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Research Article

Detection of Carbapenemase Genes in Carbapenemase-producing *Klebsiella pneumoniae* Isolated from Tay Nguyen Regional General Hospital, Vietnam

H Nuong Nie[1], Le Ka Thuy[1], Nguyen Thi Kim Loan[1], Nguyen Hong Tuoi[2]

[1]Tay Nguyen Regional General Hospital, Dak Lak, Vietnam
[2]Department of Anesthesia and Critical Care, Hanoi Medical University, Hanoi, Vietnam

Abstract

Background: Carbapenemase-producing *Klebsiella pneumoniae* (CPKP) has emerged as a major global concern due to extensive antimicrobial resistance, limited therapeutic options, and significant mortality rates. This study aimed to characterize the molecular mechanisms of carbapenem resistance and assess the antibiotic susceptibility patterns of CPKP isolates collected from Tay Nguyen Regional General Hospital, Vietnam. Methods: Seventy-nine non-duplicate K. pneumoniae isolates resistant to at least one carbapenem were analyzed using the modified carbapenem inactivation method (mCIM) and real-time PCR to detect blaKPC, blaNDM, blaVIM and blaOXA-48-like genes. Antimicrobial susceptibility was tested using the disk diffusion method following CLSI 2023 guidelines. Results: Among the 79 isolates, 62 (78.5%) were carbapenemase producers. The most prevalent carbapenemase gene was blaNDM (65.6%), followed by blaOXA-48-like (48.4%), blaKPC (35.9%) and blaVIM (32.8%). Co-harboring of multiple genes was common blaNDM+OXA-48-like (29.7%), blaKPC+NDM (21.9%). Resistance rates exceeded 90% for β-lactams, cephalosporins and quinolones, while all isolates remained susceptible to colistin. Conclusions: The predominance of blaNDM and blaOXA-48-like genes highlights the evolving resistance landscape in Vietnam. Strengthening molecular surveillance, infection control and rational antibiotic use is essential to prevent the spread of multidrug-resistant pathogens.

Keywords

Carbapenemase, Antibiotic Resistance, *Klebsiella pneumoniae*, Resistance Genes

1. Introduction

The escalating crisis of antimicrobial resistance (AMR) is one of the most formidable challenges facing modern medicine. Among the most concerning pathogens are the Carbapenem-Resistant Enterobacteriaceae (CRE), particularly *Klebsiella pneumoniae* [1]. *K. pneumoniae* is an ubiquitous Gram-negative bacterium responsible for a wide spectrum of severe infections, including pneumonia, urinary tract infections, bloodstream infections and complicated intra-abdominal infections primarily in immunocompromised and critically ill patients [2].

Carbapenems (meropenem, imipenem, doripenem and ertapenem) have long been considered the cornerstones for

*Corresponding author: nuongyk04@gmail.com (H Nuong Nie)

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treating infections caused by multidrug-resistant (MDR) Gram-negative bacteria. However, the efficacy of these agents has been severely compromised by the global dissemination of carbapenemase enzymes. These enzymes are potent beta-lactamases capable of hydrolyzing the beta-lactam ring of carbapenems, rendering them inactive [3]. The genes encoding these enzymes are frequently located on mobile genetic elements such as plasmids which facilitates their rapid horizontal transfer across different bacterial species leading to regional and global outbreaks [4]. Carbapenemases which belong to Ambler classes A, B and D include *Klebsiella pneumoniae* carbapenemase (KPC), metallo-β-lactamases such as New Delhi metallo-β-lactamase (NDM) and Verona integron-encoded metallo-β-lactamase (VIM) and oxacillin-hydrolyzing β-lactamases (OXA-48-like). These enzymes are often plasmid-encoded, allowing horizontal gene transfer across bacterial species, accelerating the global spread of resistance [5-7].

In Southeast Asia, including Vietnam, the prevalence and types of carbapenemase genes are highly dynamic and often differ from Western patterns. Studies have indicated a rising trend of CRE, with bla_{NDM} and bla_{OXA-48} emerging as the most frequent genotypes in many parts of the region, potentially displacing other older mechanisms [8, 9]. However, regional data particularly from mountainous areas like Tay Nguyen Regional General Hospital remains sparse. Understanding the specific molecular epidemiology is paramount, as the predominant gene type dictates the residual treatment options (e.g., KPC-producers might be susceptible to ceftazidime/avibactam while MBL-producers require alternative agents like colistin or fosfomycin) [10].

Tay Nguyen Regional General Hospital serves as a major tertiary referral center for a large and diverse population in the Central Highlands of Vietnam. Given the high patient turnover and complexity of cases, the institution is at high risk for the transmission and establishment of endemic MDR pathogens. There is an urgent requirement to characterize the local resistance landscape to inform evidence-based clinical and public health interventions. Despite advances in diagnostic technologies, regional molecular data remain limited, especially in Central Vietnam. This study aimed to identify carbapenemase genes and evaluate the antimicrobial susceptibility patterns of CRKP isolates from Tay Nguyen Regional General Hospital to provide essential baseline data for infection control and antibiotic stewardship.

2. Materials and Methods

2.1. Bacterial Isolates and Identification

A total of 79 non-duplicate carbapenem-resistant *K. pneumoniae* isolates were collected between January and October 2023. Samples included blood, sputum, tracheal aspirates, urine, peritoneal fluid and wound swabs. Isolates were identified using standard microbiological techniques including Gram staining, colony morphology and biochemical tests (API20E, BioMérieux, France). Only the first isolate per patient was included to ensure non-duplication.

2.2. Phenotypic Detection of Carbapenemase

Isolates exhibiting reduced susceptibility (intermediate or resistant) to any carbapenem were subjected to phenotypic confirmation of carbapenemase production using the modified Carbapenem Inactivation Method (mCIM) following CLSI 2023 guidelines [11, 12].

For each test isolate, a full 1-µL loopful of bacteria was collected from an overnight blood agar culture and inoculated into 2 mL of tryptic soy broth (TSB). The suspension was vortexed for 10–15 seconds. A 10 µL meropenem disk was fully immersed in the prepared bacterial suspension and incubated at 35 ±2 ℃ for approximately 4 hours.

Preparation of the bacterial suspension: A suspension of *Escherichia coli* ATCC 25922 (control strain) was prepared in physiological saline and adjusted to a turbidity of 0.5 McFarland.

The control strain suspension was inoculated onto Mueller–Hinton agar (MHA) using the standard disk diffusion technique. A sterile cotton swab was dipped into the suspension, then gently pressed and rotated against the inner wall of the tube to remove excess fluid. The swab was streaked evenly across the entire surface of the MHA plate. The plate was then covered and allowed to stand at room temperature for several minutes to ensure drying of the agar surface.

Following the 4-hour incubation, the meropenem disk was placed onto the inoculated MHA plate. The plate was incubated aerobically at 35 ±2 ℃ for 18–24 hours.

Interpretation of results: After incubation, the inhibition zone diameter surrounding the meropenem disk was measured:

1) Carbapenemase-positive: inhibition zone diameter <16 mm
2) Carbapenemase-negative: inhibition zone diameter >19 mm.
3) Indeterminate: inhibition zone diameter 16–18 mm or >19 mm with scattered colonies inside the inhibition zone. For isolates yielding indeterminate results, findings were documented for correlation with PCR testing.

2.3. DNA Extraction and Realtime PCR

Bacterial DNA was extracted via the boiling method and real-time PCR targeting bla_{KPC}, bla_{NDM}, bla_{VIM} and $bla_{OXA-48-like}$ genes was performed using validated primers and probe. Specific primer and probe sets for each gene were utilized. The primer and probe sequences used are listed in Table 1. Each carbapenemase-encoding genotype is shown with its expected amplicon size (bp), the forward, reverse primer and probe sequences [13].

Table 1. *Primer and Probe Sequences Used for Amplification of Carbapenemase-Encoding Genes.*

Carbapenemase genotype	Primer / Probe name	Sequence (5′–3′)	Amplicon size (bp)
bla_{KPC}	bla_{KPC}-F	GATACCACGTTCCGTCTGGA	180
	bla_{KPC}-R	GGTCGTGTTTCCCTTTAGCC	
	bla_{KPC} Probe	FAM-CGCGCGCCGTGACGGAAAGC-TAMRA	
bla_{NDM}	bla_{NDM}-F	GCGCAACACAGCCTGACTTT	155
	bla_{NDM}-R	CAGCCACCAAAAGCGATGTC	
	bla_{NDM} Probe	FAM-CAACCGCGCCCAACTTTGGC-TAMRA	
bla_{VIM}	bla_{VIM}-F	CACAGYGGCMCTTCTCGCGGAGA	132
	bla_{VIM}-R	GCGTACGTYGCCACYCCAGCC	
	bla_{VIM} Probe	FAM-AGTCTCCACGCACTTTCATGACGACCGCGTCGGCG-TAMRA	
$bla_{OXA-48-like}$	$bla_{OXA-48-like}$-F	TCTTAAACGGGCGAACCAAG	125
	$bla_{OXA-48-like}$-R	GCGTCTGTCCATCCCACTTA	
	$bla_{OXA-48-like}$ Probe	FAM-AGCTTGATCGCCCTCGATTTGG-TAMRA	

2.4. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed via disk diffusion and results were interpreted according to CLSI M100 (2023) standards.

2.5. Data Analysis

Data were analyzed descriptively using SPSS version 20.0.

3. Results

3.1. Characteristics of Clinical Isolates

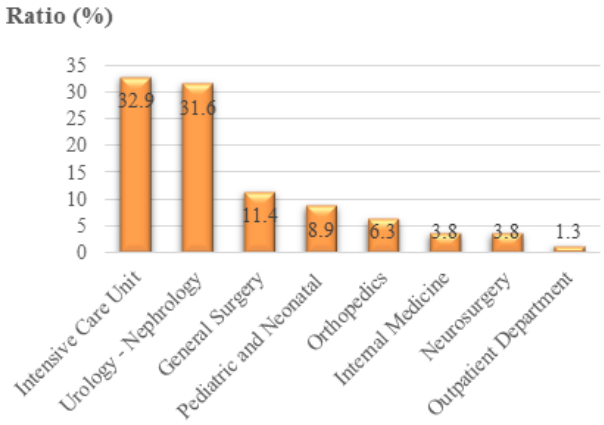

Figure 1. *Proportion of Klebsiella Pneumoniae Isolates by Clinical Department.*

A total of 79 carbapenem-resistant *Klebsiella pneumoniae* isolates were collected from various clinical departments. The highest proportions originated from the Intensive Care Unit (32.9%), the Urology–Nephrology Department (31.6%), followed by the General Surgery Department (11.4%) and the Pediatric Intensive Care Unit (8.9%) (Figure 1).

Regarding specimen type, isolates were most commonly obtained from sputum (38.0%), urine (34.1%), pus (25.3%), catheter tips and throat swabs accounted for only 1.3% each.

3.2. Distribution of Carbapenemase Production and Carbapenemase Genes

Carbapenemase activity was evaluated using the modified carbapenem inactivation method (mCIM). Among the 79 isolates, 62 (78.5%) demonstrated positive carbapenemase activity, whereas 15 isolates (19.0%) tested negative. Two isolates (2.5%) yielded inconclusive mCIM results.

Real-time PCR was performed on all 62 carbapenemase-producing isolates as well as the two indeterminate isolates, yielding 64 isolates positive for at least one carbapenemase gene. The most prevalent gene was bla_{NDM} (65.6%), followed by $bla_{OXA-48-like}$ (48.4%), bla_{KPC} (35.9%) and bla_{VIM} (32.8%). Co-existence of multiple genes was frequently observed. The most common gene combinations included $bla_{NDM+OXA-48-like}$ (29.7%), $bla_{KPC+NDM}$ (21.9%) and $bla_{NDM+VIM}$ (18.8%). Less frequent combinations included $bla_{KPC\ +\ OXA-48-like}$ and $bla_{VIM+OXA-48-like}$ (each 10.9%), $bla_{KPC+VIM}$ (7.8%). Three-gene combinations were also detected: $bla_{KPC+NDM+OXA-48-like}$ (3.1%) and $bla_{NDM+OXA-48-like\ +VIM}$ (1.6%). Among the two isolates with inconclusive mCIM

results, one harbored bla_{NDM} and the other bla_{KPC}.

Table 2. Distribution of Carbapenemase-encoding Genes in Klebsiella Pneumoniae (n = 79).

Genotype	No. of isolates	Percentage (%)
bla_{KPC}	23	35,9
bla_{NDM}	42	65,6
$bla_{OXA-48-like}$	31	48,4
bla_{VIM}	21	32,8
$bla_{NDM+OXA-48-like}$	19	29,7
$bla_{KPC+NDM}$	14	21,9
$bla_{NDM+VIM}$	12	18,8
$bla_{VIM+OXA-48-like}$	7	10,9
$bla_{KPC+OXA-48-like}$	7	10,9
$bla_{KPC+VIM}$	5	7,8
$bla_{KPC+NDM+OXA-48-like}$	2	3,1
$bla_{NDM+OXA-48-like-VIM}$	1	1,6

3.3. Antibiotic Susceptibility

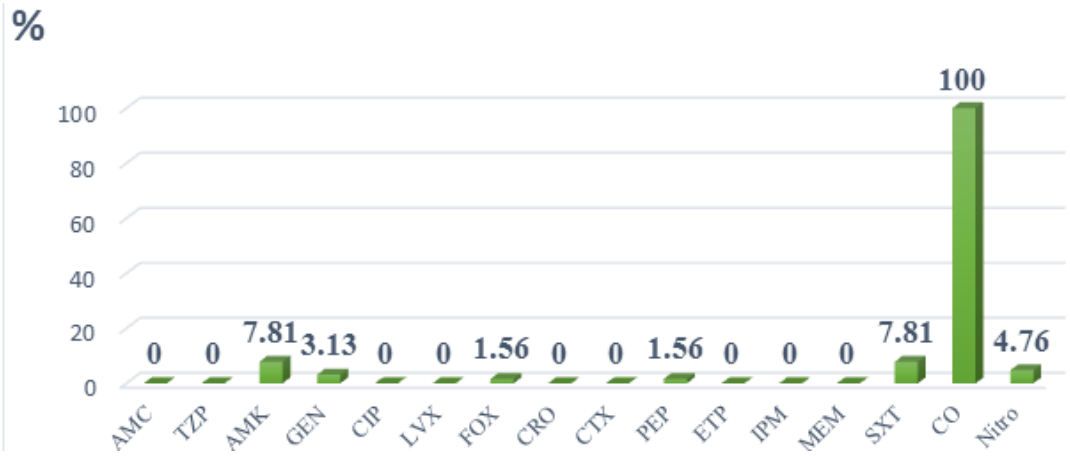

Figure 2. Susceptibility of Carbapenemase-producing Klebsiella Pneumoniae Isolates to Selected Antibiotics.

Carbapenemase-producing *Klebsiella pneumoniae* isolates exhibited resistance to most antibiotics including trimethoprim-sulfamethoxazole, cephalosporins (cefepime, cefotaxime, ceftriaxone, ceftazidime and cefoxitin), quinolones (ciprofloxacin and levofloxacin), carbapenems (ertapenem, imipenem and meropenem) and β-lactam/β-lactamase inhibitor combinations such as piperacillin-tazobactam and amoxicillin-clavulanic acid, with resistance rates 100%. Isolates recovered from urine specimens retained susceptibility to nitrofurantoin in only 4.76% of cases. All isolates remained fully susceptible (100%) to colistin.

4. Discussion

This study provides an updated molecular and antimicrobial resistance profile of carbapenemase-producing *Klebsiella pneumoniae* (CPKP) isolated from Tay Nguyen Regional General Hospital, a major referral center in the Central Highlands of Vietnam. The findings highlight the high prevalence of carbapenemase production, the dominance of bla_{NDM} and $bla_{OXA-48-like}$ genes and the alarming multidrug-resistant (MDR) phenotype characteristic of current

global trends. These results emphasize the need for strengthened antimicrobial stewardship, infection control and molecular surveillance within the region.

4.1. Carbapenemase Genes and Molecular Epidemiology

The global distribution of carbapenemase genes in *Klebsiella pneumoniae* has evolved substantially over the past decade, reflecting complex patterns of clonal expansion, plasmid transfer and antimicrobial selection pressure. Worldwide, bla_{KPC} dominates in the Americas and parts of Europe; bla_{NDM} is the leading genotype across South and Southeast Asia; $bla_{OXA\text{-}48\text{-}like}$ predominates in North Africa, the Middle East and southern Europe; while bla_{VIM} and bla_{IMP} remain more common in Mediterranean countries and East Asia, respectively [14–17]. These global trends illustrate the regional specialization of carbapenemase families shaped by population mobility, healthcare infrastructure and antibiotic consumption patterns.

In Vietnam, available surveillance data show that bla_{NDM} and $bla_{OXA\text{-}48\text{-}like}$ are now the most prevalent carbapenemase determinants among *Enterobacterales*, particularly in tertiary-care hospitals with high rates of antimicrobial usage [18–20]. Earlier national reports indicated sporadic detection of bla_{KPC}. However, recent studies from Hanoi and Ho Chi Minh City have documented an increasing presence of KPC-producing strains, often linked with high-risk clones such as ST11 and ST258 [21]. Our findings from Tay Nguyen Regional General Hospital align with these evolving epidemiological features. The predominance of bla_{NDM} (65.6%) and $bla_{OXA\text{-}48\text{-}like}$ (48.4%), followed by considerable frequencies of bla_{KPC} (35.9%) and bla_{VIM} (32.8%), underscores the simultaneous circulation of multiple carbapenemase families within a single institution. This distribution is consistent with emerging evidence that Vietnam is entering a phase of "carbapenemase convergence" in which multiple resistance mechanisms co-exist due to complex plasmid recombination and clonal persistence [19, 20]. In Southern Vietnam, the $bla_{OXA\text{-}48\text{–}like}$ family represented the most prevalent carbapenemase gene cluster (25%), with $\boldsymbol{bla_{OXA\text{-}181}}$ emerging as the leading variant. Importantly, CRE isolates co-harboring bla_{NDM} and $bla_{OXA\text{-}48\text{–}like}$ genes constituted the most common genotype (30%) [22].

Compared with regional studies, the prevalence of bla_{NDM} in our study (65.6%) is similar to rates reported in Northern Vietnam (60–70%), but higher than figures observed in certain Southeast Asian countries such as Thailand and Malaysia, where OXA-48-like enzymes tend to dominate [23, 24]. The detection of $bla_{OXA\text{-}48\text{-}like}$ in nearly half of isolates is particularly concerning as OXA-48-like enzymes often exhibit weak carbapenem hydrolysis and can escape routine laboratory detection, contributing to silent nosocomial spread. Meanwhile, the distribution of bla_{KPC} in our study (35.9%) is notably higher than earlier Vietnamese reports (<10%), suggesting recent introduction and establishment of KPC-carrying clones in the Central Highlands. This shift may reflect increased inter-hospital referrals, patient transfers and antimicrobial pressure within intensive care units.

An important finding of our study is the high proportion of isolates co-harboring multiple carbapenemase genes, particularly $bla_{NDM+OXA\text{-}48\text{-}like}$ (29.7%) and $bla_{KPC+NDM}$ (21.9%). Multi-genotype detection has been increasingly reported worldwide, particularly in India, Turkey and China, where extensive plasmid recombination facilitates the emergence of high-risk extensively drug-resistant (XDR) clones [25–27]. The presence of dual and even triple carbapenemase profiles (e.g., $bla_{KPC+NDM+OXA\text{-}48\text{-}like}$) in our cohort signals a similar evolutionary trajectory and highlights the role of plasmid-mediated horizontal gene transfer within the hospital environment. These multi-gene configurations are typically associated with increased resistance, enhanced bacterial fitness, and reduced treatment options.

4.2. Antimicrobial Resistance Profiles

The antimicrobial resistance patterns observed in this study reflect the combined effect of carbapenemase production, ESBL activity, porin mutations and efflux pump overexpression. Resistance rates exceeded 90% for nearly all β-lactams, cephalosporins and quinolones consistent with international reports of carbapenemase-producing *K. pneumoniae* (CPKP) as among the most refractory bacterial pathogens encountered in clinical practice [28]. The complete susceptibility to colistin aligns with Vietnam's current resistance landscape, where plasmid-mediated mcr genes remain relatively uncommon (29). However, reliance on colistin is not sustainable given its nephrotoxicity and global trends of emerging resistance. Moderate activity of amikacin and fosfomycin suggests potential utility in combination therapy, although rapid resistance development remains a concern.

Our findings have important clinical and public health implications. Firstly, the simultaneous circulation of NDM, OXA-48-like, KPC and VIM enzyme reflects a highly complex resistance ecosystem requiring expanded molecular diagnostics—including realtime PCR panels or whole-genome sequencing—to guide targeted infection control measures. Secondly, the shift toward multi-gene carbapenemase carriage underscores the urgency of reinforcing antimicrobial stewardship programs to curb selective pressures that drive resistance evolution. Finally, comparative epidemiological similarities with other regions of Vietnam suggest that carbapenemase spread is no longer localized but part of a broader national and regional dissemination pattern. Preserved susceptibility to colistin (100%) represents an important therapeutic window. However, global reports of plasmid-mediated mcr-gene transmission raise concern about the potential emergence of colistin resistance in Vietnam [29]. Continued monitoring is therefore essential. Nitrofurantoin susceptibility remained low (4.76%) and was confined to

isolates recovered from urine, reflecting the drug's pharmacokinetic limitations. Similar patterns have been documented across Asia and globally [30].

5. Conclusions

This study demonstrates that *bla*NDM and *bla*OXA-48-like are the dominant carbapenemase genes among *Klebsiella pneumoniae* isolates in Tay Nguyen Regional General Hospital, Vietnam, with frequent co-harboring of multiple carbapenemase determinants. These findings mirror global resistance trends, emphasizing the growing complexity of antimicrobial resistance mechanisms. The presence of hybrid genotypes such as $bla_{\text{KPC+NDM+OXA-48-like}}$ represents a serious therapeutic and epidemiological threat.

The data underscore the need for: (1) expanded molecular surveillance using multiplex PCR or realtime PCR and whole-genome sequencing, (2) routine screening of high-risk patients for carbapenemase-producing organisms and (3) antimicrobial stewardship to limit unnecessary carbapenem use. Future research should investigate the clonal distribution of high-risk *K. pneumoniae* lineages (ST147, ST307, ST11) and evaluate novel therapeutic options including β-lactamase inhibitors and phage therapy.

The emergence of multi-carbapenemase producers represents an urgent call for coordinated action among clinicians, microbiologists and policymakers to preserve the efficacy of existing antibiotics and prevent the spread of resistance within healthcare facilities and the community.

Abbreviations

CLSI	Clinical and Laboratory Standards Institute
CPE	Carbapenemase-producing Enterobacteriaceae
CRKP	Carbapenem-resistant *Klebsiella Pneumoniae*
DNA	Deoxyribonucleic Acid
IMP	Imipenemase
KPC	*Klebsiella Pneumoniae* Carbapenemase
NDM	New Delhi Metallo-β-lactamase
OXA	Oxacillin-hydrolyzing β-lactamase
PCR	Polymerase Chain Reaction
VIM	Verona Integron–encoded Metallo-β-lactamase
WGS	Whole-genome Sequencing

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This research was conducted at Tay Nguyen Regional General Hospital.

Author Contributions

H Nuong Nie: Conceptualization, Supervision, Writing – original draft.

Le Ka Thuy: Formal analysis.

Nguyen Thi Kim Loan: Methodology, Data curation.

Nguyen Hong Tuoi: Conceptualization, Writing – original draft.

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Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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Biography

H Nuong Nie is a medical doctor and Head of the Department of Laboratory Medicine at Tay Nguyen Regional General Hospital, Vietnam. She graduated from Tay Nguyen University of Medicine and Pharmacy in 2010 and obtained her Master's degree in Clinical Laboratory Medicine from Ho Chi Minh Medical University in 2016. With more than 10 years of experience in clinical laboratory diagnostics and hospital laboratory management, she has played a key role in implementing laboratory quality systems and antimicrobial resistance surveillance in the Central Highlands region of Vietnam. Dr. Nie has participated in several national research projects on infectious diseases and antibiotic resistance and has contributed to the training of laboratory staff in quality control and biosafety.

Research Field

H Nuong Nie: Antimicrobial resistance, Laboratory quality management, Clinical microbiology.