Revista Cubana de Medicina Militar
2024;53(3):e024060109

Research Article

Colistin-resistance and carbapenemase-producing among carbapenem-resistance *E. coli* and *K. pneumoniae*

Resistencia a la colistina y producción de carbapenemasas entre *E. coli* y *K. pneumoniae* resistentes a carbapenem

Ngoc Tran Bich[1] https://orcid.org/0000-0002-2255-6398
Phu Truong Thien[2]* https://orcid.org/0000-0003-0868-2811
Loc Nguyen Tran Mai[1] https://orcid.org/0000-0003-3232-3587
Dang Tran Ngoc[1] https://orcid.org/0000-0002-5287-0265
Thao Nguyen Thi Ngoc[2] https://orcid.org/0009-0004-1295-401X
Phuong Nguyen Thi Nam[2] https://orcid.org/0009-0002-5305-4726
Tuan Dang Anh[2] https://orcid.org/0009-0008-1677-2650
Van Tran Thi Hue[1] https://orcid.org/0009-0007-0677-2553
Dat Ngo Quoc[1] https://orcid.org/0000-0003-1461-0216

[1]University of Medicine and Pharmacy at Ho Chi Minh City. Ho Chi Minh City, Vietnam.
[2]Cho Ray Hospital. Ho Chi Minh City, Vietnam.

*Author for correspondence. Email: truongthienphu78@yahoo.com

ABSTRACT

Introduction: Carbapenemase-producing Enterobacterales (CPE) is one of the causes of community health-threatening, most common in *Escherichia coli* and *Klebsiella pneumoniae.* CPE has appeared in many countries, especially developing ones, but it has not been recognized in routine antibiograms. Also, assessing colistin susceptibility is a significant obstacle for both diagnostic and epidemiological because of technical difficulties. There is rare data about CPE and colistin-resistant Enterobacterales.


2024;53(3):e024060109

Objectives: Determine the prevalence of producing carbapenemase and colistin-resistance among *K. pneumoniae* and *E. coli* resistant to carbapenems.

Methods: In a cross-sectional survey, 129 *K. pneumoniae* and 31 *E. coli* strains, which demonstrated carbapenem resistance, were collected from inpatients at Cho Ray Hospital over a three-month period. Carbapenemase production was detected using the modified carbapenem inactivation method and EDTA-modified carbapenem inactivation method, and colistin resistance was detected using the broth microdilution method according to CLSI M100S34.

Results: Of 160 participants' strains, 80% of carbapenemase-producing *K. pneumoniae* and *E. coli* were detected; among these, Metallo beta-lactamase-producing was taken 69,5%. Of 31 colistin-resistant, 30 were *K. pneumoniae*, and only 1 *E. coli* was detected that was resistant to colistin.

Conclusion: Among participants' strains, carbapenemase-producing prevalence was very high. There were signs of a widespread extensively drug-resistant *K. pneumoniae. Most of E. coli* was susceptible to colistin.

Keywords: *Klebsiella pneumoniae*; *Escherichia coli*; carbapenemase; colistin; polymyxin E; drug resistance, bacterial.

RESUMEN

Introducción: Enterobacterales productoras de carbapenemasa (CPE) son una amenaza a la salud comunitaria, más común en *Escherichia coli* y *Klebsiella pneumoniae*. CPE aparecen en muchos países, especialmente en vías de desarrollo, pero no se reconocen en los antibiogramas rutinarios. Evaluar la susceptibilidad a la colistina es difícil, tanto para diagnóstico como epidemiología, por las dificultades técnicas. Hay pocos datos sobre Enterobacterales resistentes a la colistina y productoras de carbapenemasa.

Objetivos: Determinar la prevalencia de la producción de carbapenemasas y resistencia a la colistina entre *K. pneumoniae* y *E. coli* resistentes a carbapenemos.

Métodos: Estudio transversal; se recolectaron 129 cepas de *K. pneumoniae* y 31 cepas de *E. coli*, resistentes a carbapenemos, de pacientes hospitalizados en el Hospital Cho Ray, durante 3 meses. La producción de carbapenemasa se detectó mediante el método modificado de inactivación de carbapenem


2024;53(3):e024060109

y el método modificado de inactivación de carbapenem con EDTA; la resistencia a la colistina se detectó mediante el método de microdilución en caldo, según CLSI M100S34.

Resultados: De las 160 cepas se detectó el 80 % de *K. pneumoniae* y *E. coli* productoras de carbapenemasa; entre estas, se encontraron MBLs en el 69,5 %. De 31 resistentes a colistina, 30 eran *K. pneumaniae* y solo se detectó 1 *E. coli* resistente a la colistina.

Conclusión: Entre las cepas de los participantes, la prevalencia de producción de carbapenemasa fue muy alta. Hubo signos de una *K. pneumoniae* extensamente resistente a los medicamentos. La mayoría de E. coli fue susceptible a la colistina.

Palabras clave: *Klebsiella pneumoniae; Escherichia coli*; carbapenemasa; colistina; polimixina E; resistencia a los medicamentos, bacteriana.

Received: 12/06/2024

Approved: 08/07/2024

INTRODUCTION

Escherichia coli (E. coli) and *Klebsiella pneumoniae (K. pneumoniae)* are 2 of the omnipresent Gram-negative bacilli belonging to the Enterobacterales order. They are common bacterium in the colon, but they could lead to many severe infection syndromes in humans, such as meningitis, gastrointestinal and urinary tract infections, septicemia, and others. In addition, most of them are multidrug-resistant.[1]

Although carbapenems are commonly used for treating severe infections, carbapenemase-producing Enterobacterales (CPE) is also one of the causes leading to community health-threatening.[2] Carbapenemase, a beta-lactamase, includes Ambler classes A, B, and D with serin beta-lactamase (SBL) and metallo beta-lactamase (MBL) encoded by both genes in chromosome and plasmid.[3] Along with the rapid spreading of CPE, the effectiveness of these last-resort drugs is decreasing.[4] Besides ceftazidime/avibactam, the first line of CPE treatment, the return of an old antibiotic, colistin, is salvation, and combined therapeutic regimens consisting of colistin are broadly used worldwide.[5]


2024;53(3):e024060109

In recent years, CPE has appeared in many countries, especially developing ones, but it has not been recognized in routine antibiograms. Also, assessing colistin susceptibility is a significant obstacle for both diagnostic and epidemiological because of technical difficulties.[5] The size of the colistin molecular structure is quite large, so colistin resistance cannot be detected by routine methods such as Kirby-Bauer or E-tests.[5]

Consequently, there is rare data about CPE and colistin resistance Enterobacterales, so research about multidrug resistance (MDR) should be conducted to support physicians in selecting the right antibiotic. Therefore, this study surveyed colistin-resistant and carbapenemase phenotypes in carbapenem-resistant *E. coli* and *K. pneumoniae*.

This study aims to determine the prevalence of producing carbapenemase and colistin-resistance among *K. pneumoniae* and *E. coli* resistant to carbapenems.

METHODS

Study design and participants

A cross-sectional study was conducted at Cho Ray Hospital in Ho Chi Minh City, Vietnam. This is a terminal hospital with diverse patients from the Southern provinces. 160 modulated isolates of *K. pneumoniae* and *E. coli* from October 2023 to December 2023, which show resistance to at least one antibiotic of carbapenems, were collected from inpatients in the clinical departments of Cho Ray Hospital.

Data collection

The samples were cultivated in Blood agar, MacConkey agar, chocolate agar, or chromogenic agar, depending on which type of specimens, and incubated from 16 to 18 hours at 35-37 °C to assess viability and purity. Then, using the Vitek MS System (BioMérieux, France) to identify bacteria, using the Vitek-2 compact system (BioMérieux, France) to detect carbapenem resistance bacteria strains. *E. coli* ATCC 25922 and *K. pneumoniae* BAA ATCC 1705 were used for quality control for bacterial identification tests and chromogenic agar.


2024;53(3):e024060109

After bacterial strains were assessed in this study, each sample was coded with an ID, so the participants' information was secured to avoid bias errors in collecting data. Each isolate was stored in TSB 15% until used.

The modified carbapenem inactivation method and EDTA modified carbapenem inactivation method (mCIM/eCIM) were used to detect carbapenemase-producing Enterobacteriales. The test procedure and interpretation were performed according to CLSI M100 S34.[6] Bacteria would be considered to produce carbapenemase when they have positive results on the mCIM test. If these strains have positive results with eCIM, they would be detected as MBL, either, or SBL.

Colistin resistance was detected by the Broth microdilution method (BMD). The test procedure complied with the manufacturer's recommendation (Trek diagnostic), and the test interpretation followed the CLSI M100 S34.[6] Colistin is resistant to bacteria strains when MIC is > 2 µg/mL and colistin is intermediate to bacteria strains when MIC is ≤ 2 µg/mL.

Data analysis

The Student Version of IBM-SPSS version 20.0 was used to analyze data. Categorical variables about the characteristics of the study participants are presented by frequency and percentages. The Chi-square test assessed associations between carbapenemase-producing type and colistin resistance. A p-value of less than 0.05 was considered statistically significant.

Ethical considerations

The study collected frozen bacterial strains isolated from patients who met the study's selection criteria and did not have any intervention on the patients. The University of Medicine and Pharmacy Ethics Committee at Ho Chi Minh City approved this research with the number contract 142/HĐĐĐ-ĐHYD.

RESULTS

Characteristics of the study participants

In the flow chart of colony participants, 162 samples were assessed for initial eligibility and selected to participate in this study. The figure (Fig. 1) was shown in the flow chart of participants throughout the

2024;53(3):e024060109

study. Participant strains that were excluded (attached the reasons) were noted; there were 160 eligible participant strains.

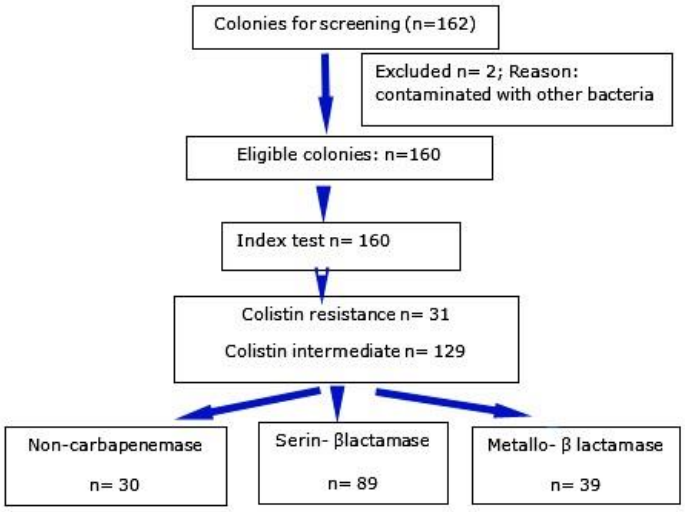

Fig. 1 - Flow chart of participants.

In table 1, of the 160 colony participants from many specimens screened in the study, most came from elders (more than 60 years old), accounting for 53.7%. Among these, the prevalence of *K. pneumoniae* was more frequent than that of E. coli (80.6% and 19.4%), and most bacteria strains were resistant to a combo of ertapenem, imipenem, and meropenem, accounting for 93.8%.

In table 2, 128 of 160 participant strains were identified as having carbapenemase with mCIM-positive results, accounting for 80% of the total sample. Specifically, of these 128 samples, there are 89 samples, accounting for 69.5% belonging to MBLs with eCIM-positive results, and others were SBLs.

Of 129 *K. pneumoniae,* 102 samples were identified as carbapenemase, accounting for 79.1%. Among these, 70 samples (68.6%) with MBLs were detected; the rest were SBLs.

Of 31 *E. coli,* 26 samples were identified as carbapenemase, accounting for 83.9 %. Among these, 19 samples (73.1%) with MBLs were detected; the rest were SBLs.

2024;53(3):e024060109

Table 1 - Characteristics of the study population

Characteristics	n	%
Ages		
0-15	1	0.6
16-45	35	21.9
46-60	38	23.8
> 60	86	53.7
Gender		
Male	93	58.1
Female	67	41.9
Specimens		
Blood	31	19.4
Urine	23	14.4
Phlegm	48	30.0
Pus	13	8.1
Body fluid	45	28.1
Bacteria		
Klebsiella pneumoniae	129	80.6
Escherichia coli	31	19.4
Resistance to carbapenems		
Ertapenem; Imipenem; Meropenem;	150	93.8
Ertapenem	1	0.6
Imipenem, Meropenem	4	2.5
Ertapenem, Imipenem	1	0.6
Ertapenem, Meropenem	1	0.6
Intermediate to Ertapenem	2	1.3
Intermediate to Ertapenem, Imipenem, Meropenem	2	0.6
Total	160	100


2024;53(3):e024060109

Prevalence of carbapenemase

Table 2 – Results of mCIM and eCIM for 160 CRE isolates

Species	mCIM		eCIM	
	Positive	Negative	Positive	Negative
K. pneumoniae	102	25	70	32
E. coli	26	5	19	7
Total	128	30	89	39

Prevalence of colistin resistance

Table 3 - Results of BMD for 160 CRE isolates

Species	Intermediate (MIC ≤ 2 µg/mL)		Resistant (MIC > 2 µg/mL)		p-value
	n	%	n	%	
K. pneumoniae	99	61.9	30	18.8	0.011
E. coli	30	18.8	1	0.6	
Total	129	80.7	31	19.4	

In table 3, 31 of 160 participant strains were identified as colistin-resistant, accounting for 19.4% of the total sample. Of 129 *K. pneumoniae,* 30 samples were identified as carbapenemase, accounting for 23.3%. Among 31 *E. coli,* only 1 sample was identified as colistin resistance, accounting for 3.2%. There were **s**tatistical differences in colistin resistance between *K. pneumoniae* and *E. coli* with p= 0.011.

2024;53(3):e024060109

Correlation between carbapenemase and colistin–resistance

Table 4 - Distributions of colistin MICs determined by BMD for 160 CRE isolates

Species	Carbapenemase type	Colistin Reference MICs (µg/mL)		p-value
		Intermediate (MIC ≤ 2 µg/mL)	Resistant (MIC > 2 µg/mL)	
Klebsiella pneumoniae	MBL	56	14	0.370
	SBL	25	7	
	Non-carbapenemase	18	9	
Escherichia coli	MBL	19	0	0.387
	SBL	6	1	
	Non-carbapenemase	5	0	
Total	MBL	75	14	0.308
	SBL	31	8	
	Non-carbapenemase	23	9	

Of the 31 participant strains with colistin-resistant results, 14 were MBLs, 8 were SBLs, and others did not have any carbapenemase; there was no statistical difference between carbapenemase types and either having or not carbapenemase (table 4).

Among 30 colistin-resistant *K. pneumoniae,* 14 were MBLs, and 7 were SBLs. Only 1 *E. coli* was resistant to colistin, which was detected as having SBLs.

DISCUSSION

The prevalence of CPE has been spreading rapidly and poses a problem with public health challenges and the healthcare system. As a developing country, Vietnam has faced the increasing prevalence of MDR bacteria, which could threaten to undermine the effectiveness of the last resort antibiotic.

This study collected samples from various specimens with 80% carbapenemase prevalence detected in *K. pneumoniae* and *E. coli*. These results aligned with *Sharma K* et al.[7] in India, in which this prevalence


2024;53(3):e024060109

showed 86.1%.[7] In Egypt and China, *Khattab S* et al.[8] and *Tsai YM* et al.[9] study has a slightly lower 70.7% and 57.1%, respectively. The causes of these differences may stem from variations in ethnicity, living conditions, and antibiotic usage habits.

Specifically, among 128 CPE, 89 (69.5%) isolates producing MBLs were detected by using the mCIM/eCIM method. This result is reasonable, considering the epidemiology of the distribution of carbapenemase types in South and Southeast Asia.[10]

Metallo-carbapenemase, known as harboring NDM, VIM, or IMP enzymes, often shows resistance to a wide range of beta-lactams, including carbapenems. Newer antibiotics and beta-lactamase inhibitors, such as ceftazidime-avibactam, are ineffective against MBLs. However, aztreonam, which MBLs do not hydrolyze in combination with avibactam, can be considered a treatment option.[11] Besides that, combination therapy can enhance treatment efficacy and help prevent the development of further resistance. In developing countries like Vietnam, combining classic antibiotics such as colistin may be effective against some metallo-carbapenemase bacteria.[11] Nevertheless, colistin-resistant bacteria are present worldwide.

Bacterial strains that are resistant to both carbapenems and colistin are called extensively drug-resistant (XDR).[12] In this study, overall colistin resistance among *K. pneumoniae* and *E. coli* is 19.4%. Current result is similar to the study of *Raunak Bir* et al. (15%).[13] Specifically, the number of colistin-resistant *K. pneumoniae* (CRKP) was significantly higher than *E. coli* (p= 0.011). Among 129 CRKP in current study, 30 strains (23,3%) resisted colistin. It was correlated with *Bui Thanh Thuyet* et al.[14] So, along with previous studies, this research was evidence of the rising rate of XDR in Vietnam's hospitals. There was only one colistin-resistant *E.coli* among participant strains, accounting for 3.3%. Therefore, initial antibiotic treatment with colistin could be considered for carbapenem-resistant *E. coli.*

Of 30 CRKP, there are 14 strains producing MBLs (45.2%) and 8 strains producing SBLs (25.8%). There was no statistical difference in colistin resistance between carbapenemase type and either having or not having carbapenemase. However, in the study of *Elaskary SA* et al.,[15] among colistin-resistant strains, there was a significant difference in carbapenemase gene prevalence, with SBLs being more predominant than MBLs in CRKP. Epidemiological differences across countries may account for this variation prevalence.


2024;53(3):e024060109

There are also some limitations. This research was about carbapenemase phenotype and could not show the exact genes that code for MBLs or SBLs. Furthermore, the sample size should come from a multicenter to be more significant in detecting MDR, especially in *E. coli*.

The prevalence of carbapenemase-producing strains among participants' strains was very high. There were signs of a widespread, extensively drug-resistant *K. pneumoniae*. Most *E. coli* strains were susceptible to colistin.

Based on the evidence from this research at Cho Ray Hospital, aztreonam combined with colistin could be recommended for treating diseases caused by CRE, especially *E. coli.*[16]

Acknowledgments

We give special thanks to the University of Medicine and Pharmacy at Ho Chi Minh City for funding and Cho Ray Hospital for assisting us with the auto-machines and facilities for the research project. Furthermore, we are honored to aid samples from the Microbiology Department, Cho Ray Hospital, in implementing our study.

BIBLIOGRAPHIC REFERENCES

1. Elshamy AA, Aboshanab KM. A review on bacterial resistance to carbapenems: epidemiology, detection and treatment options [Internet]. Future Sci OA. 2020;6(3):FSO438. DOI:10.2144/fsoa-2019-0098

2. Park SH, Yi Y, Suh W, Ji SK, Han E, Shin S. The impact of enhanced screening for carbapenemase-producing Enterobacterales in an acute care hospital in South Korea [Internet]. Antimicrob Resist Infect Control. 2023;12:62. DOI:10.1186/s13756-023-01270-8

3. Del Franco M, Paone L, Novati R, Giacomazzi CG, Bagattini M, Galotto C, et al. Molecular epidemiology of carbapenem resistant Enterobacteriaceae in Valle d'Aosta region, Italy, shows the emergence of KPC-2 producing Klebsiella pneumoniae clonal complex 101 (ST101 and ST1789) [Internet]. BMC Microbiology. 2015;15(1):260. DOI:10.1186/s12866-015-0597-z

4. Van Duin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae [Internet]. Virulence. 2017; 8(4):460-9. DOI:10.1080/21505594.2016.1222343


2024;53(3):e024060109

5. Mo Y, Lorenzo M, Farghaly S, Kaur K, Housman ST. What's new in the treatment of multidrug-resistant gram-negative infections? [Internet]. Diagnostic Microbiology and Infectious Disease. 2019; 93(2):171-81. DOI: 10.1016/j.diagmicrobio.2018.08.007

6. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing [Internet]. 34th ed. CLSI supplement M100. Wayne, PA: CLSI; 2024. [access: 28/02/2024]. Available from: https://clsi.org/standards/products/microbiology/documents/m100/

7. Sharma K, Tak V, Nag VL, Bhatia PK, Kothari N. An observational study on carbapenem-resistant Enterobacterales (CRE) colonization and subsequent risk of infection in an adult intensive care unit (ICU) at a tertiary care hospital in India [Internet]. Infect Prev Pract. 2023; 5(4):100312. DOI: 10.1016/j.infpip.2023.100312

8. Khattab S, Sweify A, Ali M, Metwally L, Elazab S, Hashem A. Detection of plasmid-mediated colistin resistance in carbapenem-resistant Escherichia coli and Klebsiella pneumoniae isolates in Suez Canal University Hospitals [Internet]. Microbes and Infectious Diseases. 2021;2(3):497-507. DOI: 10.21608/MID.2021.78200.1162

9. Tsai YM, Wang S, Chiu HC, Kao CY, Wen LL. Combination of modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM) for phenotypic detection of carbapenemase-producing Enterobacteriaceae [Internet]. BMC Microbiol. 2020; 20(1):315. DOI: 10.1186/s12866-020-02010-3

10. Boyd SE, Livermore DM, Hooper DC, Hope WW. Metallo-β-lactamases: structure, function, epidemiology, treatment options, and the development pipeline [Internet]. Antimicrobial Agents and Chemotherapy. 2020; 64(10):10-1128. DOI:10.1128/aac.00397-20

11. Hughes S, Gilchrist M, Heard K, Hamilton R, Sneddon J. Treating infections caused by carbapenemase-producing Enterobacterales (CPE): a pragmatic approach to antimicrobial stewardship on behalf of the UKCPA Pharmacy Infection Network (PIN) [Internet]. JAC-Antimicrobial Resistance. 2020; 2(3):dlaa075. DOI: 10.1093/jacamr/dlaa075

12. Fernandes MR, Moura Q, Sartori L, Silva KC, Cunha MP, Esposito F, et al. Silent dissemination of colistin-resistant Escherichia coli in South America could contribute to the global spread of the mcr-1 gene [Internet]. Euro Surveill. 2016; 21(17):30214. DOI: 10.2807/1560-7917.ES.2016.21.17.30214

13. Bir R, Gautam H, Arif N, Chakravarti P, Verma J, Banerjee S, et al. Analysis of colistin resistance in carbapenem-resistant Enterobacterales and XDR Klebsiella pneumoniae [Internet]. Ther Adv Infect Dis. 2022; 26(9):1-15. DOI:10.1177/20499361221080650

14. Thuyet BT, Nhung NTH., Huong NL, Sy BT, Minh LVN. Tỷ lệ kháng colistin của vi khuẩn Klebsiella pneumoniae đa kháng và kháng carbapenem phân lập tại Bệnh viện Trung ương Quân đội 108 từ tháng 01/2020 đến tháng 09/2020 [Internet]. Journal of 108 - Clinical Medicine and Pharmacy. 2021; 16(DB11). DOI:10.52389/ydls.v0i0.864

15. Elaskary SA, Badawy HM. Colistin Susceptibility among Carbapenem Resistant Klebsiella pneumoniae Isolated from Menoufia University Hospitals [Internet]. The Egyptian Journal of Hospital Medicine. 2023; 90(1): 475-82. DOI:10.21608/ejhm.2023.279664

16. Karaiskos I, Souli M, Galani I, Giamarellou H. Colistin: still a lifesaver for the 21st century? [Internet]. Expert Opin Drug Metab Toxicol. 2017;13(1):59-71. DOI: 10.1080/17425255.2017.1230200

Conflict of interest

The authors declare that there is no conflict of interest.

Financial information

This research was funded by the University of Medicine and Pharmacy at Ho Chi Minh City under contract number 212 /2023 HĐ-ĐHYD dated 09/22/2023

Authors' contributions

Conceptualization: *Ngoc Tran Bich.*

Data curation: *Loc Nguyen Tran Mai, Thao Nguyen Thi Ngoc, Ngoc Tran Bich.*

Formal analysis: *Dang Tran Ngoc, Ngoc Tran Bich, Loc Nguyen Tran Mai.*

Research: *Loc Nguyen Tran Mai, Thao Nguyen Thi Ngoc, Ngoc Tran Bich, Phu Thien Truong*


2024;53(3):e024060109

Methodology: *Loc Nguyen Tran Mai, Thao Nguyen Thi Ngoc, Ngoc Tran Bich, Phương Nguyen Thi Nam, Anh Dang Tuan.*

Supervision: *Phu Thien Truong, Dat Ngo Quoc.*

Validation: *Phu Thien Truong, Van Tran Thi Hue.*

Writing – original draft: *Ngoc Tran Bich, Phu Thien Truong.*

Writing – review and editing: *Ngoc Tran Bich.*

Data availability

This research data is confidential according to the applicable confidentiality agreements and regulations and, therefore, cannot be publicly displayed or shared. The data are securely stored at the Integrated Planning Department at Cho Ray Hospital. Access to these data requires proper authorization. If you have any questions or need further information, please contact Ngoc Tran Bich at bichngoctran@ump.edu.vn.