Research Article

Overexpression of bla_{OXA-58} Gene Driven by IS\textsubscript{Aba3} Is Associated with Imipenem Resistance in a Clinical Acinetobacter baumannii Isolate from Vietnam

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The aim of this study was to investigate genetic structures and expression of bla_{OXA-58} gene in five Acinetobacter baumannii clinical isolates recovered from two hospitals in southern Vietnam during 2012-2014. A. baumannii isolates were identified by automated microbiology systems and confirmed by PCR. All isolates were characterized as multidrug resistant by antimicrobial testing using the disk diffusion method. Four imipenem susceptible and one nonsusceptible isolates (MIC > 32 μg·ml\textsuperscript{-1}) were identified by E-test. PCR amplification of bla_{OXA-58} gene upstream and downstream sequences revealed the presence of IS\textsubscript{Aba3} at both locations in one multidrug-resistant isolate. Semiquantitation of bla_{OXA-51} and bla_{OXA-58} gene expression was performed by the 2\textsuperscript{-ΔΔCt} method. The bla_{OXA-51} gene expression of five isolates showed little difference, but the isolate bearing IS\textsubscript{Aba3}-bla\textsubscript{OXA-58}-IS\textsubscript{Aba3} exhibited significantly higher bla_{OXA-58} mRNA level. Higher β-lactamases activity in periplasmic than cytoplasmic fraction was found in most isolates. The isolate overexpressing bla_{OXA-58} gene possessed very high periplasmic enzyme activity. In conclusion, the A. baumannii isolate bearing IS\textsubscript{Aba3}-bla\textsubscript{OXA-58} gene exhibited high resistance to imipenem, corresponding to an overexpression of bla_{OXA-58} gene and very high periplasmic β-lactamase activity.

1. Introduction

Multidrug resistant A. baumannii constitutes a serious threat for nosocomial infection control [1]. Carbapenems are currently the antibiotics of choice against multidrug-resistant Acinetobacter infections [2], but an increasing rate of resistance to carbapenems was reported worldwide, seriously limiting therapeutic options [3]. Carbapenem-resistant A. baumannii has become an alarming health care problem, mainly in developing countries [4]. As a result, carbapenem-resistant A. baumannii is classified into the critical priority group according to the urgency of need for new antibiotic treatment and the level of reported antibiotic resistance by the World Health Organization [5].

Multiple mechanisms of carbapenem resistance have been identified in A. baumannii including low membrane permeability, mutation in its chromosome genes, overexpression of efflux pumps, and acquisition of mobile resistance genes [6]. However, the production of carbapenemases is considered the principal resistance mechanism [7, 8]. The most frequent ones are carbapenem-hydrolyzing class D β-lactamases (CHDLs) and secondly metalloenzymes (MBL) such as bla\textsubscript{NDM} [9]. In addition, class A β-lactamases such as bla\textsubscript{KPC} gene has been recently also detected in A.
baumannii [10], presenting a serious threat of expanding resistance spectrum in the bacteria.

Currently, six main groups of CHDLs found in A. bau-
mannii include blaOXA-51-like, blaOXA-23-like, blaOXA-24-like, 
blaOXA-58-like, blaOXA-143-like, and blaOXA-235-like genes [2, 11, 12]. CHDLs exhibit weak carbapenem hydrolysis; however, they can confer resistance mediated by the combi-
nation of natural low permeability and ISAbu elements located upstream of the gene possibly leading to the gene’s overexpression [2]. Overexpression of blaOXA genes usually corresponds to resistance phenotypes [13–15]. Overproduction of oxacillinases, including blaOXA-58 enzyme, results from the presence of insertion sequences such as ISAbu1, ISAbu2, ISAbu3, or IS18 which provide strong promoters for gene expression [13, 16].

In Vietnam, blaOXA-23 is the most widely disseminated class D-carbapenemase in carbapenem-resistant Acinetobacter baumannii while blaOXA-24 is not detected [17]. Even though there is not any information of blaOXA-143 and blaOXA-235 in Vietnam up to now, these genes are believed to emerge in other parts of the world [18, 19]. During 2003-2014, the majority of A. baumannii clinical isolates recovered harbored blaOXA-51 and blaOXA-23 genes. The blaOXA-58 gene was only detected in isolates recovered from 2010, after the introduction of imipenem in 2008-2009 into hospitals in Vietnam [17, 20]. The blaOXA-58-positive isolates investigated in the present study probably emerged at the same time. This recent emergence was in contrast with the striking replacement of blaOXA-58 by blaOXA-23 reported in Italy and China for the same period [21, 22]. Furthermore, isolates bearing blaOXA-58-like gene were recovered from dif-
ferent countries during outbreaks and showed remarkable conserved gene sequence [23–25]. The aim of this study was to investigate genetic structures and relative expression of blaOXA-58 gene, which lead to imipenem nonsusceptibility in clinical isolates recovered from two Vietnamese hospitals during 2012-2014.

2. Materials and Methods

2.1. Study Design. The study focused on A. baumannii iso-
lates containing blaOXA-58 gene with the purpose of deter-
imining imipenem-resistance mechanism related to the gene.

2.2. Bacterial Isolates, Microbial Identification, and Antimicrobial Susceptibility Testing. Five A. baumannii iso-
lates were chosen from a total of 252 nonduplicate Acinetobacter spp. isolates recovered from patients admitted to hospitals in southern Vietnam during 2012-2014 and were named DN and TN based on their source hospitals [17]. Microbial isolation and identification in source laboratories were performed using the Phoenix System (BD) and the API 20NE system (bioMérieux). Identification of A. baumannii isolates was confirmed by PCR amplification and sequencing of 16S-23S intergenic spacer (ITS) regions. The sequences were deposited in GenBank under accession numbers KY659325, KY659326, KY659327, KY659328, and KY659329. Antimicrobial susceptibility testing was performed by the disk diffusion method and interpreted accord-
ing to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2014). Tested antimicrobials included cef-
tazidime, cefotaxime, ceftriaxone, cefpodoxime, cefepime, piperacillin, ampicillin/sublactam, piperacillin/tazobactam, ticarcillin/clavulanic acid, and meropenem, as well as others not belonging to β-lactams such as amikacin, gentamicin, amikacin, netilmicin, ciprofloxacin, and levofloxacin. MIC values of imipenem were determined by the E-test (bioMérieux); the CLSI-approved breakpoints for imipenem ≥ 8 μg ·ml⁻¹ and ≤2 μg ·ml⁻¹ were considered resistant and suscepti-
ble, respectively.

2.3. Detection of blaOXA, blaNDM, and blaKPC Genes and Insertion Sequences. Amplification of blaOXA genes including blaOXA-51, blaOXA-23, blaOXA-24, and blaOXA-58 genes were performed and published in the previous study [17]. blaNDM and blaKPC genes were amplified in this study as previously reported [26]. The presence of ISAbu1, ISAbu2, ISAbu3, ISAbu4, and IS18 was detected as previously described [13, 27]. The sequence of all primers is shown in Table 1.

2.4. PCR Mapping of blaOXA-58 and blaOXA-51 Genes. PCR mapping of blaOXA genes upstream regions was carried out using combinations of insertion sequence-specific forward primers and blaOXA-51 and blaOXA-58 gene-specific reverse primers (Table 1). The presence of ISAbu3 downstream of 
blaOXA-58 was determined by a long-range PCR containing 1X PrimeSTAR GXL Buffer, 0.2 mmol dNTPs, 500 nmol 
OXA-58-F, 500 nmol ISAbu3C, and 0.5 U PrimeSTAR GXL 
DNA polymerase (Takara). PCR products were sent to 1-
BASE (https://order.base-asia.com/) for purification and sequencing. The sequences were analysed by BioEdit 7.0.9.0. (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), and sequence similarity was assessed using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequence of 
blaOXA-58 and its surrounding ISAbu3 was deposited in GenBank under accession number KY660721.

2.5. Analysis of blaOXA-58 and blaOXA-51 Gene Expression by Real-Time RT-PCR. The midlog phase of bacterial cultures was treated with 1 μmol ·ml⁻¹ oxacillin for 24 h and was sub-
sequently used for RNA extraction [28]. Treatment with RNase-free DNase I (Sigma) was performed at 37°C for 2 h. The concentration and DNase-free quality of RNA sam-
pies were spectrophotometrically assessed and con-
duced by the disk diffusion method and interpreted accord-
antly the Clinical and Laboratory Standards Institute guidelines (CLSI, 2014). Tested antimicrobials included cef-
tazidime, cefotaxime, ceftriaxone, cefpodoxime, cefepime, piperacillin, ampicillin/sublactam, piperacillin/tazobactam, ticarcillin/clavulanic acid, and meropenem, as well as others not belonging to β-lactams such as amikacin, gentamicin, amikacin, netilmicin, ciprofloxacin, and levofloxacin. MIC values of imipenem were determined by the E-test (bioMérieux); the CLSI-approved breakpoints for imipenem ≥ 8 μg ·ml⁻¹ and ≤2 μg ·ml⁻¹ were considered resistant and suscepti-
ble, respectively.

Antimicrobial susceptibility testing was performed by the disk diffusion method and interpreted accord-

Amplification of blaOXA-51 and blaOXA-58 and 16S rRNA was performed in a final volume of 25 μl containing random hex-

mers, MMLV reverse transcriptase (Agilent) at 42° C for 45 min.

Amplification of blaOXA-51 and blaOXA-58 and 16S rRNA was performed in a final volume of 25 μl containing random hex-

ers, MMLV reverse transcriptase (Agilent) at 42° C for 45 min.

Antimicrobial susceptibility testing was performed by the disk diffusion method and interpreted accord-

ly the Clinical and Laboratory Standards Institute guidelines (CLSI, 2014). Tested antimicrobials included cef-
tazidime, cefotaxime, ceftriaxone, cefpodoxime, cefepime, piperacillin, ampicillin/sublactam, piperacillin/tazobactam, ticarcillin/clavulanic acid, and meropenem, as well as others not belonging to β-lactams such as amikacin, gentamicin, amikacin, netilmicin, ciprofloxacin, and levofloxacin. MIC values of imipenem were determined by the E-test (bioMérieux); the CLSI-approved breakpoints for imipenem ≥ 8 μg ·ml⁻¹ and ≤2 μg ·ml⁻¹ were considered resistant and suscepti-
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ble, respectively.
mixture was incubated for 15 min at 95°C, followed by 40 cycles of 10 s at 95°C and 20 s at 60°C. Normalized expression of blaOXA-51 and blaOXA-58 genes was calculated relatively to the 16S rRNA reference gene according to the 2^ΔΔCt method [29].

2.6. Multiple-Locus Variable Number Tandem Repeat Analysis. Multiple-locus variable number tandem repeat analysis (MLVA) as previously described [17, 30, 31] was used to profiling the A. baumannii isolates in the study. The method works on eight variable number tandem repeat (VNTR) loci, namely, 3468, 1988, 3002, 845, 2396, 5350, 826, and 2240 to determine relatedness among the A. baumannii isolates.

2.7. β-Lactamase Extraction and Quantitation. Isolates were grown on LB medium supplemented with 1 μmol·ml⁻¹ oxa-
cillin for 18-24 h at 37°C in a shaking incubator. The supernatants (extracellular fraction) were collected after centrifugation of bacterial cultures and precipitated with absolute ethanol (1 : 4) in 20 min at -20°C [32]. Periplasmic fractions were recovered from cell pellets [33]. Protein concentration was determined by the Bradford method [34]. β-Lactamase activity was determined based on nitrocefin hydrolysis [35, 36]. Briefly, 1-5 μl extracellular and periplasmic fractions obtained from each isolate were incubated with 40 nmol nitrocefin dissolved in 0.1 M phosphate buffer, pH 7.0 in a total volume of 100 μl. Samples were loaded onto microtiter plates, and the absorbance at 482 nm was measured kinetically at room temperature for 2-30 minutes using an ELISA spectrophotometer. The specific β-lactamase activity was calculated and expressed as mU·mg⁻¹ of protein based on the quotient of β-lactamase activity (mU·ml⁻¹) and protein concentration (mg·ml⁻¹).

### Table 1: Primers and probes used for PCR amplification and sequencing of antimicrobial resistance genes and related genetic elements.

| Primers/probes   | Sequence (5’→3’) | Length (bp) | Tm (°C) | Product (bp) | Ref.              |
|------------------|------------------|-------------|---------|--------------|------------------|
| OXA-23-F         | CACTAGGAGAAGCCATGAAGC | 21          | 55.0    | 114          | Nguyen et al., 2017 |
| OXA-23-R         | CAGCATTACCGAACCACCATACG | 22          | 55.0    |              |                  |
| OXA-24-F         | GCTAAATGCTTTAATCGGGCTAG | 24          | 55.0    | 141          | Nguyen et al., 2017 |
| OXA-24-R         | ACTGGAACCTGCTGAACATG | 20          | 55.0    |              |                  |
| OXA-51-F         | GAAGTGAAGCGTGTTGGTTATG | 22          | 55.0    |              | Nguyen et al., 2017 |
| OXA-51-R         | GCCCTTTGCTGACAGGTTAAT | 20          | 55.0    | 148          |                  |
| OXA-51-P         | FAM-CGACATTGGGTACCGATATCGCATG-BOH1 | 27          | 61.3    |              | This study       |
| OXA-58-F         | ATATTTAATGGGGATGGAAGCC | 23          | 55.0    | 110          | Nguyen et al., 2017 |
| OXA-58-R         | CGTGCAATTTCTGATATACAGG | 23          | 55.0    |              |                  |
| OXA-58-P         | FAM-TTACATTTGGGCAAGCCATGCAAG-BHQ1 | 25          | 60.6    |              | This study       |
| 16S-rRNA-F       | CCAGTGCAAAACTGGAGGAAG | 21          | 55.5    |              |                  |
| 16S-rRNA-R       | GCTGTGTAGCAACCCCTTTGTA | 21          | 55.2    | 199          |                  |
| 16S-rRNA-P       | HEX-ACGCTCAGTCTCAGTCCTGCC-BOH1 | 25          | 61.5    |              |                  |
| HRF/ISAba1       | CACGAATTCAGAATTAGCCG | 17          | 56.0    | 520          | Segal et al., 2005 |
| HRF/ISAba1       | CGACGAATACTATAGCAC | 18          | 56.0    |              |                  |
| ISAba2A          | AATCCGAGATAAGCGCGGTTTC | 20          | 54.0    | 1200         | Poirel et al., 2006 |
| ISAba2B          | TGACACATAACCTGTGCAC | 20          | 52.1    |              |                  |
| ISAba3A          | CAATCAACATCCACACCTGC | 20          | 52.3    |              |                  |
| ISAba3C          | AGCAATACGTCTGTATACCGC | 20          | 51.8    |              |                  |
| ISAba4A          | ATTTGAACCCCATCATTGCG | 20          | 50.6    | 612          | Brown et al., 2007 |
| ISAba4B          | AATCTTATATTTTTCTTGG | 20          | 45.3    |              |                  |
| IS18A            | CAACCCAATCTGTCAGATG | 20          | 51.2    | 925          | Poirel et al., 2006 |
| IS18B            | ACCAGCCTAATATCTTCATCG | 20          | 54.7    |              |                  |
| 1512F/ITS        | GTCGTAACAGGGATGCGGTA | 20          | 54.1    | 607          | Chang et al., 2005 |
| 6R/ITS           | GGCTTYCCCCRTCRGAAT | 20          | 56.5    |              |                  |
| NDM-F            | GACGCCGTCATCTCCAA | 18          | 55.4    |              |                  |
| NDM-R            | CGCGACCACGCGCGTT | 15          | 57.0    | 52           | Yong et al., 2009; CDC 2011 |
| NDM-P            | HEX-TGATACAAAGCAGGAGAT-ZEN/IBFQ | 17          | 48.3    |              |                  |
| KPC-F            | GCCCGCGTGCGAAAC | 16          | 56.0    |              |                  |
| KPC-R            | GCCGCCAACTCTCTCA | 17          | 56.5    | 61           | Garcia et al., 2010; CDC 2011 |
| KPC-P            | 6FAM-TGATAAAGCCGCCCGCCGCAATTGT-ZEN/IBFQ | 23          | 62.2    |              |                  |
2.8. Statistical Analysis. The analysis of variance (ANOVA) was used to analyse the difference among β-lactamase activity means of isolates. A t-test was used to determine the significant difference of extracellular and periplasmic β-lactamase activity. A p value < 0.05 was considered significant.

3. Results and Discussion

blaOXA are prevalent in A. baumannii. We had previously performed blaOXA identification in A. baumannii isolates from three hospitals in Southern Vietnam and found blaOXA-23 was dominant [17]. Even though blaOXA-58 existed with a small number in Vietnamese population, the exact genetic context involving antimicrobial resistance elements remained unknown. Here, we uncovered the imipenem-resistant mechanism of blaOXA-58-positive A. baumannii isolates. The overexpression of blaOXA-58 gene has been seen in the isolate with high-resistance phenotype through relative quantification of mRNA of the corresponding gene. The specific possible-intact ISAba3 sequence upstream of blaOXA-58 gene could be the key factor for the high expression. In addition, the high β-lactamase activity in the periplasmic space observed in the study could be the outcome of the phenomenon.

3.1. Antimicrobial Susceptibility Testing. All five isolates (DN050, TN078, DN014, TN341, and TN345) were classified as multidrug resistant (MDR) since they were nonsusceptible to at least one agent in three or more antimicrobial categories including aminoglycosides, antipseudomonal carbenapens, antipseudomonal fluoroquinolones, antipseudomonal penicillins and β-lactamase inhibitors, extended-spectrum cephalosporins, folate pathway inhibitors, penicillins and β-lactamase inhibitors, polymyxins, and tetracyclines [37]. In this study, although several antimicrobials were not tested because of their availableness at different times and hospitals, all isolates satisfied the definition to be defined as MDR. Isolate DNA050 was nonsusceptible to all antimicrobials tested. The other four were all susceptible to imipenem (there were three isolates nonsusceptible to meropenem as hospitals reported), but for other antimicrobials, their susceptibility varied. Isolate TN078 and DN014 were nonsusceptible to three categories while isolates TN341 and TN345 were nonsusceptible to five categories (Table 2).

3.2. Isolate Genotyping and Profiling. All isolates were identified as A. baumannii based on 16S-23S intergenic spacer (ITS) region sequencing. Based on MLVA profiling, four different MLVA types within the five isolates reflected substantial genetic diversity in the sampled Vietnamese A. baumannii isolates, as previously described [17].

No isolate with blaCPC gene was detected, while two isolates contained blaNDM gene (DN050 and TN078). Even though the two isolates were singletons (based on MLVA types from previous study [17]) with different phenotypes, they had close relatedness with just difference in 3/8 loci surveyed and very similar resistance determinants, especially the blaNDM gene. Therefore, the difference in resistance phenotype was mostly because of the distinguished genotype with ISAba3-blaOXA-58 in isolate DN050, compared to isolate TN078. It might be necessary for blaNDM gene located in a specific genetic context to be expressed as one of the important and strong resistance determinants. The mechanism should be explored further.

Regardless of the genetic diversity of the isolates, the blaOXA-58 gene sequence analysis (data not shown) of all isolates was identical with the reported blaOXA-58 gene [38]. This was in agreement with a previous work showing a lack of diversity in this gene, probably due to its recent acquisition by A. baumannii from other species [3].

All isolates were blaOXA-58- and blaOXA-51-positive and blaOXA-23-negative and blaOXA-24-negative (Table 2). The analysis of insertion sequences revealed the presence of ISAba1 and ISAba2, but they were not located upstream of blaOXA-51 or blaOXA-58 genes in all isolates. ISAba4 and IS18 were not detected. ISAba3 was detected in all isolates (Table 2). However, only isolate DN050 possessed a blaOXA-58 gene bracketed by two ISAba3 elements (Figures 1 and 2). The promoter region of blaOXA-58 gene in this isolate (Figure 2) was similar to sequences described by Poirel and Nordmann [38]. The genetic structure of blaOXA-58 upstream sequences which led to overexpression of this gene displayed a remarkable variability [38–40]. Hybrid promoters constituting an ISAba3 sequence truncated by other insertion sequences were generally considered strong promoters [22, 41]. However, in this study, isolate DN050 bearing possible-intact ISAba3 sequence upstream of blaOXA-58 gene was not interrupted by inserted sequences, provided -35 and -10 promoter sequences as already described [38]. This structure probably drove high level carbapenemase production. The acquisition of insertion sequences by an imipenem-susceptible blaOXA-58 harboring isolate can lead to carbapenem resistance in A. baumannii [38]. Our results highlighted the threat of undetected reservoirs of carbapenem-resistant determinants and mechanisms in Vietnamese A. baumannii isolates.

3.3. Relative Quantitation of blaOXA-58 and blaOXA-51 mRNA Level. We chose three isolates (DN050, TN341, and TN345) to study the relative expression of blaOXA-51 and blaOXA-58 under condition with oxacillin as an inducer and without oxacillin induction. They all had high β-lactamase activity in periplasmic fractions as shown in the following experiment (Table 3). The mRNA level of blaOXA-58 and blaOXA-51 genes in all isolates was determined by quantitative real-time RT-PCR. Under oxacillin induction, DN050 showed a significantly higher level of blaOXA-58 mRNA expression than isolates TN341 and TN345 (Figure 3). blaOXA-51 expression was also upregulated, but not comparable to that of blaOXA-58. Interestingly, the high expression level of blaOXA-58 from DN050 could be associated with the presence of an upstream ISAba3 sequence as previously suggested [38]. Furthermore, in this study, the possible intact ISAba3 sequence might be customized to blaOXA-58 gene to drive a very strong gene expression, as seen in ISAba1 for blaOXA-23 and AmpC genes [42]. The other isolates lacked upstream ISAba3 sequence.
3.4. Analysis of Periplasmic β-Lactamase Activity in Association with bla OXA-51/58 Relative Expression. Under the condition of oxacillin induction, the bla OXA-58 expression of isolate DN050 (MICimipenem ≥ 32 μg·ml⁻¹) was also significantly higher than the expression of other four isolates, TN078, DN014, TNA341, and TN345 with MIC imipenem which were 0.5, 0.19, 0.75, and 0.5, respectively (Table 3). All isolates expressed a low level of bla OXA-51, conﬁrming that the presence of bla OXA-51, without an upstream IS Aba1, did not confer a resistance phenotype [16]. Furthermore, in variants harboring bla OXA-51 and bla OXA-58 genes, carbapenem resistance only correlated with bla OXA-58 [43], which is in agreement with the results of this study.

The enzyme activity of extracellular fractions was not signiﬁcantly different (p=0.2187) while one of the periplasmic fractions exhibited a signiﬁcant difference among isolates (p<0.0001). Extracellular fractions possessed lower enzyme activity than periplasmic fractions (p=0.0355) in most cases. The periplasmic fraction recovered from all isolates exhibited variable β-lactamase activity, with very high activity corresponding to isolate DN050. Isolates TN341 displayed the highest β-lactamase activity though weakly expressed bla OXA-58 gene. This high enzyme activity probably corresponded to other β-lactamases responsible for the multidrug resistance phenotypes of the isolate, such as...
extended-spectrum AmpCs [44]. The presence of other β-lactamases could explain the high enzyme activity in periplasmic fractions of the other isolates. Particularly, \( \text{bla}_{\text{NDM}} \) gene detected in both isolates DN050 and TN078, but the corresponding β-lactamase activities as well as the antimicrobial susceptibilities were different between the two isolates. The mechanism that a strain carrying a \( \text{bla}_{\text{NDM}} \) gene is not resistant to carbapenems needs to be discovered further in \( A. \) baumannii. It might need a unique genetic structure for \( \text{bla}_{\text{NDM}} \) gene to be expressed as seen in \( K. \) pneumoniae [45]. In a transformed \( A. \) baumannii strain with a \( \text{bla}_{\text{OXA-58}} \) plasmid-borne vector, this carbapenemase is selectively released via outer membrane vesicles (OMV) after periplasmic

**Figure 2:** Promoter structure of \( \text{bla}_{\text{OXA-58}} \) gene from isolate DN050. The -35 and -10 putative promoter sequences and the +1 transcription initiation site within IS\( \text{Aba3} \) are boxed. The \( \text{bla}_{\text{OXA-58}} \) start and stop codons, ATG (M) and TAA (*), respectively, are underlined. Upstream IS\( \text{Aba3} / \text{bla}_{\text{OXA-58}} \) sequences and downstream IS\( \text{Aba3} / \text{bla}_{\text{OXA-58}} \) gene junctions are indicated by arrows. Full sequences obtained are deposited in GenBank (accession number KY660721).

**Table 3:** Relative quantitation of \( \text{bla}_{\text{OXA-51}} \) and \( \text{bla}_{\text{OXA-58}} \) mRNA level and β-lactamase activity in five \( A. \) baumannii isolates.

| Isolate | MIC imipenem (µg·ml\(^{-1}\)) | Relative expression of \( \text{bla}_{\text{OXA-51}} \) (ΔCt) | Relative expression of \( \text{bla}_{\text{OXA-58}} \) (ΔCt) | Total β-lactamase activity (U·mg\(^{-1}\)) |
|---------|-------------------------------|-------------------------------------------------|--------------------------------------------------|------------------------------------------|
|         |                               | ΔCt                                             | ΔCt                                             | Extracellular                           |
|         |                               | (Expression time)                               | (Expression time)                               |                                         |
| DN050   | ≥32                           | 8.87 ± 0.39 (0.76-1.31)                          | 4.18 ± 1.18 (11.3-58.37)                        | 10.8 ± 3.3                              |
| TN078   | 0.5                           | 12.87 ± 0.30 (0.05-0.08)                         | 8.64 ± 0.48 (0.84-1.64)                        | 17.7 ± 5.2                              |
| DN014   | 0.19                          | 7.54 ± 0.66 (1.58-3.98)                          | 7.99 ± 2.23 (0.39-8.61)                        | 13.9 ± 4.1                              |
| TN341   | 0.75                          | 9.64 ± 0.71 (0.36-0.96)                          | 8.45 ± 0.53 (0.93-1.93)                        | 10.8 ± 3.3                              |
| TN345   | 0.5                           | 8.80 ± 0.60 (0.69-1.59)                          | 7.28 ± 0.24 (2.55-3.56)                        | 12.9 ± 3.8                              |
translocation through Sec-dependent system [32]. Furthermore, overexpression of blaOXA-58 gene increases its periplasmic enzyme concentration and extracellular release leading to efficient carbapenem hydrolysis [32]. The blaOXA-58 high mRNA level and high periplasmic β-lactamase activity of the DN050 isolate in this study suggested a similar overexpression, periplasmic translocation, and release mechanism of blaOXA-58 carbapenemase, even though our experimental work did not directly show the selection of OMV after being translocated to a periplasmic space. The high periplasmic β-lactamase activity of the isolates, especially TN341 in this study, also suggested a possible translocation and release of other β-lactamases with a mechanism similar to that identified with blaOXA-58. Further studies should be carried out to prove the suggested mechanism in clinical isolate similar to the transformed A. baumannii strain. To the best of our knowledge, our study is the first report on the overexpression of blaOXA-58 gene of A. baumannii clinical isolates from Vietnam.

This study had some limitations. The first limitation involved the small sample size due to the low prevalence of clinical isolates harboring blaOXA-58 gene in the population surveyed. The screening has been done in previous studies [17]. Secondly, we did not characterize other resistance mechanisms in A. baumannii such as the overexpression of efflux pump genes or existence of multicity blaOXA-58 gene [7, 11, 46]. In addition, the presence of other β-lactamase genes such as blaIMP, blaVIM, blaGES, blaOXA-143, and blaOXA-235 was not excluded. Furthermore, we did not carry out an alternative experimental approach, such as western blotting against blaOXA-58 to unequivocally determine if the increase in β-lactamase activity is mainly due to this protein.

4. Conclusions

This study identified a mechanism of imipenem resistance related to the overexpression of blaOXA-58 gene preceded by ISaba3 and its corresponding periplasmic enzyme present at high concentration in a multidrug-resistant clinical isolate recovered from a hospital in Vietnam.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials

Figure S1: result of imipenem E-test for five clinical isolates of A. baumannii (blaOXA-58). Figure S2: electrophoresis results of PCR screening for the presence/absence of ISAba1, ISAba2, ISAba3, ISAba4, and IS18 in five clinical isolates of A. baumannii (blaOXA-58). Figure S3: electrophoresis results of PCR for the presence/absence of insertion sequence (IS) upstream of blaOXA-58 gene. Figure S4: duplex real-time RT-PCR analysis of the blaOXA-51 and blaOXA-58 mRNA relative expression compared with 16S rRNA in five A. baumannii isolates. Figure S5: Bradford assay standard curve of concentration versus absorbance for protein.

![Figure 3: Duplex real-time RT-PCR analysis of the blaOXA-51 and blaOXA-58 mRNA relative expression in three A. baumannii isolates. The error bars represent the deviation for the normalized fold expression of blaOXA-51 and blaOXA-58 in three isolates which were positive or negative for the ISaba3 upstream of the blaOXA-58 gene. -: not induced; +: induced.](image-url)
quantification. Figure S6: nitrocefin standard curve. Table S1: duplex real-time RT-PCR analysis of the blaOXA-51 and blaOXA-58 mRNA relative expression in three A. baumannii isolates under conditions with oxacillin as an inducer or without oxacillin induction. Table S2: results for protein quantification of supernatant and periplasmic fractions. Table S3: results for \( \beta \)-lactamase activity of supernatant and periplasmic fractions. (Supplementary Materials)

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