BRIEF REPORT

REVISED *Klebsiella pneumoniae* with capsule type K64 is overrepresented among invasive disease in Vietnam

[version 2; peer review: 1 approved, 2 approved with reservations, 1 not approved]

Bich Vu Thi Ngoc[1], Sylvain Brisse[2-4], Trinh Dao Tuyet[5], Dung Vu Tien Viet[1], Kathryn E Holt[6], Trung Nguyen Vu[5], Huong Tran Thi Kieu[1], Diep Nguyen Thi Ngoc[1], H Rogier van Doorn[1,7], Heiman F L Wertheim[1,8]

[1]Oxford University Clinical Research Unit - Hanoi, Hanoi, Vietnam
[2]French National Centre for Scientific Research, Paris, France
[3]Microbial Evolutionary Genomics, Institut Pasteur, Microbial Evolutionary Genomics, Paris, France, Paris, France
[4]Institut Pasteur, Université Paris Cité, Biodiversity and Epidemiology of Bacterial Pathogens, F-75015, Paris, France
[5]National Hospital of Tropical Diseases, Hanoi, Vietnam
[6]Department of Biochemistry & Molecular Biology, Centre for Systems Genomics, University of Melbourne, Melbourne, Australia
[7]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK
[8]Department of Medical Microbiology and Radboudumc Center for Infectious Diseases, Radboudumc, Nijmegen, The Netherlands

v2 **First published:** 08 Jun 2021, **10**:454
https://doi.org/10.12688/f1000research.52799.1
Latest published: 17 Jan 2025, **10**:454
https://doi.org/10.12688/f1000research.52799.2

Abstract

Introduction

Recent reports indicate the emergence of community-acquired pneumonia associated with K64- *Klebsiella pneumoniae (K. pneumoniae)* . Here, we identify the capsular types and sequence type of invasive and commensal *K. pneumoniae* isolates from Vietnam.

Methods

We included 93 *K. pneumoniae* isolates from patients hospitalized at the National Hospital for Tropical Diseases, Hanoi between 2007 and 2011; and 110 commensal isolates from throat swabs from healthy volunteers living in rural and urban Hanoi in 2012. We determined sequence types (STs) by multi-locus sequence typing (MLST) and capsule typing for seven K types by PCR. Antibiotic susceptibility testing was performed using disk diffusion.

Results

The most common detected capsule types were K1 (39/203, 19.2%, mainly ST23) and K2 (31/203, 15.3%, multiple STs: ST65, ST86, ST380).

Open Peer Review

Approval Status ? ✓ ✗ ?

	1	2	3	4
version 2 (revision) 17 Jan 2025		✓ view	✗ view	? view
version 1 08 Jun 2021	? view	? view		

1. **John L. Kiley**, Brooke Army Medical Center, San Antonio, USA
2. **Frank R. DeLeo**, National Institutes of Health, Hamilton, USA
3. **Ning Dong**, Zhejiang University School of Medicine, Hangzhou, China
4. **Neris García-González**, Instituto de Biomedicina de Valencia (Ringgold ID: 54426), Valencia, Spain

Any reports and responses or comments on the

We found significantly more K2 isolates among invasive in comparison to commensal isolates (22.6% vs 9%, p = 0.01) but no significant difference was observed between invasive and commensal K1 isolates (14.5% vs 24.7%, p = 0.075). K64 with varying sequence types were predominantly seen among invasive *K. pneumoniae* (8 vs. 3) and were isolated from sepsis and meningitis patients. Among K64 isolates, one was carbapenem-resistant with ST799.

Conclusion

Our study confirms that capsule type K64 *K. pneumoniae* is associated with community-acquired invasive infections in Vietnam. Research is needed to unravel the mechanisms of virulence of capsule type K64 in both community and hospital settings.

Keywords
Klebsiella pneumoniae, K64, capsule type, community-acquired infections, carbapenem-resistant

article can be found at the end of the article.

This article is included in the Pathogens gateway.

Corresponding author: Bich Vu Thi Ngoc (bichvtn@oucru.org)

Author roles: Vu Thi Ngoc B: Data Curation, Methodology, Writing – Original Draft Preparation; **Brisse S**: Conceptualization, Writing – Review & Editing; **Dao Tuyet T**: Methodology, Resources; **Vu Tien Viet D**: Formal Analysis; **Holt KE**: Conceptualization; **Nguyen Vu T**: Methodology; **Tran Thi Kieu H**: Methodology; **Nguyen Thi Ngoc D**: Methodology; **van Doorn HR**: Supervision, Writing – Review & Editing; **Wertheim HFL**: Conceptualization, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: Wellcome Trust OxTREC, 49-12
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Vu Thi Ngoc B, Brisse S, Dao Tuyet T *et al.* ***Klebsiella pneumoniae* with capsule type K64 is overrepresented among invasive disease in Vietnam [version 2; peer review: 1 approved, 2 approved with reservations, 1 not approved]** F1000Research 2025, **10**:454 https://doi.org/10.12688/f1000research.52799.2

First published: 08 Jun 2021, **10**:454 https://doi.org/10.12688/f1000research.52799.1

REVISED **Amendments from Version 1**

The major differences between the old and new versions include updates based on recent understanding of *K. pneumoniae* and the K64 type, as well as revisions made in response to reviewer feedback. The methods section has been clarified, particularly regarding antibiotic susceptibility and the statistical methods applied in the analysis. Additionally, Table 1 has been revised to correct a typo that led to bias in the selection of one strain for the study. In the discussion, we address the study's limitations and further suggest the role of the K64 type in *K. pneumoniae* pathogenicity.

Any further responses from the reviewers can be found at the end of the article

Introduction

In low and middle-income countries in Asia, like Vietnam, *K. pneumoniae* is an important cause of severe community-acquired infections, including pneumonia, liver abscesses and sepsis.[1] Multidrug-resistance in *K. pneumoniae*, especially among hospital acquired infections, is an emerging problem associated with high morbidity and mortality.[2] *K. pneumoniae* is classified across two main virulence phenotypes, classical (cKp) and hypervirulent (hvKp). Most *K. pneumoniae* are associated with uncomplicated community acquired infections and nocosomial infections. However, hvKp results in more severe community – acquired infections with manifestations such as pyogenic liver abscess, meningitis, endophthalmitis, and necrotizing fasciitis.[3] The polysaccharide capsule is perhaps the most well-known virulence factor of K. pneumoniae, including for the hvKp phenotype. The capsule surrounding *K. pneumoniae* cells can be divided into at least 79 capsular types (K1 to K79), among which K1, K2, and K64 are common serotypes of the MDR-hvKp group, which is commonly found in Asia.[4,5] A genomic analysis of diversity and population structure of 288 human and animal *K. pneumoniae* isolates from six countries, spanning four continents, has shown that K64 was among the important capsule types associated with community acquired pneumonia in Vietnam (n = 3) and Singapore (n = 1).[5] In addition to cases and outbreaks reported on severe *K. pneumoniae* infections by K64 capsular type with the convergence of carbapenem-resistant phenotypes,[6] in one case report, K64-ST1764 *K. pneumoniae* was found to be a cause of pyogenic liver abscess and endogenous endophthalmitis. *K. pneumoniae* can asymptomatically colonize the gastrointestinal (proportion between 40% to 66%)[7] and upper respiratory tract of healthy humans (14.1%)[8] but carriage of K64 *K. pneumoniae* in healthy persons has rarely been described. According to previous studies, K1 and K2 are known to be highly virulent capsule types associated with community-acquired and hospital-acquired infections. K54 and K57 are mainly found in the human microbiome, particularly in the upper respiratory tract of healthy individuals. Our literature review indicates that K5 and K20 have been sporadically reported as pathogens, although they are less common. As for K64, reported by K. Holt et al. in 2015, it appears to be an emerging cause of severe disease.[5,9] Our study aims to investigate the diversity of *K. pneumoniae* in both community and hospital settings by classifying the diversity of capsular types. Here, we found K64-*K. pneumoniae* to be more common among invasive isolates as compared to commensal isolates isolated from Vietnamese individuals.

Methods

Klebsiella pneumoniae isolates and antibiotic susceptibility testing

From a total of 589 *K. pneumoniae* that were isolated from patients hospitalized at the National Hospital of Tropical Diseases between 2007 and 2011, 332 isolates were recovered after re-cultivation. Of those, 30 isolates with lacking clinical metadata were excluded, leaving the remaining 302 isolates for downstream analysis. Ninety-three were isolated from otherwise sterile sites, including blood (n = 70), cerebrospinal fluid (CSF) (n = 7), and pus (n = 16). These were re-cultured and re-confirmed using biochemical test strips (API 20E, Biomérieux, Marcy l'Étoile, France). Antibiotic susceptibility testing (AST) using disk diffusion was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2019. A phenotypic confirmatory double-disk test was performed for confirmation of ESBL production using CTX (30 mg) and CAZ (30 mg) disks alone and in combination with CA (10 mg) (Mast Diagnostic a GmbH, Reinfeld, Germany).

We collected clinical data from patients infected with these isolates (invasive isolates) and classified their infections based on the definition of healthcare-associated and community-associated infections (https://arpsp.cdc.gov/profile/infections?tab=nhsn). To ensure that the isolates were from community-acquired infections, we used a time difference of ≥48 hours between the time of admission and the time of specimen collection. To compare invasive with commensal isolates, we used randomization tools (https://www.randomizer.org/) to select 110 of 331 *K. pneumoniae* isolates from throat swabs of healthy volunteers living in rural (Bavi) and urban (DongDa district), Hanoi in 2012. The epidemiology of these healthy volunteers has been described in our previous study which was designed to investigate *K. pneumoniae* oropharyngeal carriage and rick factors in Vietnam.[8] Commensal isolates were tested and analysed in the same manner as invasive isolates.

Molecular typing

Invasive and commensal isolates were tested to identify their capsule types (for capsule types K1, K2, K5, K20, K54, K57, and K64) by polymerase chain reaction (PCR) according to previously described methods.[10,11] A specific K64 PCR was developed to detect capsule type K64 that was reported to be common in Southeast Asia[5] with the following primers: Forward (5′TTC TTT AAG TCT TCT GGG TAT CA3′) and Reverse (5′AGT CTT TAA TCG CCT TCT3′). The PCR cycling program for K64 consisted of 95°C for 15 min, followed by 30 cycles of 95 °C for 30 sec, 60 °C for 30 sec, 72 °C for 1 min 20 sec and the final elongation step was performed for 7 min at 72 °C. The PCR products were loaded on agarose (1.5%) gel electrophoresis. Samples contained PCR products with size equivalent to 782 bp as K64 positive.

Multi-locus sequence typing (MLST) was performed by sequencing the PCR products of seven house-keeping genes including (gapA, infB, mdh, pgi, phoE, rpoB, tonB). The sequence of these genes was analysed using the BIGSdb-Pasteur website (https://bigsdb.pasteur.fr/) for determining the sequence types. Sequence types (STs) were grouped into clonal complexes (CC) as described previously.[12] A clonal complex is defined as a group of STs with at least 6 identical alleles with at least one other member of the group. STs that did not fall within a CC were defined as singletons.

We used Statistical Package of Social Sciences (SPSS) version 25 (IBM corporation, Armonk (NY), USA) for analysis,[13] p values < 0.05 were considered significant (2-sided).

Ethics statement

This study was approved by the Oxford University Tropical Research Ethics Committee (Oxtrec, 49-12) and the National Hospital for Tropical Diseases Institutional Review Board. Before participation, written informed consent from subjects or, in case of minors, their caregivers, was obtained on a standard study consent form.

Results

Among 203 *K. pneumoniae* isolates, 100 (49.2%) were positive with one of the seven tested capsule (K) types (K1, K2, K5, K20, K54, K57, K64). The most common K types were K1 (n = 39) and K2 (n = 31). Whereas 36/39 (92.3%) K1 isolates belonged to STs that were classified into clonal complex, CC23, K2 isolates were more diverse: the most frequent clonal complex was CC65 (n = 18), followed by CC86 (n = 8) (Table 1). While K2 isolates were more prevalent among invasive than among commensal isolates (22.6% vs 9%, Chi-square, p = 0.01), K1 was relatively equally distributed (14.5% vs 24.7%, p = 0.075), and K57 (n = 18) was detected mostly among commensal isolates (15.4% vs 1%, p < 0.0001). We detected seven isolates with K64, five of which were invasive (p < 0.001). Among five invasive

Table 1. Clonal complex (CCs) as determined by multi-locus sequence typing (MLST) and distribution of capsular types among invasive isolates and commensal isolates of *Klebsiella pneumoniae* in Vietnam. P-value were determined by Chi-square (2-sides) test.

Clonal Complex (CC)	Overall	Commensal (n, %)	Invasive (n, %)	p-value
23	63 (31)	35 (31.8)	28 (28)	0.791
65	23 (11.3)	6 (5.5)	17 (18.2)	0.004
231	4 (2)	0 (0)	4 (4.3)	
412	9 (4.4)	8 (7.3)	1 (1)	
806	6 (3)	1 (1)	5 (5.3)	
86	8 (4)	3 (2.7)	5 (5.3)	
Others CCs	23 (11.3)	15 (13.6)	7 (7.5)	0.251
Singleton	68 (34)	42 (38.1)	26 (27.9)	0.083
Capsular type				
K1	39 (19.2)	16 (14.5)	23 (24.7)	0.075
K2	31 (15.3)	10 (9)	21 (22.6)	0.01
K5	1 (0.5)	0 (0)	1 (1)	0.458
K20	2 (1)	2 (1.8)	0 (0)	0.5
K54	2 (1)	1 (1)	1 (1)	1
K57	18 (9)	17 (15.4)	1 (1)	<0.0001
K64	7 (3.4)	2 (1.8)	5 (5.4)	<0.0001

Table 2. Comparison of the proportion of antibiotic resistance between invasive versus commensal *Klebsiella pneumoniae* isolates in Vietnam. P-value were determined by Chi-square (2-sides) test.

	Overall	Commensal (n, %)	Invasive (n, %)	p-value
ESBL	15 (7.4)	4 (3.6)	11 (11.8)	0.025
CIP (Ciprofloxacin)	7 (3.4)	0 (0)	7 (7.5)	0.002
AMC (Amoxicillin - clavulanate)	16 (7.8)	0 (0)	16 (17.2)	0.004
AMP (Ampicillin)	196 (96.5)	104 (94.5)	92 (98.5)	0.339
FEP (Cefepime)	9 (4.4)	0 (0)	9 (9.6)	0.001
GEN (Gentamicin)	N/A	N/A	14 (15)	N/A
TZP (Piperacillin -tazobactam)	6 (2.9)	0 (0)	6 (6.5)	0.008
SXT (Trimethoprim-sulfamethoxazole)	29 (14.3)	9 (8.1)	20 (21.5)	<0.001
IMP (Imipenem)	1 (0.5)	0 (0)	1 (1)	0.458

K64 *K. pneumoniae*, two were isolated from sepsis patients, one from meningitis, one from sepsis-meningitis, and one from the blood of a patient with hospital-acquired pneumonia. Most of these invasive K64 isolates (4/5) were from patients on Intensive Care Units (ICU). Of those patients, two had fatal community acquired pneumonia. The seven K64 isolates (two from commensal, five from invasive isolates) were genotyped by MLST: four belonged to the CC231 (ST231, ST799, ST807) and the other to CC65 (ST692).

Overall, antimicrobial resistant proportions of commensal isolates differed significantly from invasive *K. pneumoniae* (Table 2). Among K64 isolates, one invasive ST799 isolate from a patient with hospital-acquired pneumonia was multi-drug resistant, with resistance to imipenem, ciprofloxacin, trimethoprim/sulfamethoxazole, piperacillin-tazobactam and gentamicin. Of the remaining K64 isolates, four invasive isolates were non-carbapenem resistant but they either were resistant to trimethoprim/sulfamethoxazole or piperacillin-tazobactam. Whilst, the two commensal isolates with ST1331 and ST1347, were susceptible to all tested antibiotics.[14]

Discussion and conclusion

In addition to the emergence of carbapenem-resistant *K. pneumoniae* worldwide, previous studies have shown that infections caused by hypervirulent carbapenem susceptible *K. pneumoniae* can also be considered a threat to public health.[15] Our study suggested that besides capsule type K2, capsule type K64 was associated with invasive strains (5.4% vs 1.8%, Chi-square, $p < 0.001$), consistent with previous studies.[5,16]

The capsular type K64 has been reported worldwide, especially from clinical settings in Asia, Europe, and North America and is one of the K types associated with hypervirulent strains, that have shown simultaneous expression of virulence and carbapenem-resistance genes, posing a treatment challenge.[17] ST11-K64 is a common type in China, possibly leading to pyogenic liver abscesses.[9] Contrarily, our K64 strains were mainly found in sepsis and meningitis patients with varying STs, including: ST231, ST692, ST799, and ST807. Moreover, it is worth noting that K64 has been common in *Klebsiella pneumoniae* carbapenemase (KPC) producing ST11 strains in China, and the shift from K47 to K64 has been associated with increased virulence in this strain.[18] In our study, the carbapenem-resistant K64-ST799 was isolated from the blood of a hospital-acquired patient in 2011, and was not detected in subsequent years in surveillance efforts.[19] Likely, the K64-ST799 strain might have acquired a mobile element carrying a carbapenemase-producing gene.

In particular, K64 has been recently recognized as a capsular type potentially associated with hypervirulence and invasive disease. Indeed, the presence of K64 with several STs isolated from bacteraemia and meningitis patients in Vietnam and a pyogenic liver abscess patient in China[20] provides further evidence that strains with this capsule type are virulent.[21]

The present study has several limitations. Because of the retrospective nature of the analyzed data collection, we missed some clinical data (exposures, alcohol history, out come after treatment) of the patients. The results of this study lack evidence to support the hypothesis that the risk factor of infections may be *K. pneumoniae* colonizers. In this study, the relatively small number of K64 isolates provided only limited data about the serotype's convergent virulence and carbapenem resistance. Also, we lack the whole genome sequence data of these K64 isolates for further understanding the molecular basis of hypervirulence and the phylogenetic positioning of this rare ST within the genomic taxonomy of *K. pneumoniae*.[22] However, our results led support to the hypothesis that K64 is associated with severe invasive community acquired *K. pneumoniae* infections, including sepsis and meningitis. In addition, here, only seven capsule

types were selected to investigate the diversity of *K. pneumoniae* isolates. We recognize that this is one of the limitations of our study. Further studies are needed to unravel the mechanisms of virulence of capsule type K64 in *K. pneumoniae.*

Data availability

Underlying data

Dryad: *Klebsiella pneumoniae* with capsule type K64 is overrepresented among invasive disease in Vietnam. https://doi.org/10.5061/dryad.h44j0zpjv[14]

- Table 1 (Kp_All_AST) provides the detailed information of 203 *Klebsiella pneumoniae* isolates including source of isolates, date of collection, antibiotic susceptibility profiles, K-serotypes and MLST profiles.

- Serotype_Clinical isolates.rar and Serotype_Community isolates.rar: These folders contain the photographs of the PCR products of agarose gel electrophoresis. Maps of samples on agarose plates are described in two Excel files (Electrophoresis_map.xlsx and Isolate ID on Electrophoresis.gel.xlsx for Clinical isolates and Commensal isolates, respectively).

Consent

Before participation, written informed consent from subjects or, in case of minors, their caregivers, was obtained on a standard study consent form.

References

1. Peto L, Nadjm B, Horby P, *et al.*: **The bacterial aetiology of adult community-acquired pneumonia in Asia: a systematic review.** *Trans R Soc Trop Med Hyg.* 2014 Jun; **108**(6): 326–37.
PubMed Abstract | Publisher Full Text | Free Full Text

2. Cillóniz C, Dominedò C, Torres A: **Multidrug Resistant Gram-Negative Bacteria in Community-Acquired Pneumonia.** *Crit Care.* 2019; **23**(1): 79.
PubMed Abstract | Publisher Full Text | Free Full Text

3. Catalán-Nájera JC, Garza-Ramos U, Barrios-Camacho H: **Hypervirulence and hypermucoviscosity: Two different but complementary Klebsiella spp. phenotypes?** *Virulence.* 2017; **8**(7): 1111–23.
PubMed Abstract | Publisher Full Text | Free Full Text

4. Zhu J, Wang T, Chen L, *et al.*: **Virulence Factors in Hypervirulent Klebsiella pneumoniae.** *Front Microbiol.* 2021; **12**: 642484. [published Online First: 2021/04/27]
PubMed Abstract | Publisher Full Text | Free Full Text

5. Holt KE, Wertheim H, Zadoks RN, *et al.*: **Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health.** *Proc Natl Acad Sci U S A.* 2015; **112**(27): E3574–E81.
PubMed Abstract | Publisher Full Text | Free Full Text

6. Zhang X, Ouyang J, He W, *et al.*: **Co-occurrence of Rapid Gene Gain and Loss in an Interhospital Outbreak of Carbapenem-Resistant Hypervirulent ST11-K64 Klebsiella pneumoniae.** *Front Microbiol.* 2020; **11**: 579618.
PubMed Abstract | Publisher Full Text | Free Full Text

7. Huynh BT, Passet V, Rakotondrasoa A, *et al.*: **Klebsiella pneumoniae carriage in low-income countries: antimicrobial resistance, genomic diversity and risk factors.** 2020 Sep 2; **11**(5): 1287–99.
PubMed Abstract | Publisher Full Text | Free Full Text

8. Dao TT, Liebenthal D, Tran TK, *et al.*: **Klebsiella pneumoniae Oropharyngeal Carriage in Rural and Urban Vietnam and the Effect of Alcohol Consumption.** *PloS one.* 2014; **9**(3): e91999.
PubMed Abstract | Publisher Full Text | Free Full Text

9. Zhang Y, Jin L, Ouyang P, *et al.*: **Evolution of hypervirulence in carbapenem-resistant Klebsiella pneumoniae in China: a multicentre, molecular epidemiological analysis.** *J Antimicrob Chemother.* 2020 Feb 1; **75**(2): 327–36.
PubMed Abstract | Publisher Full Text

10. Yeh KM, Lin JC, Yin FY, *et al.*: **Revisiting the importance of virulence determinant magA and its surrounding genes in Klebsiella pneumoniae causing pyogenic liver abscesses: exact role in serotype K1 capsule formation.** *J Infect Dis.* 2010 Apr 15; **201**(8): 1259–67.
PubMed Abstract | Publisher Full Text

11. Pan Y-J, Lin T-L, Chen Y-H, *et al.*: **Capsular Types of Klebsiella pneumoniae Revisited by wzc Sequencing.** *PloS one.* 2013; **8**(12): e80670.
PubMed Abstract | Publisher Full Text | Free Full Text

12. Diancourt L, Passet V, Verhoef J, *et al.*: **Multilocus Sequence Typing of Klebsiella pneumoniae Nosocomial Isolates.** *J Clin Microbiol.* 2005; **43**(8): 4178–82.
PubMed Abstract | Publisher Full Text | Free Full Text

13. IBM SPSS Statistics for Windows: **Version 25.0. ed.** Armonk, NY: IBM Corp; 2017.

14. Bich VTN: **Supplementary of: "*Klebsiella pneumoniae* with capsule type K64 is overrepresented among invasive disease in Vietnam."** *DRYAD Dataset.* 2021.
Publisher Full Text

15. Choby JE, Howard-Anderson J, Weiss DS: **Hypervirulent Klebsiella pneumoniae - clinical and molecular perspectives.** 2020 Mar; **287**(3): 283–300.
PubMed Abstract | Publisher Full Text | Free Full Text

16. Yeh K-M, Kurup A, Siu LK, *et al.*: **Capsular serotype K1 or K2, rather than magA and rmpA, is a major virulence determinant for Klebsiella pneumoniae liver abscess in Singapore and Taiwan.** *J Clin Microbiol.* 2007 2007/02//; **45**(2): 466–71.
PubMed Abstract | Publisher Full Text | Free Full Text

17. Yang Q, Jia X, Zhou M, *et al.*: **Emergence of ST11-K47 and ST11-K64 hypervirulent carbapenem-resistant Klebsiella pneumoniae in bacterial liver abscesses from China: a molecular, biological, and epidemiological study.** *Emerg Microbes Infect.* 2020; **9**(1): 320–31.
PubMed Abstract | Publisher Full Text | Free Full Text

18. Zhou K, Xiao T, David S, *et al.*: **Novel Subclone of Carbapenem-Resistant Klebsiella pneumoniae Sequence Type 11 with Enhanced Virulence and Transmissibility, China.** *Emerg Infect Dis.* 2020 Feb; **26**(2): 289–97.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Wyres KL, Nguyen TNT, Lam MMC, *et al.*: **Genomic surveillance for hypervirulence and multi-drug resistance in invasive Klebsiella pneumoniae from South and Southeast Asia.** *Genome Med.* 2020 2020/01/16; **12**(1): 11.
PubMed Abstract | Publisher Full Text | Free Full Text

20. Zhao B, Hu R, Gong L, *et al.*: **Pyogenic Liver Abscess and Endogenous Endophthalmitis Due to K64-ST1764 Hypervirulent Klebsiella pneumoniae: A Case Report.** *Infect Drug Resist.* 2021; **14**: 71–7.
PubMed Abstract | Publisher Full Text | Free Full Text

21. Marr CM, Russo TA: **Hypervirulent Klebsiella pneumoniae: a new public health threat.** *Expert Rev Anti Infect Ther.* 2019 Feb; **17**(2): 71–3.
PubMed Abstract | Publisher Full Text | Free Full Text

22. Palma F, Hennart M, Jolley KA, *et al.*: **Bacterial strain nomenclature in the genomic era: Life Identification Numbers using a gene-by-gene approach.** *bioRxiv.* 2024: 2024.03.11.584534.
Publisher Full Text

Open Peer Review

Current Peer Review Status: ? ✓ ✗ ?

Version 2

Reviewer Report 09 August 2025

https://doi.org/10.5256/f1000research.176630.r393103

?

Neris García-González
Instituto de Biomedicina de Valencia (Ringgold ID: 54426), Valencia, Valencian Community, Spain

Peer Review for "*Klebsiella pneumoniae* with capsule type K64 is overrepresented among invasive disease in Vietnam [version 2]"
The manuscript explores the distribution of *Klebsiella pneumoniae* capsule types, with a focus on K64, among invasive and commensal isolates in Vietnam. While the topic is relevant and the data valuable, I believe the conclusions are overstated given the limited number of K64 isolates. Several aspects of the methodology and analysis require clarification or revision, particularly to address the overstatement of key conclusions. Additionally, the overall writing—especially the methods section—would benefit from careful editing for clarity.

Major Comments

1. The conclusion that "Our study confirms that capsule type K64 *K. pneumoniae* is associated with community-acquired invasive infections in Vietnam" is, in my view, overstated given the data presented. Of the 100 isolates with a successfully determined capsular type, only 11 were K64 (5 from invasive, 2 from commensal samples). This is a very small number, and the application of statistical tests to such low counts does not support a definitive conclusion.

Similarly, the claim that K64 is "associated with sepsis and meningitis" is unsupported. While some K64 isolates came from such cases, no analysis was done to test specific disease associations. This should be rephrased more cautiously (e.g., "were observed in").

1. The statement that "K64-ST799 might have acquired a mobile element carrying a carbapenemase gene" is speculative and not backed by molecular data. Without sequencing or resistance gene analysis, this claim should be softened.
2. The authors tested for only seven capsule types (K1, K2, K5, K20, K54, K57, K64). It's unclear why these were chosen—are they the most common in Vietnam, or just associated with hvKp globally? This should be clarified, especially since over half of the isolates were untypeable. Without knowing whether additional relevant types were missed, it is difficult to interpret the significance of the K64 frequency.
3. The invasive isolates were collected between 2007–2011, while the commensal isolates were collected in 2012. The invasive and commensal isolates were collected in different time periods (2007–2011 vs. 2012), which could introduce temporal bias. This limitation should be

acknowledged explicitly.

The manuscript requires substantial revision for clarity. Several sentences are difficult to parse, and the structure of some sections hinders comprehension. For example:

- The methodology does not clearly explain how the 589 *K. pneumoniae* isolates were selected. Were these all from invasive infections? If so, and only 49.2% were typeable, a large proportion of relevant invasive capsule types may have been missed. Alternatively, if this set included both invasive and commensal isolates, it would improve the comparison by providing community data from the same time period. This should be clearly stated and clarified in the manuscript.
- The distinction between the two datasets (invasive and commensal) is clear in the abstract but not well explained in the main text. I suggest rephrasing to something similar to: "To compare invasive with commensal isolates, we included commensal isolates from a previous study. These were throat swabs from healthy volunteers in rural and urban Hanoi in 2012. ..."
- The statement: "To ensure that the isolates were from community-acquired infections, we used a time difference of ≥48 hours⋯" is confusing. Community-acquired infections are typically defined as those occurring within 48 hours of hospital admission. This should be reworded for clarity.

Minor Comments

- Define MDR (multidrug-resistant) on first mention.
- In Tables 1 and 2, "Overall" column should also have (n, %)
- Correct spelling of "nocosomial" to "nosocomial."

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: microbial genomics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 06 June 2025

https://doi.org/10.5256/f1000research.176630.r383866

Ning Dong
Zhejiang University School of Medicine, Hangzhou, China

In this study on *Klebsiella pneumoniae*, the authors analyzed 203 clinical isolates and found that capsular K64 was significantly enriched in invasive infections, mainly associated with sepsis and meningitis, and some K64 strains (such as ST799) were multidrug resistant. This study suggests that K64 is an important pathogen of community-acquired invasive infections in Vietnam. I suggest the following directions for further improvement of the study:
The theoretical basis for capsule type selection was supplemented, and the latest literature (such as studies on the global prevalence of K64 after 2023) was cited to complete the discussion section.
The sample size was small and underrepresented. The sample size of K64 strains was only 7 strains (5 invasive strains and 2 commensal strains), which was too small to support the conclusion that K64 was significantly enriched in invasive infections, which may lead to limited reliability of statistical results. Second, the commensal strains lack sample coverage from multiple regions and time points to fully reflect the epidemiological characteristics of community-acquired infections in Vietnam.
The results "K64 with varying sequence types were predominantly seen among invasive *K. pneumoniae* (8 vs. 3)" was inconsistent with the table data in the text (5 invasive strains, 2 commensal strains).
The whole genome sequencing data was supplemented to analyze the virulence genes (such as *rmpA* and *magA*) and drug resistance genes (such as *KPC* and *NDM*) of K64 strain, and to clarify its pathogenic mechanism at the molecular level.
There is a logical jump between the conclusion and the data. The title and conclusion use the word "overrepresented", which is misleading. Because the proportion of K64 in invasive strains was only 5.4% (5/93), which was much lower than that of K1 (24.7%) and K2 (22.6%). Second, it was mentioned that K64 was associated with carbapenem resistance (such as the ST799 strain). However, the finding of only one drug-resistant strain cannot support the inference that the K64 strain is generally resistant to carbapenem, and more data are needed to verify it.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Epidemiology of infectious diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 28 January 2025

https://doi.org/10.5256/f1000research.176630.r360165

Frank R. DeLeo
Laboratory of Bacteriology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, 59840, USA

Revised version is appropriate.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bacterial pathogenesis and host defense. Primary focus on Staphylococcus aureus, Klebsiella pneumoniae, and human neutrophil biology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 28 June 2024

https://doi.org/10.5256/f1000research.56121.r287539

?

Frank R. DeLeo
Laboratory of Bacteriology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, 59840, USA

Ngoc et al. report capsule type and multilocus sequence type of *Klebsiella pneumoniae* clinical isolates from patients hospitalized at National Hospital for Tropical Diseases in Hanoi from 2007 to 2011. The authors compared these isolates to commensal isolates obtained by throat swab from healthy volunteers in 2012. The study is interesting and adds to our understanding of the distribution of the *K. pneumoniae* capsule types associated with human infections. I have a few comments for the authors to consider.

1. Please verify numbers of isolates in Table 1 compared with what is stated in the text. By my count, there are 110 commensal isolates (correct in the text) and 94 (stated as 93 in the text) invasive isolates. These numbers are concordant with the sum of the numbers in the Overall column, which total 204 isolates (the text indicates 203). Please verify for accuracy.
2. It would be optimal to provide text in the Table 1 legend that states what the p-value refers to from a comparison standpoint, and indicate the test used here as well.
3. I recommend providing the rationale for selecting the seven capsule types used to screen isolates in this study. Why were these seven capsule types chosen among others?
4. A conclusion of the study is that “capsule type K64 is overrepresented among invasive strains”. In reality there are only seven K64 isolates out of 204 total isolates and only five of these were from invasive disease. This is 5.4% of the total number of invasive isolates and much less than either K1 or K2. In addition, the *Conclusion* text on the title page states that K64 *K. pneumoniae* is associated with community-acquired invasive infections in Vietnam.” Please provide the criteria in the Methods section that were used to determine whether an infection is a bona fide community-acquired infection. It would be fair to state that K64 contributes to invasive disease and is among the top three capsule types tested that are associated with invasive infections. However, the term “overrepresented among invasive strains” might best be restated as “there were significantly more capsule type K64 isolates recovered from patients with invasive infections compared to healthy individuals with commensal isolates” or something along those lines.

Minor points.
Introduction: “A genomic analysis of diversity and population structure of 288 human and animal *K. pneumoniae* isolates from six countries, spanning four continents, has shown that K64 mostly found in Vietnam (n = 3) and Singapore (n = 1), was among the important capsule types associated with community acquired pneumonia.” is not easy to read.

Consider revising to:
"A genomic analysis of diversity and population structure of 288 human and animal *K. pneumoniae* isolates from six countries, spanning four continents, has shown that K64 was among the important capsule types associated with community acquired pneumonia in Vietnam (n = 3) and Singapore (n = 1)."

Introduction, penultimate sentence: change K64 capsular to K64 capsule types

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bacterial pathogenesis and host defense. Primary focus on Staphylococcus aureus, Klebsiella pneumoniae, and human neutrophil biology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 12 October 2021

https://doi.org/10.5256/f1000research.56121.r93803

?

John L. Kiley

Brooke Army Medical Center, San Antonio, TX, USA

This paper describes the epidemiology of K64 *Klebsiella pneumoniae* isolates with a particular focus on K64 from a hospital in Vietnam.

Introduction

- "...but K64 capsular have rarely been described in healthy carriers." in the introductory paragraph reads a bit unclearly.

- Adding a bit more about the importance of K64 and the differences between classic and hypervirulent types of *Klebsiella* spp. (as well as how capsules might play a role in this) would better situate the authors' opening argument as to why they are performing this current study.

Methods

- "302 *K. pneumoniae* were isolated..." are these all isolates from the hospital during this time period?

Results

- I note that ESBL production was determined in Table 2 - please clarify how you determined this in the methods.

Discussion and conclusion

- I think some discussion about the small numbers of K64 isolates would also be helpful in the limitations of the paper. The second paragraph starts off by suggesting K64 has "been little reported so far," but I think the data from China would argue against this.

    - Zhang *et al*., 2020 reported Chinese epidemiological data on K64[1].

    - Walker *et al*., 2020 for general discussion of hypervirulence and association with capsular type[2].

    - Catalán-Nájera *et al*., 2017 for hypermucoviscious, hypervirulent differences/discussion[3].

Great paper overall!

References

1. Zhang Y, Jin L, Ouyang P, Wang Q, et al.: Evolution of hypervirulence in carbapenem-resistant Klebsiella pneumoniae in China: a multicentre, molecular epidemiological analysis. *Journal of Antimicrobial Chemotherapy*. 2020; **75** (2): 327-336 Publisher Full Text
2. Walker KA, Miller VL: The intersection of capsule gene expression, hypermucoviscosity and hypervirulence in Klebsiella pneumoniae.*Curr Opin Microbiol*. **54**: 95-102 PubMed Abstract | Publisher Full Text
3. Catalán-Nájera J, Garza-Ramos U, Barrios-Camacho H: Hypervirulence and hypermucoviscosity: Two different but complementaryKlebsiella spp. phenotypes?. *Virulence*. 2017; **8** (7): 1111-1123 Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Multi drug resistant Gram-negative infections

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 04 Jan 2025

Bich Vu Thi Ngoc

We would like to thank reviewer for your valuable comments. We have addressed all the reviewers' comments in the revised manuscript, including with additional information, and responded in the point-to-point rebuttal below. We hope our revisions are adequate and make our manuscript clear and publishable.

Reviewer 1

This paper describes the epidemiology of K64 *Klebsiella pneumoniae* isolates with a particular focus on K64 from a hospital in Vietnam.

Comment 1: Introduction

"...but K64 capsular have rarely been described in healthy carriers." in the introductory paragraph reads a bit unclearly.

Response: Thank you very much for your comment. We have now revised the sentence in the updated version as below:

"...but carriage of K64 *K. pneumoniae* in healthy persons has rarely been described."

Comment 2:

Adding a bit more about the importance of K64 and the differences between classic and hypervirulent types of Klebsiella spp. (as well as how capsules might play a role in this) would better situate the authors' opening argument as to why they are performing this current study.

Response: Thank you very much for your recommendation. We have now included the following sentences in the introduction of the manuscript:

K pneumoniae is classified across two main virulence phenotypes, classical (cKp) and hypervirulent (hvKp). Most *K. pneumoniae* are associated with uncomplicated community

acquired infections and nocosomial infections. However, hvKp results in more severe community – acquired infections with manifestations such as pyogenic liver abscess, meningitis, endophthalmitis, and necrotizing fasciitis. The polysaccharide capsule is perhaps the most well-known virulence factor of *K. pneumoniae*, including for the hvKp phenotype. The capsule surrounding *K. pneumoniae* cells can be divided into at least 79 capsular types (K1 to K79), among which K1, K2, and K64 are common serotypes of the MDR-hvKp group, which is commonly found in Asia.

Comment 3: Methods

"302 *K. pneumoniae* were isolated..." are these all isolates from the hospital during this time period?

Response:

Thank you very much for your question. The total number of isolates from the hospital during this time was 589 isolates; however, because this was a retrospective study, we performed re-culture, and 332/589 isolates were recovered. Of those, 30 isolates had no associated clinical metadata, and were excluded from the study.

We now have clarified this in the manuscript as below:

“From a total of 589 *K. pneumoniae* that were isolated from patients hospitalized at the National Hospital of Tropical Diseases between 2007 and 2011, 332 isolates were recovered after re-cultivation. Of those, 30 isolates with lacking clinical metadata were excluded, leaving the remaining 302 isolates for downstream analysis.”

Comment 4: Results

I note that ESBL production was determined in Table 2 - please clarify how you determined this in the methods.

Response:

Thank you very much for your comment. We have added the information into the updated version as below:

“A phenotypic confirmatory double-disk test was performed for confirmation of ESBL production using CTX (30 mg) and CAZ (30 mg) disks alone and in combination with CA (10 mg) (Mast Diagnostic a GmbH, Reinfeld, Germany).”

Comment 5: Discussion and conclusion

I think some discussion about the small numbers of K64 isolates would also be helpful in the limitations of the paper. The second paragraph starts off by suggesting K64 has "been little reported so far," but I think the data from China would argue against this.

Response: Thank you for your recommendation.

We agree that data from China would argue against our statement in the second paragraph starts off by suggesting K64 has "been little reported so far,". We now have revised the sentence as below:

“The capsular type K64 has been reported worldwide, especially from clinical settings in Asia, Europe, and North America and is one of the K types associated with hypervirulent strains, that have shown simultaneous expression of virulence and carbapenem-resistance genes, posing a treatment challenge.”

Regarding the small numbers of K64 isolates in our study, we have added into the limitation:

“In this study, the relatively small number of K64 isolates provided only limited data about the serotype's convergent virulence and carbapenem resistance. Also, we lack the whole genome sequence data of these K64 isolates for further understanding of the molecular

basis of hypervirulence and the phylogenetic positioning of this rare ST within the genomic taxonomy of *K. pneumoniae*. However, our results led support to the hypothesis that K64 is associated with severe invasive community acquired *K. pneumoniae* infections, including sepsis and meningitis. In addition, here, only seven capsule types were selected to investigate the diversity of *K. pneumoniae* isolates. We recognize that this is one of the limitations of our study. Further studies are needed to unravel the mechanisms of virulence of capsule type K64 in *K. pneumoniae*."

Competing Interests: We do not have any competing interests to disclose