





BRIEF REPORT

Klebsiella pneumoniae with capsule type K64 is overrepresented among invasive disease in Vietnam [version 1; peer review: 1 approved with reservations]

Bich Vu Thi Ngoc¹, Sylvain Brisse^{2,3}, Trinh Dao Tuyet⁴, Dung Vu Tien Viet¹, Kathryn E Holt⁵, Trung Nguyen Vu⁴, Huong Tran Thi Kieu¹, Diep Nguyen Thi Ngoc¹, H Rogier van Doorn^{1,6}, Heiman F L Wertheim^{1,7}

¹Oxford University Clinical Research Unit and Wellcome Trust Major Asia Programme, Hanoi, Vietnam

²Microbial Evolutionary Genomics, Institut Pasteur, Paris, France

³French National Centre for Scientific Research, Paris, France

⁴National Hospital of Tropical Diseases, Hanoi, Vietnam

⁵Department of Biochemistry & Molecular Biology, Centre for Systems Genomics, University of Melbourne, Melbourne, Australia

⁶Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK

⁷Department of Medical Microbiology and Radboudumc Center for Infectious Diseases, Radboudumc, Nijmegen, The Netherlands

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Abstract

Introduction: Recent reports indicate the emergence of community-acquired pneumonia associated with K64-*Klebsiella 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). 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.

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Approval Status ?

1

version 1

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?
view

1. **John L. Kiley**, Brooke Army Medical Center, San Antonio, USA

Any reports and responses or comments on the article can be found at the end of the article.

Keywords

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



This article is included in the **Pathogens** gateway.

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

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Introduction

In low and middle-income countries in Asia, like Vietnam, *Klebsiella 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 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.³ In addition to cases and outbreaks reported on severe *K. pneumoniae* infections by K64 with the convergence of carbapenem-resistant phenotypes,⁴ in one case report, K64-ST1764 *K. pneumoniae* was found to be a cause of pyogenic liver abscess and endogenous endophthalmitis. *K. pneumoniae* can asymptotically colonize the gastrointestinal (proportion between 40% to 66%)⁵ and upper respiratory tract of healthy humans (14.1%)⁶ but K64 capsular have rarely been described in healthy carriers. 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

302 *K. pneumoniae* were isolated from patients hospitalized at the National Hospital of Tropical Diseases from 2007 to 2011. 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. 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 risk factors in Vietnam.⁶ 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.^{7,8} A specific K64 PCR was developed to detect capsule type K64 that was reported to be common in Southeast Asia³ 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.⁹ 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,¹⁰ 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.

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)	8 (8.6)	0.251
Singleton	68 (34)	42 (38.1)	26 (29)	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

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.¹¹

Table 2. Comparison of the proportion of antibiotic resistance between invasive versus commensal *Klebsiella pneumoniae* isolates in Vietnam.

	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

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.¹² Our study showed that besides capsule type K2, capsule type K64 was overrepresented among invasive strains (5.4% vs 1.8%, Chi-square, $p < 0.001$), consistent with previous studies.^{3,13}

The capsular type K64 has been little reported so far, but some reports show simultaneous possession of carbapenem-resistance genes, which poses a treatment challenge.¹⁴ ST11-K64 is a common type in China, possibly leading to pyogenic liver abscesses.¹⁵ 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.¹⁶ 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.¹⁷ 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 China¹⁸ provides further evidence that strains with this capsule type are virulent.¹⁹

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 addition, we lack the whole genome sequence data of these K64 isolates for further understanding the molecular basis of hypervirulence. However, our results confirm that K64 is associated with severe invasive community acquired *K. pneumoniae* infections, including sepsis and meningitis. Further studies are needed to unravel the mechanisms of virulence by 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>¹¹

- **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).

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Consent

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

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Reviewer Report 12 October 2021

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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

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

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

Results

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

Discussion and conclusion

- o 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.
 - o Zhang *et al.*, 2020 reported Chinese epidemiological data on K64¹.
 - o Walker *et al.*, 2020 for general discussion of hypervirulence and association with capsular type².
 - o Catalán-Nájera *et al.*, 2017 for hypermucoviscous, hypervirulent differences/discussion³.

Great paper overall!

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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.

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