of infection.

The researchers also examined the prevalence of *A. baumannii* infections among different age and gender groups [Figure 1]. The results showed that elderly patients (60+ years) had the highest positive rate for *A. baumannii* infections, with no infections observed among children aged 0–17 years. In addition, the prevalence of *A. baumannii* infections was higher among females than males, with 51.85% of all cases occurring in females ($P > 0.05$).

Furthermore, the study explored the distribution of *A. baumannii* infections in different medical departments, with the ICU showing the highest positive rate (62.9%), followed by the DIM (12.5%) and the DOE (13.3%). This emphasizes the importance of identifying departments with high-positive rates to facilitate targeted infection control measures [Figure 2].

3.2. Profile of Antibiotic Resistance Phenotypes of *A. baumannii* Strains

This study investigated the resistance of *A. baumannii* strains to various classes of antibiotics. A total of 135 *A. baumannii* strains were analyzed, and they were classified based on their susceptibility to different antibiotics [Figure 3]. The results showed that a high proportion of strains were resistant to most of the antibiotics tested, including carbapenems, penicillins, cephalosporins, aminoglycosides, and fluoroquinolones. In the carbapenems class, more than 86% of the strains were resistant to imipenem, ertapenem, and meropenem. Similarly, in the penicillins class, more than 85% of the strains were resistant to ampicillin/sulbactam and piperacillin/tazobactam. All

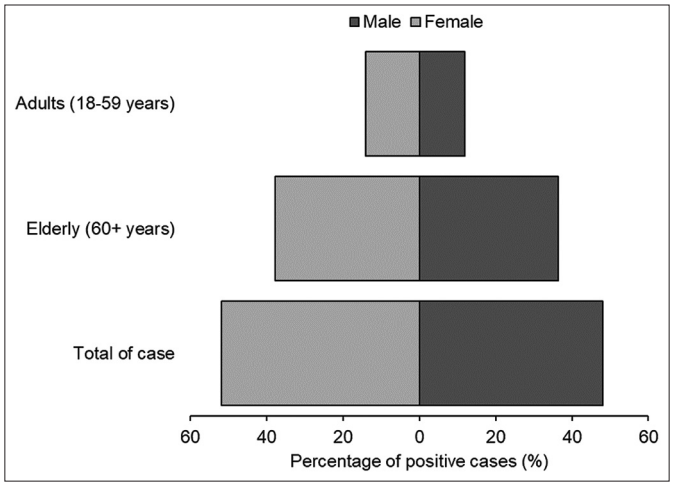

Figure 1: Positive percentage of *Acinetobacter baumannii* infections by gender and age.

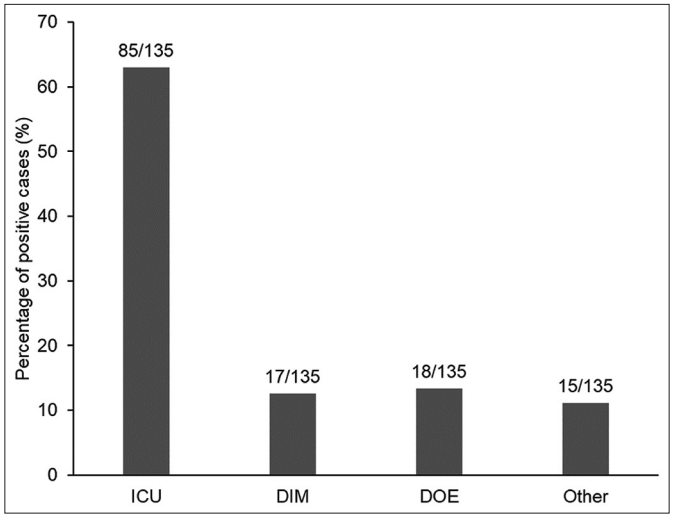

Figure 2: Prevalence of *Acinetobacter baumannii* infections by department.

strains in the cephalosporin class were resistant to cefazolin, while almost 90% were resistant to ceftazidime, ceftriaxone, and cefepime. A high proportion of strains were also resistant to aminoglycosides and fluoroquinolones. Only colistin and trimethoprim/sulfamethoxazole showed some efficacy, but even so, some strains were resistant to them.

The distribution of antibiotic-resistant strains was presented, and among the 135 clinical isolates, 77.04% were found to be XDR, while 9.63% were MDR. Nine isolates were pan-drug-resistant (PDR), and the remaining nine were non-resistant. The high prevalence of XDR strains suggests that *A. baumannii* infections may be difficult to treat, and alternative treatment options are needed. This study highlights the urgent need for the development of new antibiotics to combat antibiotic-resistant *A. baumannii* strains. Figure 3 shows the susceptibility of *A. baumannii* strains to various antibiotics, and Figure 4 shows the distribution of antibiotic-resistant strains.

3.3. Antibiotic Resistance Phenotyping and Carbapenemase-encoding Genes Profile of CRAB Strains

Carbapenem-resistant *A. baumannii* (CRAB) infections pose a major challenge due to antibiotic resistance. In this study, 117 CRAB strains were genotyped for resistance to imipenem and meropenem antibiotics, with 86.7% (n = 117) found to be resistant to both antibiotics. Analysis of antibiotic resistance polymorphism in four different hospital departments showed that the ICU department had the highest prevalence of XDR strains (63.2%), while the DOE department had the highest prevalence of MDR strains (3.41%). Overall, the findings indicate the need for increased surveillance and improved infection control measures in the ICU department to prevent the spread of antibiotic-resistant CRAB strains. In addition, the results suggest that tailored treatment options are necessary for patients infected with CRAB strains in different hospital departments, based on observed patterns of antibiotic resistance polymorphism [Figure 5].

Further, analysis of the CRAB strains revealed that 76.9% carried at least one of the tested resistance genes, with *bla*OXA-23 and *bla*OXA-51 genes being the most prevalent. The combination of *bla*OXA-23 and *bla*OXA-51 genes was detected in 83.76% of the strains. XDR strains were found to have the most common profile of the presence of *bla*OXA-23 and *bla*OXA-51 genes, followed by the *bla*OXA-23/*bla*OXA-51 combination, and the *bla*OXA-23/*bla*OXA-51/*bla*IMP combination. Among the MDR strains, only the *bla*OXA-23/*bla*OXA-51/*bla*IMP combination was detected in one strain, and among the PDR strains, the most common profile was the *bla*OXA-23/*bla*OXA-51 combination, followed by the *bla*OXA-23/*bla*OXA-51/*bla*IMP combination [Figure 6].

4. DISCUSSION

In this study, respiratory specimens were positive for *A. baumannii*, *Pseudomonas* sp., and *Enterobacteriaceae*. These groups of bacteria are reported by the WHO to be in the critical group in hospital-associated infections [2]. ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) are bacteria involved in nosocomial infections of concern worldwide [19]. Among them, carbapenem resistance was most commonly reported in *A. baumannii* [20]. The carbapenem resistance phenotype is very common for MDR and XDR *A. baumannii* strains [21]. The immune system in the elderly is not responding as strongly as in young adults as determined by impaired B- and T-cell production in the bone marrow and thymus and impaired function of mature lymphocytes in secondary lymphoid tissues [22]. Most of the patients over 60 years of age in the study were hospitalized inpatients. Weakened immune system, underlying medical conditions, and hospital stay increase the risk of HAP in patients [23]. The intensive care unit was reported to have a high prevalence of this bacterium [24,25]. Patients admitted to the ICU are in poor health, often require prolonged hospital stays, undergo invasive procedures, and are treated with broad-spectrum antibiotics [11,25]. In addition, the PDR *A. baumannii* strains were isolated from the patient treated for SARS-CoV-2 in the ICU. Cases of secondary infection with SARS-CoV-2 were reported in several studies. *A. baumannii* was reported to account for a high proportion of secondary Gram-negative bacteremia [26]. *A. baumannii* carrying the *bla*OXA-23 gene has caused outbreaks in patients positive for SARS-CoV-2 in multiple intensive care units in Japan [26].

Carbapenems (especially meropenem and imipenem) are considered the first-line treatment for *A. baumannii* infections. However, the non-guided use of antibiotics led to the emergence of CRAB. CRAB has spread to Asia-Pacific, the Indian continent, North America, Latin America, and Europe [27]. The mechanism of antibiotic resistance in

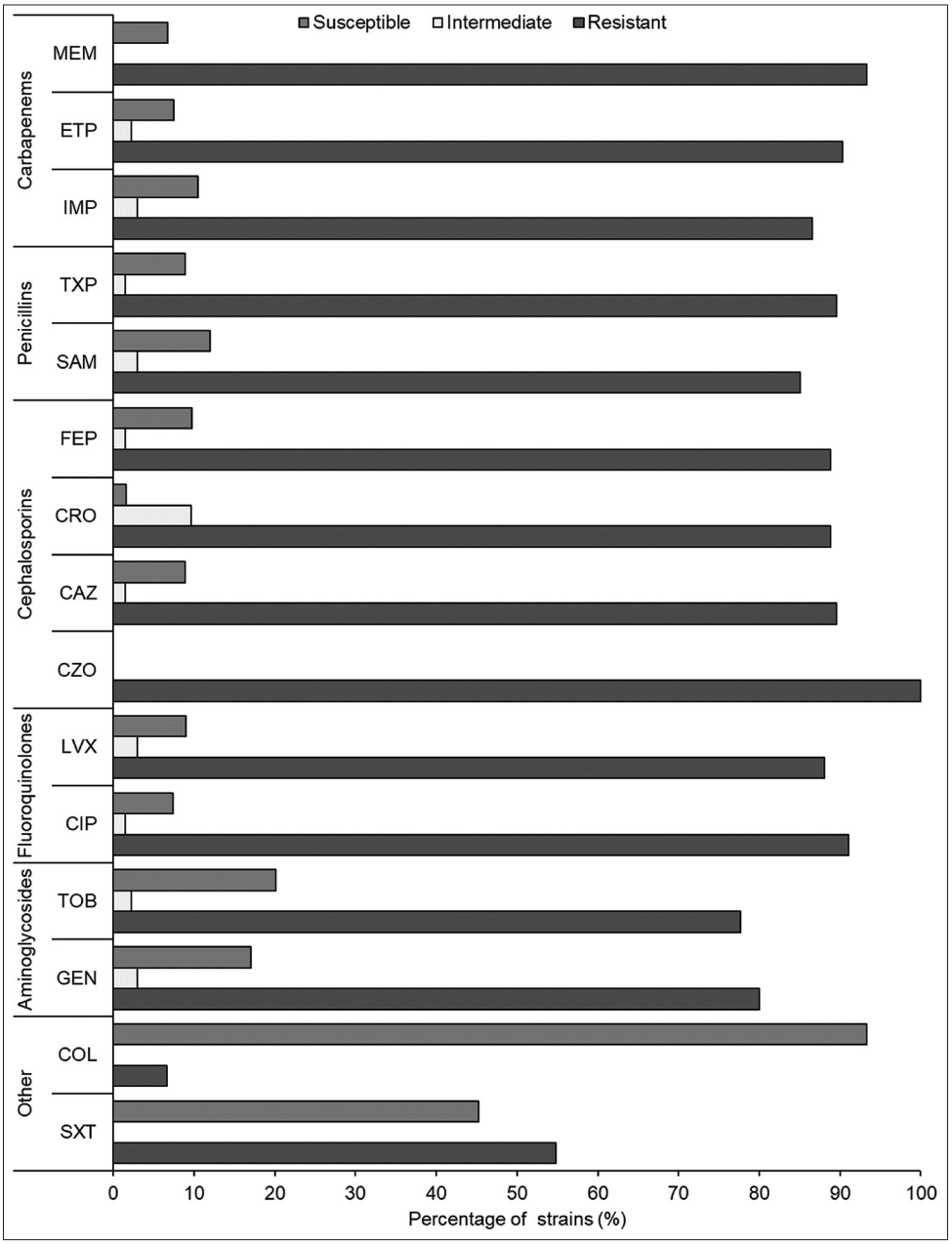

Figure 3: Antibiotic susceptibility profiles of bacterial phenotypes (%).

A. baumannii is complex, and coexist multiple virulence factors [7]. Resistance of *A. baumannii* can be influenced by different virulence factors, including – but not limited to, beta-lactam-degrading enzymes, and/or non-enzymes [25]. Genes encoding Group D carbapenemases could be found on chromosomes and plasmids but not on integrons [7]. Group B carbapenemases are a group of metal-bound enzymes. These enzymes were encoded by mobile genetic elements (plasmid and integron) [26]. Genes encoding class D beta-lactamases are common in *A. baumannii*, including *bla*OXA-23, ISAb1-*bla*OXA-51, *bla*OXA-58, *bla*OXA-40, and *bla*OXA-143 considered as the mechanism responsible for carbapenem resistance in *A. baumannii* [20,28].

Non-enzymatic mechanisms are primarily related to changes in membrane permeability, and/or to the action of membrane protein pumps/channels. The activity of reducing membrane permeability, and the presence of capsular polysaccharides, lipopolysaccharides, phospholipases, and outer membrane vesicles, were reported to influence the multidrug resistance of *A. baumannii* [24,25]. Reduction in the number of porin channels in the outer membrane, overexpression of MDR pumps, and changes in the characteristics of penicillin-binding proteins, increased multidrug resistance in *A. baumannii* [7,24].

In this study, *A. baumannii* strains showed a high rate of resistance to testing antimicrobial agents. The isolates were resistant to most antibiotics belonging to the groups of penicillins, cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones. Carbapenem resistance in *A. baumannii* is increasing in many areas. Ababneh reported 90.6% of *A. baumannii* isolates in Jordanian hospitals as CRAB, of which 10.4% were MDR and 84.4% were XDR. The percentage of strains carrying the *bla*OXA-51 gene was

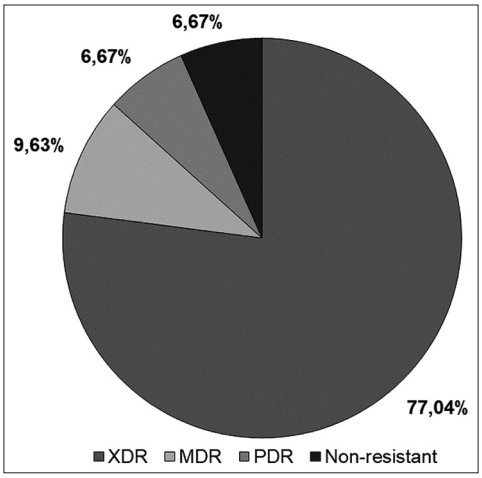

Figure 4: Antibiotic resistance polymorphism (%) in clinical isolates. The percentage of each category is presented, including extensively drug-resistant, multidrug-resistant, pandrug-resistant, and non-resistant isolates.

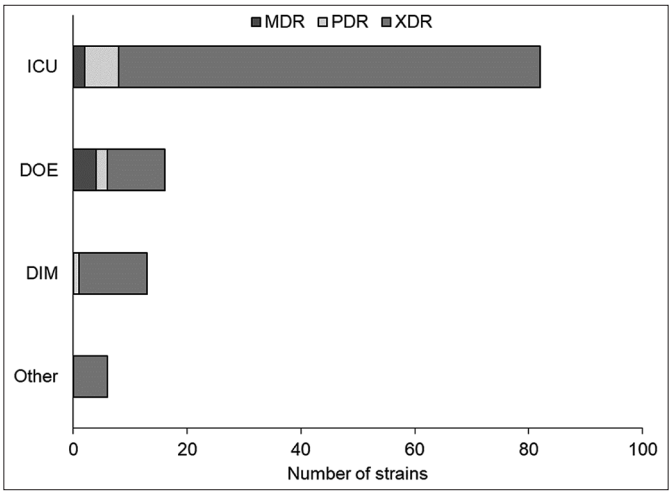

Figure 5: Antibiotic resistance polymorphism of carbapenem-resistant *Acinetobacter baumannii* strains by department. The three categories of antibiotic resistance are multidrug-resistant, pandrug resistant, and extensively drug resistant (XDR). The figure reveals that the intensive care unit department has the highest number of XDR strains, followed by the department of internal medicine and the department of endocrinology the departments. The other department has only six strains, and all of them fall under the XDR category.

highest (89,5%), followed by *bla*OXA-23 (88.3%) and New Delhi Metallo-β-lactamase (*bla*NDM-1) (10.4%) [29]. In other studies, the *bla*OXA-23 gene was reported to be the most common and dominant gene in CRAB [21,28]. In particular, the spread of the *bla*OXA-23 gene is reported to gradually replace the prevalence of *bla*OXA-58 in *A. baumannii* in the ICU in several regions of Italy [28]. Most of the isolates carried the *bla*OXA-23 gene. Pulsed-field gel electrophoresis analysis showed cross-contamination of *A. baumannii* in patients and the environment. In Vietnam, a marked increase in carbapenem-resistant and MDR *A. baumannii* has been recorded in many different hospitals. Drug resistance of clinical *A. baumannii* strains is mainly due to the action of the *bla*OXA-23 gene [30-32]. In addition, the factor ISaba1 was reported to increase the expression of *bla*OXA genes in *A. baumannii* strains [32,33]. MDR in *A. baumannii*,

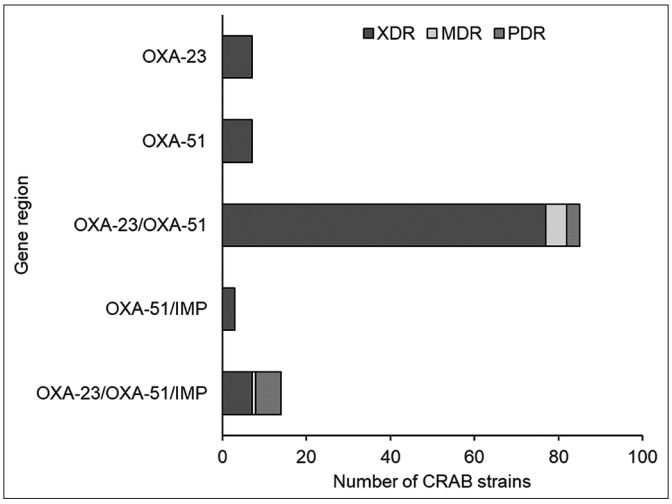

Figure 6: Antibiotic resistance polymorphism of carbapenem-resistant *Acinetobacter baumannii* strains by gene region. OXA-23 is *bla*OXA-23, OXA-51 is *bla*OXA-51, and IMP is *bla*IMP.

especially in Vietnam, represents a concern in controlling this pathogen. Therefore, an assessment of antibiotic resistance patterns in each medical facility is necessary.

5. CONCLUSIONS

The study showed that *A. baumannii* is the leading cause of infections, particularly in patients over 60 years old in both genders, indicating the need for effective infection control measures, especially in ICUs. Colistin, a member of the polymyxin class of antibiotics, was found to be effective against *A. baumannii*, but resistance to various classes of antibiotics, including carbapenems, was also observed. The prevalence of carbapenem-resistant *A. baumannii* (CRAB) strains was highest in the ICU, with a significant proportion of XDR strains. The *bla*OXA-23 and *bla*OXA-51 genes were found to be the most prevalent resistance genes in CRAB strains, with the combination of *bla*OXA-23 and *bla*OXA-51 genes being the most common. This study emphasizes the importance of developing tailored treatment options for CRAB strains based on observed patterns of antibiotic resistance polymorphism in different hospital departments.

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7. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval

of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

The ethical approval details are given in materials and methods section.

11. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

12. PUBLISHER'S NOTE

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