Open Access

Journal of Clinical Immunology and Microbiology

Research Article

Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam

Van Thi Thu Ha[1,2], Hieu Duc Nguyen[1], Minh Ngoc Nghiem[1], Son Thai Nguyen[2], Tram Bao Nguyen[1], Duc Minh Nguyen[1], Cuong Xuan Hoang[3], Thuy Thi Bich Vo[1*]

[1]Institute of Genome Research, Vietnam Academy of Science and Technology, Hanoi, Vietnam

[2]Military Hospital 103, Vietnam Military Medical University, Hanoi, Vietnam

[3]Vietnam Military Medical University, Hanoi, Vietnam

[*]**Corresponding Author:** Thuy Thi Bich Vo, Institute of Genome Research, Vietnam Academy of Science and Technology, Hanoi, Vietnam; Email: thuytbvo@igr.ac.vn

Received Date: 05-02-2022; **Accepted Date:** 21-02-2022; **Published Date:** 28-02-2022

Abstract

Background: The over using of antibiotics in hospitals, communities and agriculture has raised drug resistance in Vietnam.

Objective: The study was to identify the multi antimicrobial resistance gene expression associated with extensively drug resistance phenotype in *Klebsiella pneumonia* (*K.pneumoniae*) isolated from clinical samples in the Military Hospital 103 in Vietnam.

Material and Method: Seventeen extensively drug resistant (XDR) strains were identified and measured antibiotic susceptibility by using Vitek 2 Compact System. Genes related to the antimicrobial resistant were screened using in-house multiplex PCR at Vietnam Institute of Genome Research.

Results: *K.pneumoniae* showed resistance to almost fifteen antibiotics with very high rates of over 70%, only sensitive to colistin 100%. There is a correlation between carpabenem resistance and XDR status in the *K.pnemoniae* group. Most of the *K.pneumoniae* isolates harbored the widely distributed ESBL gene groups. All the isolates carried more than 3

Ha VTT | Volume 3; Issue 1 (2022) | JCIM-3(1)-041 | Research Article

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101

antibiotic resistance genes, 10 of which even carried 7 to 10 of the total 13 tested antibiotic resistance genes, equivalent to more than 13 antibiotic resistant phenotypes of all types.

Conclusion: Our study adds the significant of antibiotic resistance gene expressions that are showing the genotype and phenotype relations of XDR *K.pneumoniae* isolates. Future research should focus on the impact of these gene sources on the multidrug resistant phenotype of the patients and the genotypes can be used to predict the antibiotic sensitive phenotype of XDR *K.pneumoniae* isolates in hospital.

Keywords

Klebsiella pneumonia; Antibiotic Resistance Genes; Genotypic; Phenotype Resistances

Abbreviations

K.pneumoniae: *Klebsiella pneumonia*; XDR: Extensively Drug Resistant; MDR: Multiple Drug Resistance; PDR: Pan Drug Resistant; ESBL: Extended- Spectrum Beta-Lactamases; KPC: K.Pneumonia Pneumonia Carbapenemase; NDM: New Delhi Metallo-B-Lactamase; OXA: Oxacillinase; BMD: Broth Microdilution; MIC: Minimum Inhibitory Concentration; CLSI: Clinical And Laboratory Standard Institute; ICU: Intensive Care Unit; RT-PCR: Reverse Transcription Polymerase Chain Reaction; cDNA: Complementary Deoxyribonucleic Acid

Introduction

In the near decades, the increasing clinical incidence of antibiotic resistant bacteria is a serious issue for global health care [1]. The rapid dissemination of Multiple Drug Resistance (MDR) and Extensively Drug-Resistant (XDR) or Pan Drug Resistant (PDR) *K.pneumoniae* has recently recognized as an urgent public health threat, thus requiring immediate and aggressive actions [2]. Some clinical *K.pneumoniae* isolates can acquire of antibiotic resistance genes and intrinsic resistance of bacteria that has consequently limited treatment options for infections [3]. Currently, *K.pneumoniae* strains producing Extended-Spectrum Beta-Lactamases (ESBLs) and carbapenemases have spread globally [4]. The frequent outbreak of nosocomial infections due to these strains that is attributed to multiple drug resistance mechanisms [5]. The mechanisms involved when β-lactamase hydrolyzes β-lactam antibiotics or inactivates β-lactams have been investigated [6]. However, no precise molecular biological mechanisms underlying the antimicrobial drug resistance of *K.pneumoniae* have been reported [7]. In the previous several studies indicated that antibiotic resistance genes originate in microorganisms and subsequently integrate into the genome of other pathogens through transduction and/or

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101

transformation to produce a multitude of different antibiotic resistance strains that are especially dangerous for the course of treatment for patients [8-10]. Antimicrobial resistance genes in *K.pneumoniae* are also associated with high rates of morbidity and mortality in clinical patients [11]. Therefore, understanding the mechanisms involved in the antibiotic resistance of *K.pneumoniae* are highly required to warrant the development of novel antibiotics or alternative treatment strategies to decrease mortality of patients infected with *K.pneumoniae*.

Globally, β-lactam antibiotics are the most widely used in the treatment of many infections. However, the formation of the ESBLs group has made the problem of antibiotic resistance in bacteria very worrisome [12]. There are three main types of ESBL such as TEM, SHV and CTX-M, which are considered to be the most common and influential. These types of ESBLs are constantly evolving; increasing in number and more complex in characteristic. Currently, more than 600 different ESBL enzyme variants have been discovered, corresponding to more than 600 different ESBL-encoding genotypes [12,13]. Carbapenem is the widest antimicrobial spectrum and stable activity to against bacteria that produce AmpC β-lactamase and ESBL, therefore, this antibiotic is considered last treatment options in the treatment of infections caused by multiple antibiotics resistant gram-negative bacteria [14]. However, the rapid dissemination of carbapenemase genes, namely, *K.pneumonia* Carbapenemase (KPC), New Delhi Metallo-b-lactamase (NDM) and Oxacillinase (OXA) type carbapenemases is the major contributing factor to the rapid global spread of carbapenem resistance [15]. Therefore, it is necessary to have measures to manage the rational use of antibiotics in both hospitals and communities to limit antibiotic resistance, especially multidrug resistance, which is increasing in the community.

Methodology

Sample Collection

The seventeen XDR *K.pneumoniae* isolates were randomly selected from over 1000 total clinical isolates between 2015 and 2017 in the Military Hospital 103. The identification of *K.pneumoniae* isolate was done by analyzing colony morphology in special culture media, microscopic examination, performing biochemical testing, and Vitek 2 system (bioMérieux, Marcy l'Étoile, France).

Resistant Antibiotic Phenotype Identification

Antimicrobial susceptibilities were performed for samples by the Vitek 2 system and the AST-GN card, E-test and Broth Microdilution (BMD) methods (bioMérieux, Marcy l'Étoile, France). Colistin susceptibility assay was performed according to recommendations of joint

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101

CLSI-EUCAST guidelines [16]. The Minimum Inhibitory Concentration (MIC) values of the antibiotics carbapenem was measured for all isolates using E-test (bioM'erieux) according to the manufacturer's instructions. The results were used to classify the isolates as either susceptible, intermediate or resistant to the tested antibiotics, based on Clinical and Laboratory Standard Institute (CLSI) breakpoints [17].

DNA Extraction and Species Identification

The DNA was extracted using a GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific) according to the manufacturer guideline. The quality and quantity of DNA were assessed before sequencing. The 16S rRNA gene of seventeen isolates was sequenced in the ABI3500 system (Applied Biosystem). The isolate sequences in this study and 23 published reference sequences including twenty two Enterobacteriae strains and one out group strain were aligned using MUSCLE algorithms. The maximum likelihood tree with T92+G substitution model and bootstrap 1000 were built to determine the genetic relationship. All analysis processes were performed using MEGA6 software [18].

Quantitative Reverse Transcription-PCR

For molecular analysis of antibiotic resistance determinants of XDR *K.pneumoniae*, cDNA samples and primers of target genes (Table 1) were prepared for RT-PCR according to the protocol description. Briefly, total RNA was extracted from bacterial culture media using Trizol (Thermo Fisher Science, USA). The concentration and quality of each RNA samples were determined by measuring absorbance at 260 nm. Complementary DNA (cDNA) was synthezed from 1 µg of total RNA. The volume of 1 µl cDNA was used for PCR performed using standard conditions: denatured at 95°C for 30 seconds, annealed at 52-60^{o}C for 30 seconds, extended at 72^{o}C for 1 min, and performed using 30 cycles. The PCR product of 16S rRNA (a housekeeping gene) was used as a control for variations in mRNA concentrations in the RT-PCRs. A volume of 8 µl of PCR product was loaded on 2% agarose gel and stained with ethidium bromide. The gel photograph was scanned using Quantity One program (Gel Doc EQ; Bio-Rad,Hercules, CA).

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101

Gene Name	Sequence (5' - 3')		Size (bp)	Melting temperature
blaNDM	Fwd	CACCTCATGTTTGAATTCGCC	984	58ºC
	Rev	CTCTGTCACATCGAAATCGC		
blaKPC	Fwd	TGTCACTGTATCGCCGTC	1011	58ºC
	Rev	CTCAGTGCTCTACAGAAAACC		
blaOXA	Fwd	ATGCGTGTATTAGCCTTATCG	888	55ºC
	Rev	AACTACAAGCGCATCGAGCA		
blaCTX-M	Fwd	GTGCAGTACCAGTAAAGTTATGG	538	55ºC
	Rev	CGCAATATCATTGGTGGTGCC		
blaTEM	Fwd	CATTTCCGTGTCGCCCTTATTC	800	55ºC
	Rev	CGTTCATCCATAGTTGCCTGAC		
bla SHV	Fwd	ATGCGTTATATTCGCCTGTG	753	58ºC
	Rev	TGCTTTGTTATTCGGGCCAA		
QnrB	Fwd	CCTGAGCGGCACTGAATTTAT	409	55ºC
	Rev	GTTTGCTGCTCGCCAGTCGA		
OqxA	Fwd	CTCGGCGCGATGATGCT	393	55ºC
	Rev	CCACTCTTCACGGGAGACGA		
OqxB	Fwd	ACCAACACGCCGAATACC	945	55ºC
	Rev	CATCAGGACCACCAGACCC		
Sul1	Fwd	CGGCGTGGGCTACCTGAACG	433	55ºC
	Rev	GCCGATCGCGTGAAGTTCCG		
Sul2	Fwd	CGGCATCGTCAACATAACCT	721	55ºC
	Rev	TGTGCGGATGAAGTCAGCTC		
aac(6')-Ib-cr	Fwd	TTGCGATGCTCTATGAGTGGCTA	482	52ºC
	Rev	CTCGAATGCCTGGCGTGTTT		
rmtB	Fwd	GCTTTCTGCGGGCGATGTAA	173	52ºC
	Rev	ATGCAATGCCGCGCTCGTAT		
16S rRNA	Fwd	GCCTACGGGAGGCAGCAG	550	55ºC
	Rev	CCGTCAATTCMTTTGAGTTT		

Table 1: Primer sets used for screening target genes.

Results

Characteristics of Clinical Samples

K.pneumoniae isolates were isolated from 14 male (84.4%) and 03 female (17.6%) patients. Most of *K.pneumoniae* were isolated from patients aged from 23 to 92 years old (median age

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101

at 59.53). Besides, *K.pneumoniae* samples were mostly isolated from Intensive Care Unit (ICU) (71.4%) and non-ICU (9.6%) (Table 2).

The 16S rRNA-based subspecies analysis was conducted to determine the genetic relationships of the strains using in research. The Fig. 1 shows phylogenetic species of all *K.pneumoniae* isolates closely related in a group of *K.pneumoniae* strains ATCC_13883, 11296, 13884 (77 - 97 bootrap values), and belong to the family *Enterobacteriaceae*. Thus, all strains of *K.pneumoniae* isolated at the hospital have highly genetically similarity level with *K.pneumoniae* ATCC strains.

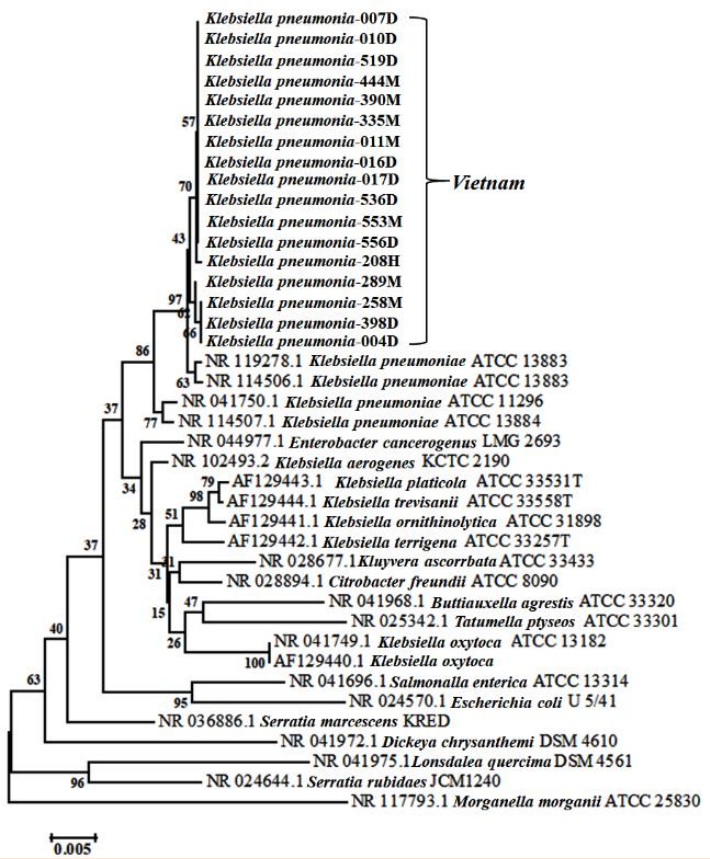

Figure 1: Phylogenetic tree was built using Maximum-Likelihood algorithm, bootstrap 1000. "Vietnam": mean the seventeen multidrug-resistance *K.pneumoniae* isolated in the Military Hospital.

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101

ID	Collection Year	Age (years)	Gender	Location	Source of Sample	Ward
KP-208H	2015	76	M	inx	sputum	Ort
KP-258M	2015	92	M	ICU	blood	ICU
KP-289M	2015	59	M	inx	blood	Nep
KP-335M	2016	23	F	ICU	blood	ICU
KP-390M	2016	63	M	inx	blood	Abd
KP-398D	2016	88	M	ICU	bili	ICU
KP-444M	2016	32	M	ICU	blood	ICU
KP-519D	2016	46	M	ICU	sputum	ICU
KP-536D	2017	47	M	ICU	sputum	ICU
KP-004D	2017	65	F	ICU	sputum	ICU
KP-007D	2017	49	M	inx	blood	inf
KP-010D	2017	75	M	inx	urine	inf
KP-011M	2017	57	M	inx	urine	inf
KP-016D	2017	67	M	ICU	wound	ICU
KP-017D	2017	54	F	ICU	sputum	ICU
KP-553M	2017	56	M	ICU	sputum	ICU
KP-556D	2017	63	M	inx	sputum	out

M: Male; F: Female; Inx: Inpatient (non-ICU); ICU: Intensive care unit; Ort: orthopedic; Nep: nephrology; Abd: abdominal surgery; Inf: infection department; Out: outpatient department

Table 2: Sociodemographic characteristics associated with 17 *K. pneumoniae* bacterial isolates from patients who were admitted to the Military Hospital 103.

Antibiotic Sensitivity and MIC of Isolates Studied

The isolates were screened against with 18 antibiotics. The results showed that all *K.pneumoniae* isolates were still susceptible to colistin (100%); highly resistant to ampicillin, sulfamethoxazole/trimethoprim, and ertapenem (82.4 - 94.1%); and completely resistant (100%) to ampicillin, piperacillin/tazobactam, cefotaxime, certapenem, cefepime, ciprofloxacin, and norfloxacin. In particular, one isolate (KP-208H) was resistant to 17/18 tested antibiotics, and 2 to 6 isolates were resistant to 12 from 15 antibiotics (accounting for 88.89 %), and the lowest resistance was also 8/18 of the antibiotics tested (KP-007D isolate).

Besides, a study was designed to determine *in-vitro* efficacy of Modified Hodge Test (MHT) for detection of carbapenemase production in *K.pneumoniae* isolates [19]. Out of a total 17 isolates which were showing intermediate or susceptible zone, i.e. 6 mm - 23 mm for meronem, 14/17 (82.4%) were positive for carbapenemase production by MHT. In addition, the strains used in the study all had similar MICs with imipenem, meropenem and ertapenem and all were

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101

very high compared to the CLSI recommended standard, up to 32 µg / mL (Supplemental Table 1).

Antibiotic Resistance Gene Profile

From the analysis of RT-PCR results, genes responsible for conferring aminoglycoside resistance, carbapenem resistance, beta-lactam resistance, fluoroquinolone, and folate pathway antagonist were presented across all of *K.pneumoniae* isolates (Fig. 2, Table 3). Briefly, the highest occurrence were genes belonging to the group of combined resistance to fluoroquinolone and folate pathway antagonist (OqxB gene appeared in 16/17 isolates, accounting for 94.12%; OqxA, Sul-1, and Sul-2 genes were 74.47, 58.82 and 29.41%, respectively), followed by aminoglycoside and fluoroquinolone combination resistance (aac(6')-Ib-cr gene, appearing in 15/17 isolates, approximately 88.24%), and then the group with combined carbapenem and beta-lactam resistance (the blaNDM gene appeared in 9/17 isolates, accounting for 52.94%, other blaKPC and blaOXA genes were 41.18 and 11.76%, respectively). The highest resistance genes in single groups such as beta-lactam, or fluoroquinolone, or aminoglycoside were blaCTX-M (70.59%), blaSHV and qnrB (both of them 58.82%), blaTEM (35.29%), and the lowest was rmtB gene (only approximately 5.88%).

Analysis of carbapenem resistance gene expression (Table 4) showed that all XDR *K.pneumoniae* isolates possess carbapenem resistance genes, which could appear independently or in combination of multiple genes in the same ESBL-producing bacteria. In particular, all five genes (blaNDM, blaOXA, blaCTX-M, blaTEM, and blaSHV) were found in KB-016D isolate; four genes occurred in 3 isolates (KB-017D, KB-553M, and KB-011D). There were 13 isolates containing from 1 to 3 carbapenem resistance genes, of which 3 isolates carried 3 genes (17.65 %), 8 isolates carried 2 genes (47.06 %), and only 1 isolate (KB-289M) carrying a gene blaCTX-M (5.88%). The results showed an association between the prevalence of antibiotic resistant gene expression and drug resistant phenotype in some isolates of *K.pneumoniae*. However, there was also the opposite, KB-007D carried 4 genes for carbapenem resistance but was 100% sensitive to the antibiotics of this group, therefore, these antibiotic resistance gene expressions did not correlate with the number of antibiotics to which a particular isolate has shown resistance. The cross-resistance to antibiotics is generally a combination of mechanisms, osmolarity, targets and enzymatis changes. Therefore, further studies are needed.

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101

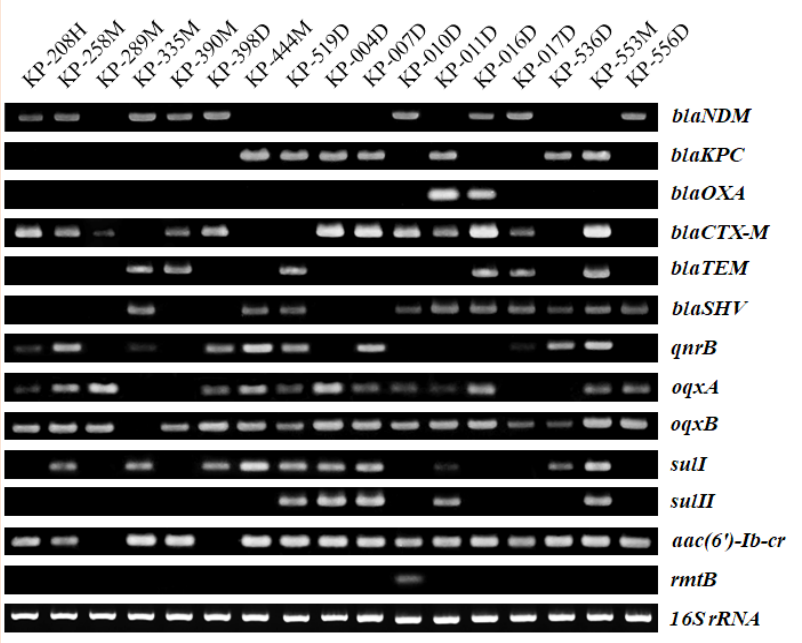

Figure 2: List of antibiotic resistance gene expression.

Group of Antibiotic Resistance	Target gene	Ratio	(%)
Carbapenem; Beta-lactam	*blaNDM*	9/17	52.94
	blaKPC	7/17	41.18
	blaOXA	2/17	11.76
Beta-lactam	*blaCTX-M*	12/17	70.59
	blaTEM	6/17	35.29
	blaSHV	10/17	58.82
Fluoroquinolone	*qnrB*	10/17	58.82
Folate pathway antagonist; Fluoroquinolone	*OqxA*	13/17	76.47
	OqxB	16/17	94.12
	Sul-1	10/17	58.82
	Sul-2	5/17	29.41
Aminoglycoside	*aac(6')-Ib-cr*	15/17	88.24
Fluoroquinolone			
Aminoglycoside	*rmtB*	1/17	5.88

Table 3: Distribution of multiple resistance genes identified among the isolates.

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101

Number of Antimicrobial Resistance Gene	Antimicrobial Resistance Gene Pattern	Number of Isolates (%)
One	CTX-M	1/17 (5.88)
Two	NDM+CTX	3/17 (17.65)
	NDM+SHV	1/17 (5.88)
	KPC+SHV	2/17 (11.76)
	KPC+CTX	2/17 (11.76)
Three	NDM+CTX+SHV	1/17 (5.88)
	NDM+TEM+SHV	1/17 (5.88)
	NDM+CTX+TEM	1/17 (5.88)
	KPC+TEM+SHV	1/17 (5.88)
Four	NDM+CTX+TEM+SHV	1/17 (5.88)
	KPC+CTX+TEM+SHV	1/17 (5.88)
	KPC+OXA+CTX+SHV	1/17 (5.88)
Five	NDM+OXA+CTX+TEM+SHV	1/17 (5.88)

Table 4: The multiple carbapenem resistance gene profile of *K.pneumoniae* isolates.

Discussion

In this study, most of the XDR *K.pneumoniae* isolates were collected from male patients. There may be a association in poor lifestyle choices, smoking and alcoholism between male and female [20]. In addition, most of *K.pneumoniae* in this study were isolated from patients aged 23 to 92 years old. The differences in age distribution of patients may be related to the response of the immune system, such as under 40 years have stronger immune systems, and more pressure to fight to *K.pneumoniae* [21]. Besides, an increasing age leads to increase the comorbid illness cause of a higher risk of *K.pneumoniae* infection [22]. These results also indicated that *K.pneumoniae* isolates were mainly isolated from ICU and respiratory specimens (namely is sputum samples). John and Dawson emphasized that *K.pneumoniae* typically colonizes human mucosal surfaces of the oropharynx [23]. For this reason, *K.pneumoniae* is considered to be the most common cause of hospital acquired pneumonia in the Vietnam [24].

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101

Antimicrobial resistance bacteria are commonly associated with nosocomial infections cause of the overuse of antibiotics, and without monitoring or control [25]. In our study, most of *K.pneumoniae* was showed a fully resistant to ampicillin, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, ciprofloxacin and norfloxacin, whereas the most sensitive with colistin, amikacin, nitrofurantoin, and fosfomycin. The finding was higher than other results, Cepas, et al., reported that 40% of *K.pneumoniae* strains were resisted to ciprofloxacin and amoxicillin-clavulanic acid [26]. Manjula, et al., showed that 90.2% of *K.pneumonia* isolates were MDR, and high resistance to penicillin, cephalosporin, fluoroquinolone, aminoglycoside, and sulfonamide [27]. These MDR microbes are causing a great challenge in controlling infections, thus it is very important to monitor and optimize antibiotic use through antibiotic management programs [28].

The analysis of antibiotic resistance gene expression and the relationship between genotypes and multi-drug resistance phenotype of the *K.pneumoniae* isolates were performed. This will provide a clearer and more indepth look at about the XDR capacity of these strains. Here, *K.pneumoniae* isolates had 13 difference resistance genotypes separating in four antibiotic phenotypes. Among the resistance against carbapenems *K.pneumoniae* isolates, the results have shown a predominant of blaCTX-M gene, then blaSHV, blaNDM, blaKPC, blaTEM, and a low percentage of blaOXA gene. In the previous studies, blaSHV and blaTEM were the two most common ESBL-coding genes in ESBL-producing bacteria. However, in recent years the blaCTX-M gene groups have become dominant [12,13]. The distribution of ESBL genes in our study was similar to the results of some recent studies in Vietnam [29,30]. Thereby, it shows that the distribution trend of genes encoding ESBL production in Vietnam is similar in the world, especially the common and widespread distribution of blaCTX-M instead of blaTEM and blaSHV, from there can confirm the flexible, unpredictable and difficult to control variation of antibiotic resistance in bacteria. In addition, there were isolates harboring about from 3 to 5 resistance genes encoding ESBL, also carrying on 2 to 6 other antibiotic resistance genes, and all were phenotypically resistant to 13 and 14 antibiotics of the tested groups. The presence of many genes encoding ESBL and other antibiotic resistance genes on the same strain of bacteria has changed the antibiotic resistance phenotype in a more complicated direction, resistance to multiple antibiotics at the same time, and dangerous multidrug resistance [31]. According to some previous studies, because it is located on a plasmid, ESBL is easily transmitted from one species to another or within the same species. Besides, ESBL-carrying plasmids also carry other antibiotic resistance genes, creating co-resistance, so bacteria with ESBL are not only resistant to β-lactams but also resistant to many antibiotics of other groups such as quinolones, sulphonamides and aminoglycosides [13].

The study results also showed a similarity in genotypic and phenotypic antibiotic resistances. Specifically, 16 isolates of XDR *K.pneumoniae* had 100% resistant phenotype with CIP and NOR (fluoroquinolone antibiotic group) all haboured OpxB genotype. The blaCTX-M genotype indicating for CTX-resistant phenotype was presented in 12/17 isolates that were

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101

completely resistant to CTX (beta-lactam antibiotic group). The similar isolates with 100% phenotype resistance to CAZ (beta-lactam group) also appeared 2 genes indicating CAZ resistance, which were blaSHV and blaTEM. The level of resistance to AN (an antibiotic belonging to the aminoglycoside group) was low (23.5%), equivalent to rmtB gene also appeared in only one isolate. The formation of resistance phenotypes in microorganisms is due to genetic changes in chromosomes or due to the reception of plasmids carrying resistance genes. This diverse mechanism of genetic information transmission contributes to the rapid spread of antibiotic resistance genes within species and to other bacterial species [13]. This leads to great difficulties for the selection of specific antibiotics in clinical treatment, although generations of antibiotics are constantly being researched and updated. Obviously, the emergence of strains of XDR *K.pneumoniae* resistant in the hospital is a dilemma for clinicians.

Conclusion

Taken together, our study has added a systematic analysis of 13 antibiotic resistance genes expression (belong to Carbapenem, Beta-lactam, Fluoroquinolone, Folate pathway antagonist, and Aminoglycoside groups), the association between multiple genotypes and XDR phenotypes in 13 *K.pneumoniae* isolates, and other clinical changes in antibiotic resistance of XDR *K.pneumonia* isolates in hospital. From the research results, there may be new suggestions to improve drug resistance and increase the effectiveness of treatment.

Acknowledgement

The authors thank to Department of Medical Microbiology, Military Hospital 103 for collecting samples. Vietnam Academy of Science and Technology support this research under grant number NCVCC40.06/22-22.

Conflicts of Interest

The authors declare that have no competing interest and not any conflict of interest.

References

1. Di Tella D, Tamburro M, Guerrizio G, Fanelli I, Sammarco ML, Ripabelli G. Molecular epidemiological insights into colistin-resistant and carbapenemases-producing clinical *Klebsiella pneumoniae* isolates. Infect Drug Resist. 2019;12:3783-95.

2. Centers for Disease Control and Prevention CDC. Antibiotic resistance threats in the United States, 2019 (2019 ar threats report). Atlanta, ga: us : division of healthcare quality promotion, national center for emerging and zoonotic Infectious Dis. 2019.
3. Elemam A, Rahimian J, Mandell W. Infection with panresistant *Klebsiella pneumoniae*: a report of 2 cases and a brief review of the literature. Clin Infect Dis. 2009;49(2):271-4.
4. Ensor VM, Jamal W, Rotimi VO, Evans JT, Hawkey PM. Predominance of CTX-M-15 extended spectrum beta-lactamases in diverse *Escherichia coli* and *Klebsiella pneumoniae* from hospital and community patients in Kuwait. Int J Antimicrob Agents. 2009;33(5):487-9.
5. Rastogi V, Nirwan PS, Jain S, Kapil A. Nosocomial outbreak of septicaemia in neonatal intensive care unit due to extended spectrum beta-lactamase producing *Klebsiella pneumoniae* showing multiple mechanisms of drug resistance. Indian J Med Microbiol. 2010;28(4):380-4.
6. Sekowska A, Janicka G, Klyszejko C, Wojda M, Wroblewski M, Szymankiewicz M. Resistance of *Klebsiella pneumoniae* strains producing and not producing ESBL (extended-spectrum beta-lactamase) type enzymes to selected non-beta-lactam antibiotics. Med Sci Monit. 2002;8(3):BR100-4.
7. Sanchez GV, Master RN, Clark RB, Fyyaz M, Duvvuri P, Ekta G, et al. *Klebsiella pneumoniae* antimicrobial drug resistance, United States, 1998-2010. Emerg Infect Dis. 2012;19(1):133-6.
8. Boggio SB, Roveri OA. Catalytic properties of an endogenous beta-lactamase responsible for the resistance of *Azospirillum lipoferum* to beta-lactam antibiotics. Microbiol (Reading). 2003;149(Pt 2):445-50.
9. Makoka MH, Miller WC, Hoffman IF, Cholera R, Gilligan PH, Kamwendo D, et al. Bacterial infections in Lilongwe, Malawi: aetiology and antibiotic resistance. BMC Infect Dis. 2012;12:67.
10. Uraz G, Simsek H, Celik B. beta-Lactamase activities and resistance to antibiotics of *Haemophilus influenzae*, *H. parainfluenzae* and *H. aphrophilus* strains identified in throat cultures from children. Drug Metabol Drug Interact. 2000;16(3):217-28.
11. Koczura R, Mokracka J, Krzyminska S, Kaznowski A. Virulence properties and integron-associated antibiotic resistance of *Klebsiella mobilis* strains isolated from clinical specimens. J Med Microbiol. 2010;60(3):281-8.
12. Bradford PA. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology and detection of this important resistance threat. Clin Microbiol Rev. 2001;14(4):933-51.
13. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev. 2005;18(4):657-86.
14. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspect Medicin Chem. 2014;6:25-64.
15. Berglund B, Hoang NTB, Tarnberg M, Le NK, Nilsson M, Khu DTK, et al. Molecular and phenotypic characterization of clinical isolates belonging to a KPC-2-producing strain of ST15 *Klebsiella pneumoniae* from a Vietnamese pediatric hospital. Antimicrob Resist Infect Control. 2019;8:156.
16. Roe CC, Vazquez AJ, Esposito EP, Zarrilli R, Sahl JW. Diversity, Virulence and antimicrobial resistance in isolates from the newly emerging *Klebsiella pneumoniae* ST101 lineage. Front Microbiol. 2019;10:542.
17. Clinical and Laboratory Standards Institute Antimicrobial CLSI. M100 performance standards for antimicrobial susceptibility testing, 30th Edition. PA, USA2020.
18. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30(12):2725-9.
19. Amjad A, Mirza I, Abbasi S, Farwa U, Malik N, Zia F. Modified Hodge test: A simple and effective test for detection of carbapenemase production. Iran J Microbiol. 2012;3(4):189-93.
20. Osagie RN, Eyaufe AA, Iserhienrhien O, Okodua M, Onuabonah F, Daibo OO. Antibiotic susceptibility profile of *Klebsiella pneumoniae* isolated from sputum samples amongst hospitalized adults in parts of Edo State, South-South, Nigeria. Merit Research J Medicine and Medical Sci. 2017;5(8):378-83.
21. Gunn JS, Bakaletz LO, Wozniak DJ. What's on the outside matters: the role of the extracellular polymeric substance of gram-negative biofilms in evading host immunity and as a target for therapeutic intervention? J Biol Chem. 2016;291(24):12538-46.
22. Meatherall BL, Gregson D, Ross T, Pitout JD, Laupland KB. Incidence, risk factors and outcomes of *Klebsiella pneumoniae* bacteremia. Am J Med. 2009;122(9):866-73.

Ha VTT | Volume 3; Issue 1 (2022) | JCIM-3(1)-041 | Research Article

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101

23. John VA, Dawson A. *Klebsiella Pneumonia*. In: StatPearls. Treasure Island (FL): StatPearls Publishing. 2021;2021.
24. Dao TT, Liebenthal D, Tran TK, Ngoc Thi Vu B, Ngoc Thi Nguyen D, Thi Tran HK, et al. *Klebsiella pneumoniae* oropharyngeal carriage in rural and urban Vietnam and the effect of alcohol consumption. PLoS One. 2014;9(3):e91999.
25. Stephan H, Hanan HB, Herman G, Vincent J, Jan K, Ramanan L, et al. Antimicrobial resistance: one world, one fight! Antimicrob Resist Infect Control. 2015;4:49.
26. Cepas V, Lopez Y, Munoz E, Rolo D, Ardanuy C, Marti S, et al. Relationship between biofilm formation and antimicrobial resistance in gram-negative bacteria. Microb Drug Resist. 2018;25(1):72-79.
27. Manjula NG, Girish C, Math KN, Shripad AP, Subhashchandra MG, Channappa TS. Antibiotic susceptibility pattern of esβl producing *Klebsiella pneumoniae* isolated from urine samples of pregnant women in Karnataka. J Clin Diagn Res. 2014;8(10):8-11.
28. Lee CR, Lee JH, Park KS, Jeon JH, Kim YB, Cha CJ, et al. Antimicrobial resistance of hypervirulent *Klebsiella pneumoniae*: epidemiology, hypervirulence-associated determinants, and resistance mechanisms. Front Cell Infect Microbiol. 2017;7:483.
29. Hoang PH, Awasthi SP, P DON, Nguyen NL, Nguyen DT, Le NH, et al. Antimicrobial resistance profiles and molecular characterization of Escherichia coli strains isolated from healthy adults in Ho Chi Minh City, Vietnam. J Vet Med Sci. 2017;79(3):479-85.
30. Nakayama T, Ueda S, Huong BT, Tuyen le D, Komalamisra C, Kusolsuk T, et al. Wide dissemination of extended-spectrum beta-lactamase-producing *Escherichia coli* in community residents in the Indochinese peninsula. Infect Drug Resist. 2015;8:1-5.
31. Li B, Sun JY, Liu QZ, Han LZ, Huang XH, Ni YX. High prevalence of CTX-M beta-lactamases in faecal *Escherichia coli* strains from healthy humans in Fuzhou, China. Scand J Infect Dis. 2011;43(3):170-4.

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101

Supplementary Files

ID (MIC/ CLSI)	VITEK													ETEST				Colistin COL (BMD)	Carba pene m-ase
	AM	AMC	TZP	CTX	CAZ	FEP	AN	GM	CIP	NOR	FOS	NIT	SXT	ETP	IMP	MEM	DOR		
KP-208H	> 16	>16	>64	>32	>32	>32	>32	>8	>2	>8	>128	>256	160	>4	>32	>32	>32	< 0.25	22
	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	(-)
KP-258M	> 16	>16	>64	>32	>32	16	>32	>8	>2	>8	64	256	40	>4	2	6	4	< 0.25	6
	R	R	R	R	R	R	R	R	R	R	S	R	S	R	I	R	R	S	+
KP-289M	> 16	16	>64	>32	>16	8	>32	>8	>2	>8	<=16	32	>160	<0.5	0.19	0.032	0.032	< 0.25	24
	R	R	R	R	R	R	R	R	R	R	S	S	R	S	S	S	S	S	(-)
KP-335M	> 16	>16	>64	>32	>32	>32	16	>8	>2	>8	32	128	>160	>4	16	16	12	< 0.25	6
	R	R	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	S	+
KP-390M	> 16	>16	>64	>32	>16	>32	<=2	<=1	>2	>8	>128	128	>160	>4	8	8	>32	< 0.25	6
	R	R	R	R	R	R	S	S	R	R	R	S	R	R	R	R	R	S	+
KP-398D	> 16	>16	>64	>32	>32	>32	>32	>8	>2	>8	>128	>256	40	>4	2	8	8	< 0.25	6
	R	R	R	R	R	R	R	R	R	R	R	R	S	R	I	R	R	S	+
KP-444M	> 16	>16	>64	>32	>32	16	<=2	>8	>2	>8	>128	64	>160	>4	4	6	1.5	< 0.25	6
	R	R	R	R	R	R	S	R	R	R	R	S	R	R	R	R	I	S	+
KP-519D	> 16	16	32	>32	>32	4	<=2	>8	>2	>8	>128	128	>160	<0.5	4	4	4	< 0.25	23
	R	R	R	R	R	R	S	R	R	R	R	S	R	S	R	R	R	S	(-)
KP-536D	> 16	16	>64	4	>32	>32	<=2	<=1	>2	>8	>128	128	>160	>4	8	3	2	< 0.25	6
	R	R	R	R	R	R	S	S	R	R	R	S	R	R	R	I	I	S	+
KP-004D	> 16	>16	>64	>32	>32	>32	<=2	>8	>2	>8	>128	256	>160	>4	2	4	3	< 0.25	6
	R	R	R	R	R	R	S	R	R	R	R	R	R	R	I	R	I	S	+

ID (MIC/ CLSI)	VITEK													ETEST				Colistin COL (BMD)	Carba penem -ase
	AM	AMC	TZP	CTX	CAZ	FEP	AN	GM	CIP	NOR	FOS	NIT	SXT	ETP	IMP	MEM	DOR		
KP-007D	> 16	4	>64	>32	>32	>32	<=2	<=1	>2	>8	<=16	64	>160	<0.5	0.125	0.047	0.032	< 0.25	22
	R	I	R	R	R	R	S	S	R	R	S	S	R	S	S	S	S	S	(-)
KP-010D	> 16	>16	>64	>32	>32	>32	16	<=1	>2	>8	<=16	>256	<=20	>4	> 32	> 32	> 32	< 0.25	6
	R	R	R	R	R	R	S	S	R	R	S	R	S	R	R	R	R	S	+
KP-011M	> 16	>16	>64	>32	>32	>32	16	>8	>2	>8	64	64	>160	>4	> 32	> 32	> 32	< 0.25	6
	R	R	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	S	+
KP-016D	> 16	>16	>64	>32	>16	>32	<=2	<=1	>2	>8	<=16	>256	>160	>4	2	12	6	< 0.25	6
	R	R	R	R	R	R	S	S	R	R	S	R	R	R	I	R	R	S	+
KP-017D	> 16	>16	>64	>32	>32	>32	16	>8	>2	>8	<=16	128	>160	>4	2	4	4	< 0.25	6
	R	R	R	R	R	R	S	R	R	R	S	S	R	R	I	R	R	S	+
KP-553M	> 16	>16	>64	>32	>32	>32	16	>8	>2	>8	>128	64	>160	>4	6	2	2	< 0.25	6
	R	R	R	R	R	R	S	R	R	R	R	S	R	R	R	I	I	S	+
KP-556D	> 16	>16	>64	>32	>32	>32	16	>8	>2	>8	<=16	128	>160	>4	8	4	4	< 0.25	6
	R	R	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	S	+
R (%)	100	94.1	100	100	100	100	23.5	70.6	100	100	47.1	35.3	82.4	82.4	64.7	76.4	64.7	0	(-)23.5
S (%)	0	0	0	0	0	0	76.5	29.4	0	0	52.9	64.7	17.6	17.6	11.8	11.8	11.8	100	(+)76.5
I (%)	0	5.9	0	0	0	0	0	0	0	0	0	0	0	0	23.5	11.8	23.5	0	

AM: Ampicillin, AMC: Ampicillin/ Clavulanic acid, TZP: Piperacillin/tazobactam, CTX: Cefotaxime, CAZ: Ceftazidime, FEP: Cefepime, AN: Amikacin, GM: Gentamicin, CIP: Ciprofloxacin, NOR: Norfloxacin, FOS: Fosfomycin, NIT: Nitrofurantoin, SXT: Sulfamethoxazole/Trimethoprim, ETP: Ertapenem, IPM: Imipenem, MEM: Meropenem, DOR: Doripenem, MIC: Minimum Inhibitory Concentration, CLSI: Clinical and Laboratory Standard Institute, BMD: Broth microdilution, R – Resistant, S – Susceptible, I – Susceptible according to CLSI guidelines

Supplemental Table 1: Phenotypic antimicrobial resistance pattern of the 17 *K. pneumoniae* isolates.

Ha VTT | Volume 3; Issue 1 (2022) | JCIM-3(1)-041 | Research Article

Citation: Vo TTB, et al. Analysis of Antibiotic Resistance Genes of the Extensively Drug Resistant Clinical *Klebsiella pneumonia* Isolates in Vietnam. J Clin Immunol Microbiol. 2022;3(1):1-15.

DOI: http://dx.doi.org/10.46889/JCIM.2022.3101