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Original Article

Application of Duplex Polymerase Chain Reaction and Whole Genome Sequencing in Analyzing Carbapenem Resistance Genes in Clinical *Klebsiella pneumoniae* Isolates

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Abstract: *Klebsiella pneumoniae* is a Gram-negative, encapsulated bacterium with the potential for high virulence and antibiotic resistance. Its ability to develop multidrug resistance poses a global threat, particularly in clinical settings. The study aimed to assess the effectiveness of two molecular-based methods, duplex polymerase chain reaction (PCR) and whole genome sequencing (WGS), in determining the prevalence of four carbapenem resistance genes, including bla_{KPC}, bla_{NDM}, bla_{VIM}, and bla_{OXA-48}, in 41 *K. pneumoniae* isolates collected from intensive care units. As a result, while PCR detected the presence of the oxacillinase 48 (OXA-48) gene well, WGS did not. In contrast, WGS revealed the presence of an OXA-48-like variant, OXA-181. Notably, regarding the co-occurrence of carbapenemase types, the PCR method identified two isolates carrying all three types, while WGS analysis did not detect this. Both methods showed the absence of bla_{VIM} in all isolates. Interestingly, *Klebsiella pneumoniae* carbapenemase (KPC) was the least frequently detected carbapenemase type, despite earlier reports highlighting the prevalence of KPC-producing *Enterobacterales*. Both methods similarly identified a high prevalence of of bla_{OXA}-carrying *K. pneumoniae* strains, aligning with global trends. In conclusion, both PCR and WGS have distinct advantages and limitations in detecting antibiotic resistance genes (ARGs). PCR is efficient and straightforward but limited in its ability to detect all gene variants. In contrast, WGS is more resource-intensive yet offers comprehensive insights into gene diversity, making it particularly valuable for capturing the full spectrum of ARGs.

Keywords: Antibiotic resistance genes; carbapenem resistance; intensive care units; *Klebsiella pneumoniae*; polymerase chain reaction; whole-genome sequencing.

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1. Introduction

Klebsiella pneumoniae (*K. pneumoniae*), a member of the family *Enterobacteriaceae*, is a Gram-negative, encapsulated, and non-motile microorganism commonly found in the environment. The bacterium can exhibit significant levels of virulence and antibiotic resistance once it enters the host. The capacity of *K. pneumoniae* to develop multidrug resistance contributes to the global threat of transmission and unsuccessful treatment outcomes. As a result, significantly fewer treatment options for multidrug-resistant *K. pneumoniae* infections have been offered in most clinical settings, even for common illnesses, but especially for patients in intensive care units (ICUs) [1-3].

Since the detection of extended-spectrum β-lactamases (ESBLs) producing *K. pneumoniae*, carbapenems have been the preferred treatment for infections caused by ESBL-producing *K. pneumoniae*. However, the bacterium began to develop carbapenem resistance decades ago [4]. Vietnam has the second-highest prevalence of *K. pneumoniae* strains producing ESBLs globally, with 67% of isolates associated with urinary tract infections [5]. The data from 2017 to 2018 revealed a high prevalence of ESBL-positive, carbapenem-resistant *Klebsiella pneumoniae* in ICUs in Vietnam, with 64% of isolates carrying between two and four different ESBL- and carbapenemase-encoding genes [6].

A wide range of carbapenemases in *K. pneumoniae* has been identified, encompassing classes A, B, and D β-lactamases according to the Ambler classification [7]. Class A *Klebsiella pneumoniae* carbapenemases (KPCs) have spread rapidly since their initial discovery.

One of the most prevalent families of class B metallo-β-lactamases includes New Delhi metallo-β-lactamase (NDM) and Verona integron-encoded metallo-β-lactamase (VIM) [8]. Class D serine-β-lactamases can hydrolyze and confer resistance to oxacillin and penicillin, hence the designation "oxacillinases" and the prefix *OXA* [9]. Due to their similarity to ESBLs caused by point mutations, *OXA-48*-type producers are among the most difficult carbapenemase producers to detect, making it challenging to accurately estimate their true prevalence.

For the detection of carbapenemase, molecular methods have proven to be practical tools. Recently, multiplex polymerase chain reaction (PCR) and microarray technologies have been developed to detect multiple carbapenemase-encoding genes in a single assay. Among these, multiplex PCR offers distinct advantages by providing rapid results, thereby supporting timely clinical decision-making and optimal treatment strategies.

Concurrently, over the past two decades, advances in sequencing technologies have revolutionized microbiology. The rapid development of next-generation sequencing (NGS) has greatly accelerated this progress, leading to an exponential increase in available genomic data. A key application of this genomic revolution is the identification of virulence genes, which provides crucial insights into the mechanisms underlying bacterial pathogenesis. Whole-genome sequencing (WGS) has already demonstrated its transformative potential by enabling high-resolution characterization of bacterial isolates during major multinational outbreaks. Its unprecedented strain-level resolution positions WGS to replace conventional typing methods in the near future [10]. A compelling example comes from Lieberman et al., [11], who employed WGS to reconstruct transmission networks of *Burkholderia dolosa* in cystic fibrosis patients, their mutation chronology analysis not only differentiated transmission donors from recipients but also revealed multiple airway-to-bloodstream transmission events within individual hosts. As an increasing number of pathogen strains from diverse diseases and ecological sources are sequenced, deeper insights into bacterial pathogenesis are being uncovered. Much of this work focuses on

the detection of resistance genes in *Enterobacteriaceae*, particularly within carbapenemase subgroups. Notably, NDM and VIM from the Class B metallo-β-lactamases, along with the once under-appreciated Class D serine carbapenemase OXA-48, have emerged as highly significant. Their widespread dissemination via plasmids has revolutionized several molecular detection and typing approaches [12-14]. While KPC, NDM, OXA-48, and VIM genes in *K. pneumoniae* are primarily harboured by plasmids, sporadic chromosomal localization has been observed, often mediated by mobile genetic elements [15]. Coupled with continuous technological improvements and cost reductions, WGS is rapidly transitioning from a research tool to a cornerstone technology for clinical diagnostics and pathogen surveillance. Comparative evaluation of PCR and WGS is critical to delineate their respective strengths in gene detection, especially carbapenem resistance genes. Key points remain in understanding their diagnostic concordance, cost-effectiveness in clinical settings, and ability to detect emerging or co-occurring resistance patterns, necessitating systematic experimental validation to guide optimal implementation.

In this study, PCR and WGS were systematically evaluated for identifying four clinically significant carbapenemase- encoding genes including *bla*$_{\text{OXA-48}}$, *bla*$_{\text{KPC}}$, *bla*$_{\text{VIM}}$, and *bla*$_{\text{NDM}}$ in *K. pneumoniae* isolates. The investigation focused on 41 *K. pneumoniae* clinical specimens obtained from ICU patients at the 108 Military Central Hospital (108 MCH), a tertiary care facility serving a high-risk patient population.

2. Experimental

2.2. Clinical Isolates

Forty-one *K. pneumoniae* isolates were obtained from the Microbiology department, 108 MCH, Hanoi, Vietnam. The isolates were obtained through random sampling across multiple ICUs during a 2023 study, without stratification by collection time, patient comorbidities, or age. Each was sub-cultured on a blood agar plate and underwent DNA extraction using Monarch Genomic DNA Purification Kit NEB #T3010 (New England Biolabs, USA). The obtained DNA samples were utilized for duplex PCR and WGS.

2.2. PCR Amplification of Carbapenemase-Encoding Genes

Several carbapenemase-encoding genes were studied, including *bla*$_{\text{OXA-48}}$, *bla*$_{\text{KPC}}$, *bla*$_{\text{VIM}}$, and *bla*$_{\text{NDM}}$. Primer sequences were applied from a previous study [16] and purchased from Eurofins Genomics (Eurofins Genomics, Germany). The yielded PCR product sizes of *bla*$_{\text{OXA-48}}$, *bla*$_{\text{KPC}}$, *bla*$_{\text{VIM}}$, and *bla*$_{\text{NDM}}$ are 438 bp, 798 bp, 390 bp, and 621 bp, respectively [16]. Two duplex PCR combinations, *bla*$_{\text{OXA-48}}$ with *bla*$_{\text{KPC}}$ and *bla*$_{\text{VIM}}$ with *bla*$_{\text{NDM}}$, were performed to identify the presence of these genes. PCR reaction contained Taq DNA polymerase (Qiagen, Germany) and associated reagents (ThermoScientific, USA). PCR program included 35 cycles of 30s at 95 °C, 30s at 52 °C, and 30s at 72 °C. Products were analyzed on 1.5% agarose gels with Tris-Acetate-EDTA 1X buffer (Merck KgaA, Germany) under electric potential of 300V for 40 minutes, visualized using ethidium bromide dye (Thermo Fisher Scientific, USA) under UV light using the Invitrogen™ E-Gel™ imager system with E-Gel™ adaptor (Fisher Scientific, USA). Four in-house positive controls for the four resistance genes including *bla*$_{\text{OXA-48}}$, *bla*$_{\text{KPC}}$, *bla*$_{\text{VIM}}$, and *bla*$_{\text{NDM}}$ were used (Molecular Biology Department, 108 MCH, Vietnam). DNA from *K. pneumoniae* ATCC 700603 and water added in the PCR mix served as negative controls in every batch.

2.3. Whole-genome Sequencing, Genome Assembly, and Bioinformatic Analysis

The genomic DNA underwent magnetic bead purification before library construction. Consequently, DNA was mixed with an

equivalent volume of AMPure XP bead solution (Beckman Coulter) and left to react on a rotator mixer at room temperature (RT). Subsequently, the beads were collected and rinsed twice with freshly made 70% ethanol. Genomic DNA was eluted by exposing the beads to MilliQ DNAse-free water at RT. DNA concentrations were determined using the Qubit BR assay kit (Thermo Fisher Scientific, USA) on a Qubit 4 fluorometer (Thermo Fisher Scientific, USA). The preparation of libraries for nanopore sequencing was carried out using the Rapid Barcoding Kit 96 V14 (Q20+) (catalog no. SQK-RBK114.96; Oxford Nanopore Technologies, UK) following the instructions provided by the manufacturer (Oxford Nanopore Technologies, UK).

Before sequencing, the flow cells were subjected to a quality control check. DNA sequencing was performed using a MinION device (Oxford Nanopore Technologies, UK) using R10.4.1 flow cells (Oxford Nanopore Technologies, UK). In MinKNOW, the minimum fragment length was configured to be 200 bp. The data underwent base-calling and demultiplexing using Guppy 6.0.6, which was set up for base-calling on an R10.4.1 flow cell.

All commands were executed with the default parameters unless otherwise specified in parentheses. After base calling, the FASTQ file was filtered using Filtlong (https://github.com/rrwick/Filtlong), and then Flye (https://github.com/fenderglass/Flye) was utilized for de novo assembly. All assemblies generated from long-read data were refined using Medaka (https://github.com/nanoporetech/medaka). polishing to achieve the ultimate consensus assemblies. The Abricate package (https://github.com/tseemann/abricate) was applied to conduct mass screening of contigs for the presence of ARGs or virulence genes.

2.4. Data Processing and Analysis

The data analysis was performed using Excel (Microsoft, USA), and the results were displayed as numerical values and percentages (%). The PCR product images were captured using the Invitrogen™ E-Gel™ imager system with an E-Gel™ adaptor from Fisher Scientific, USA, then saved as files in HEIC format and formatted using Powerpoint (Microsoft, USA).

3. Results and Discussion

3.1. Distribution of Carbapenem Resistance Genes Detected using Duplex PCR

Using duplex PCR, the data showed that NDM was the most common carbapenemase type (n = 29) (Figure 1). Figure 2 illustrates an example of samples positive with carbapenem resistance genes ($bla_{\text{OXA-48}}$, bla_{KPC}, and bla_{NDM}) detected using duplex PCR.

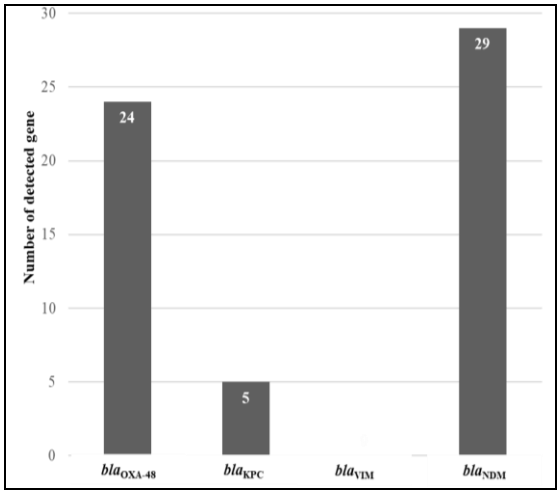

Figure 1. Prevalence of carbapenem resistance genes detected with duplex PCR in *K. pneumonia* isolates. NDM was the most common carbapenemase type (29/41, 70.7%), followed by OXA-48 (24/41, 58.5%) and KPC (5/41, 12.2%).

Sizes of expected amplicons of $bla_{\text{OXA-48}}$, bla_{KPC}, bla_{VIM}, and bla_{NDM} are 438 bp, 798 bp, 390 bp, and 621 bp, respectively [17].

Only 4.9% of strains expressed three carbapenemase types through duplex PCR (one of them, sample *KP* 76, shown in Figure 2). The most common expression found in this method was harboring two types of carbapenemase- encoding genes ($bla_{\text{OXA-48}}$ — bla_{NDM}, n = 17; $bla_{\text{OXA-48}}$ — bla_{KPC}, n = 1), which accounted for 43.9% in all samples (Figure 3).

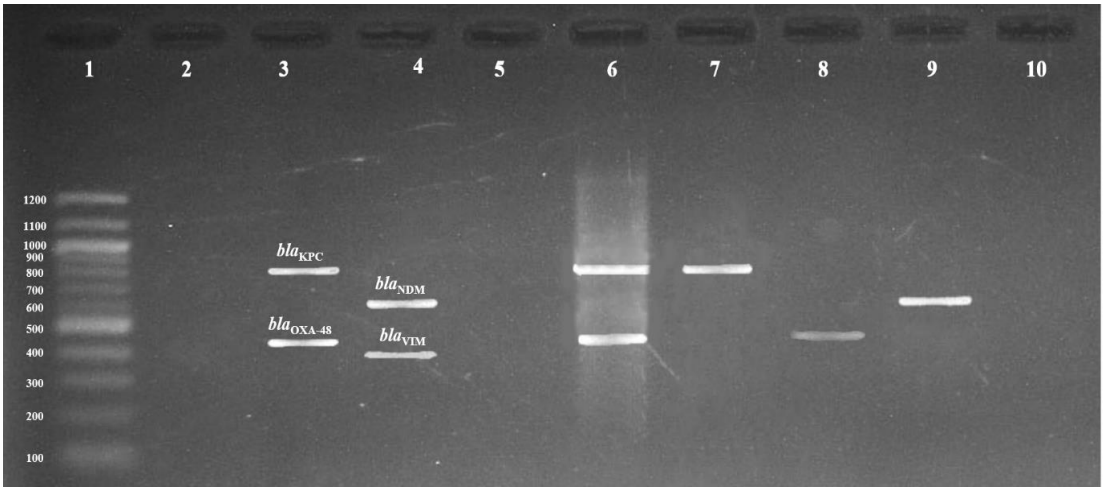

Figure 2. Example of carbapenem resistance genes detected with duplex PCR captured under UV light. Lane 1, Invitrogen™ 100 bp DNA ladder (Thermo Fisher Scientific, USA). Each lane was loaded with 20µl of a 25 µl PCR mixture. Lane 2, first negative control using nuclease-free water substituted DNA sample; lane 3, first pair of in-house positive controls; lane 4, second pair of in-house positive controls; lane 5, second negative control using *K. pneumoniae* ATCC 700603; lanes 6 and 9, *K. pneumoniae* sample 76; lane 7, *K. pneumoniae* sample 1; lane 8, *K. pneumoniae* sample 28; lane 10, *K. pneumoniae* sample 4.

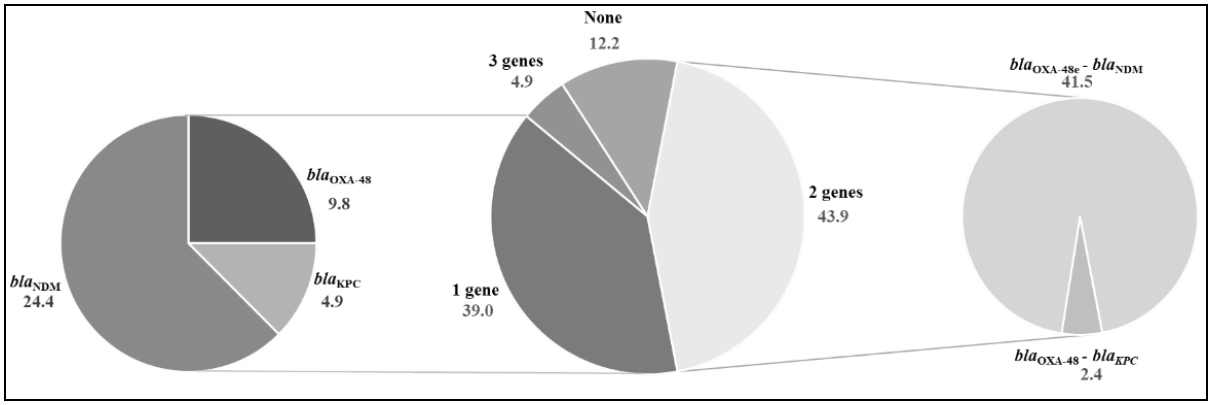

Figure 3. The proportion of carbapenemase- encoding genes ($bla_{\text{OXA-48}}$, bla_{KPC}, and bla_{NDM}) was detected with duplex PCR. The most dominant expression found was harboring two types of gene (43.9%), including $bla_{\text{OXA-48}}$ — bla_{NDM} and $bla_{\text{OXA-48}}$ — bla_{KPC}. Samples harboring one gene accounted for 39% of the total (NDM, 24.4%; OXA-48, 9.8%; KPC, 4.9%). Samples with no gene detected accounted for 12.2% in total. Two samples, which accounted for 4.9%, harbor all three carbapenemase-encoding genes.

3.2. Distribution of Carbapenem Resistance Types Detected using WGS-based Databases

OXA (including sub-types) was the most common carbapenemase type found in the ResFinder database (n = 45) (Figure 4). Among *K. pneumoniae* isolates expressing the OXA type, seven cases were found to carry two OXA subtypes simultaneously: 6 of them harbored both $bla_{\text{OXA-1}}$ and $bla_{\text{OXA-181}}$, while 1 isolate contained $bla_{\text{OXA-1}}$ and $bla_{\text{OXA-9}}$.

When using the ResFinder database, $bla_{\text{OXA-181}}$ was the most common type (n = 25) among *K. pneumoniae* isolates expressing the OXA type, while of the isolates harboring the bla_{NDM} gene, $bla_{\text{NDM-4}}$ accounted for the most (n = 25) (Figure 6). On the other hand, $bla_{\text{KPC-2}}$ was the only type of KPC carbapenemase found in this database.

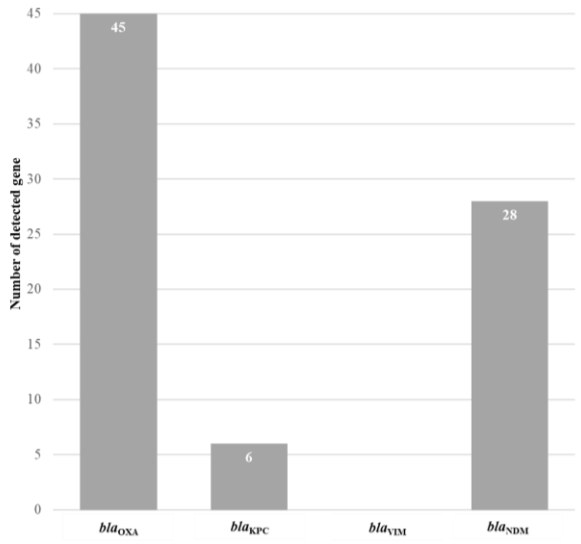

Figure 4. Prevalence of carbapenem resistance genes detected using WGS in *K. pneumoniae* isolates. The ResFinder database was chosen to screen for the presence of ARGs or virulence genes. OXA (including sub-types) was the dominant carbapenemase type (n = 45), followed by NDM (n = 28) and KPC (n = 6).

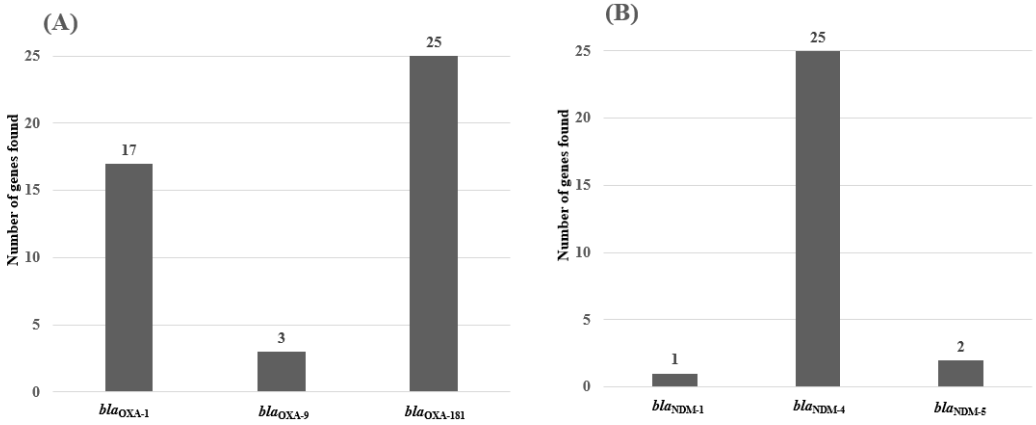

Figure 5. Prevalence of types of bla_{OXA} gene (A) and bla_{NDM} gene (B) detected using WGS. With the Resfinder database, $bla_{OXA-181}$ was the most common type (n = 25) among *K. pneumoniae* isolates expressing OXA type, followed by bla_{OXA-1} (n = 17) and bla_{OXA-9} (n = 3). Of the isolates harboring the bla_{NDM} gene, bla_{NDM-4} accounted for the most (n = 25), followed by bla_{NDM-5} (n = 2) and bla_{NDM-1} (n = 1).

Surprisingly, despite the expansion of KPC-producing *Enterobacterales* and bla_{KPC-2}, which was the most common carbapenemase-encoding gene reported in Hanoi [6, 18], in this study, the *KPC* gene was the least commonly found carbapenemase type, with a relatively low proportion in all methods. On the other hand, we found a high prevalence of bla_{OXA}-carrying *K. pneumoniae* strains. Overall, bla_{OXA-1} variants were widespread throughout the world. The bla_{OXA-9} variants were most common in North America. Meanwhile, the bla_{OXA-48} variants were primarily found in Europe and Asia, which was consistent with earlier investigations [19, 20]. Interestingly, as $bla_{OXA-181}$ was the most prevalent one in our study. Its appearance in many places of the world has been recorded, and such a gene was shown to be most likely underreported due to challenges with laboratory detection of this enzyme [21].

The second prevalence among carbapenemase-encoding genes was *bla*$_{\text{NDM-4,}}$ which was also reported to be well-recognized in Vietnam [22].

Moreover, none of the isolates expressed all three types of carbapenemase (Figure 6). The proportion of isolates expressing two carbapenemase types was 73.2% (OXA sub-types – NDM sub-types, n = 25; OXA sub-types – KPC-2, n = 5). The proportion decreased to 24.4% for isolates harboring one type of gene. In detail, *bla*$_{\text{OXA}}$, including sub-types, was the dominant one, accounting for six isolates (14.6%).

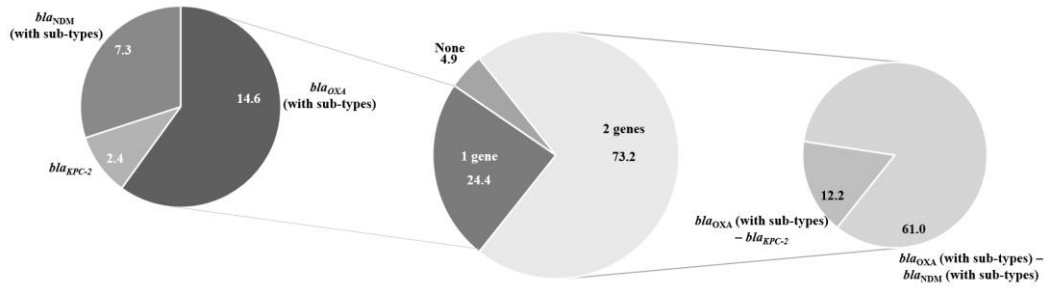

Figure 6. The proportion of carbapenemase- encoding genes (*bla*$_{\text{OXA-48}}$, *bla*$_{\text{KPC}}$, and *bla*$_{\text{NDM}}$) was detected using WGS with the ResFinder database. The most dominant expression found was harboring two types of gene (73.2%), *bla*$_{\text{OXA-48}}$ — *bla*$_{\text{NDM}}$ and *bla*$_{\text{OXA-48}}$ — *bla*$_{\text{KPC}}$. Samples harboring one gene accounted for 24.4% of the total (OXA, 14.6%; NDM, 7.3%; KPC, 2.4%). Samples with no gene detected accounted for 4.9% in total.

3.3. Comparison between Duplex PCR and WGS-based Method in Detecting Carbapenem Resistance Genes

This study employed two distinct molecular-based techniques, duplex PCR and WGS, to examine the prevalence of carbapenem resistance genes in *Klebsiella pneumoniae*. Of the 41 *K. pneumoniae* isolates, 36 (87.8%) were found to contain carbapenemase genes by using duplex PCR, and this percentage increased to 97.6% (40/41) with WGS analysis using the Resfinder database. Specifically, *bla*$_{\text{OXA,}}$ including its subtypes, *bla*$_{\text{KPC}}$, and *bla*$_{\text{NDM,}}$ including its subtypes, were detected by using both methods, with a difference of about one gene between the two methods. None of the isolates were confirmed to have the *bla*$_{\text{VIM}}$ gene.

Notably, the PCR method detected only the OXA-48 gene, while with the WGS-based method, OXA-48-like variants, such as the OXA-181 gene, were detected but not the OXA-48 gene. Due to the OXA-48 gene frequently located on a plasmid between two identical insertion sequences [23, 24], the difference in finding the *bla*$_{\text{OXA}}$ between the two methods could be explained by the fact that the PCR method was more specific for the OXA-48 gene than its variants. Without technical replication, this point accounted for one of the drawbacks of this study in screening resistance genes with the WGS-based method, which made the data incomplete and hard to fully compare with the PCR method. Thus, one suggested optimal approach is hybrid sequencing, combining different sequencing methods, which can explain our finding of *bla*$_{\text{OXA-181}}$.

Another point is that each variant differs from OXA-48 by one to five amino acid substitutions or deletions. Based on their hydrolytic profiles, OXA-48 hydrolyzed penicillin at a high level but was low in carbapenem and exhibited poor efficacy against expanded-spectrum cephalosporins [12]. On the other hand, OXA-181 demonstrated better capacity to hydrolyze carbapenems, whereas OXA-232 displayed reduced hydrolytic ability to carbapenems when compared to OXA-48 and OXA-181 [25]. This revealed that distinct OXA-48-like variations exhibit varying

antibiotic susceptibilities, which might influence treatment options. Rapid and precise diagnosis of OXA-48-like carbapenemases is critical for controlling their spread. The current approach for differential identification of OXA-48-like variants carried by carbapenem-resistant *Enterobacteriaceae* is PCR, followed by direct sequencing to determine the location of the mutation. Because of the high degree of similarity amongst OXA-48-like variants, DNA sequencing is presently the most accurate approach for identifying OXA-48 variations. However, the method still has a main disadvantage: Despite access to the complete genome sequence, the evidence remains inadequate to classify or interpret the identified variants. This is primarily due to the additional variants detected through WGS, many located in non-coding regions with insufficient evidence for proper classification. The interpretation of these non-coding variants often depends solely on in silico predictions, creating significant challenges and confusion in identifying pathogenic variants.

On the other hand, the PCR method pointed out an interesting result: two isolates carried all three carbapenemase types (OXA, KPC, and NDM), while none of the cases were reported with the WGS-based method. The co-occurrence of multiple antimicrobial resistance determinants within individual bacterial strains has emerged as a frequent phenomenon in clinical isolates [26-28]. Intriguingly, a comprehensive genomic analysis investigating carbapenem-resistant *K. pneumoniae* (CRKP) strains revealed an unexpected resistance profile: among 100 clinically isolated CRKP strains, neither KPC nor VIM carbapenemase genes were detected [29]. Instead, these isolates predominantly carried OXA-48 and NDM-type carbapenemases, while simultaneously demonstrating elevated rates of colistin resistance, a concerning observation given colistin's role as a last-resort antibiotic [29]. On the other hand, the coexistence of NDM with other carbapenemases is commonly documented globally for *K. pneumoniae* [28, 30, 31]. Some *K. pneumoniae* isolates were reported to harbor four carbapenemases (NDM, KPC, OXA-48, and VIM type) [32]. However, the frequency of co-producing among these three carbapenemase types (OXA, KPC, and NDM) has never been reported in Vietnam.

In PCR and WGS-based methods, most isolates possessed the co-occurrence of two carbapenemase types. However, the two methods found no combination between the KPCs and NDMs. It aligned with previous studies showing that although the dual $bla_{\text{KPC-2}}$–$bla_{\text{NDM-1}}$ genotype has been seen previously in Hanoi [18, 33], including in one of the 2015 outbreak isolates, until now, it has been rare. Among the duals reported in all methods, the *K. pneumoniae* isolates co-producing NDMs and OXAs appeared the most. The data aligned with previous research claimed that CRKP strains containing bla_{NDM} and $bla_{\text{OXA-48-like}}$ have already demonstrated a worldwide proliferation trend [34]. Significantly, the co-occurrence of NDM-4/OXA-181 accounted for 36.58% (15/41) of the isolates. The co-existence of dual carbapenemases between NDMs and OXAs in carbapenemase-producing *Enterobacterales* has been reported, for example, NDM-1 and OXA-232 in *K. pneumoniae*, and NDM-5 and OXA-181 in *E. coli* [35, 36]. However, the $bla_{\text{NDM-4}}$ and $bla_{\text{OXA-181}}$ coproducing CRKP strains are seldom identified in Vietnam, and their spread remains unknown.

Multiple carbapenemase producers pose a higher threat to public health as they are more challenging to regulate and may operate as a reservoir of carbapenemase-encoding genes for other bacterial pathogens. A key contributing factor is that bacteria producing multiple carbapenemases demonstrate enhanced antimicrobial resistance profiles, thereby gaining a selective advantage against therapeutic interventions. Clinically, such strains have been associated with nosocomial transmission events and have precipitated several hospital outbreaks [37]. In the past twenty years, OXA-48 has emerged as one of the dominant carbapenemases across Europe [38, 39], Northern Africa [21], the Mediterranean region [40, 41], and the Middle

East [20, 42]. However, the clinical detection of OXA-48-like enzymes presents unique challenges – their frequently low rate of carbapenem resistance may lead to underestimation of their true prevalence through conventional phenotypic methods. The occurrence of dual carbapenemase-producing strains, particularly those combining OXA-48 with other carbapenemases, might be similarly underreported in current surveillance data. Thus, further studies into the processes driving the co-occurrence of numerous carbapenemase-encoding genes are required to avoid their global spread.

In general, while phenotypic methods provided high diagnostic accuracy, they share the significant limitation of requiring bacterial isolation and overnight incubation, which increases turnaround time. The time-saving advantage of duplex PCR in this study is currently offset by its dependence on cultured isolates, which may delay critical treatment decisions. Implementing direct PCR testing of clinical samples could overcome this bottleneck, enabling faster detection of resistance genes without compromising accuracy. On the other hand, Nanopore sequencing represents a transformative technology that could democratize nucleic acid sequencing, making it widely accessible across research and clinical settings. However, its widespread adoption faces a significant barrier: the current requirement for advanced bioinformatics expertise in data interpretation. For example, a key challenge in Nanopore sequencing is that the raw current signal represents multiple overlapping bases, which base-callers must probabilistically decode. Variability in nucleic acid translocation speed complicates single-base resolution, while repetitive sequences and long homopolymers exceed the discriminatory capacity of the nanopore sensing region [43]. However, the ongoing development of user-friendly analytical pipelines, coupled with increasing computational literacy among biologists, promises to overcome these limitations and unlock the full potential of this disruptive sequencing technology. While these assays have recognized limitations, they continue to play an essential role in clinical microbiology owing to their distinct benefits and established performance in particular diagnostic scenarios.

4. Conclusion

The results highlighted the strengths and weaknesses of each molecular method. PCR techniques are robust, reliable, and user-friendly, with duplex PCR being particularly efficient in simultaneously detecting multiple genes or alleles. However, PCR has limitations, such as the potential for DNA polymerase errors leading to mutations in results and susceptibility to contamination. With careful handling and specialized materials, PCR can yield accurate outcomes compared to high-throughput genome sequencing. Nevertheless, PCR's limitation in detecting only targeted genes hinders its ability to identify all carbapenemase-encoding gene variations. PCR is suitable for situations requiring quick results without detailed gene variant information. On the other hand, while WGS is labor-intensive, time-consuming, and costly due to advanced materials, it has emerged as a powerful tool for evaluating transmission patterns and potential outbreaks. Unlike PCR, WGS is ideal for cases necessitating precise identification of resistant gene types and where time is not a critical factor.

Our findings indicated that using WGS in conjunction with Nanopore technology is considered one of the most effective methods for monitoring antibiotic-resistant bacteria in Vietnamese hospital settings.

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Author's Contributions

Giang Thi-Chau Phan, Mai Thi-Anh Pham and Anh Phuong Nguyen carried out the experiments. Giang Thi-Chau Phan obtained, analyzed, and interpreted the data. Giang Thi-Chau Phan, My Nhat Truong, and Hoai Thi-Thu Nguyen designed, wrote and revised the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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