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Clonal dissemination of carbapenem-resistant *Klebsiella pneumoniae* ST16 co-producing NDM-1 and OXA-232 in Thailand

Ryuichiro Abe[1,2], Yukihiro Akeda[1,3,4]*, Dan Takeuchi[1], Noriko Sakamoto[1,5], Yo Sugawara[1,5], Norihisa Yamamoto[3], Anusak Kerdsin[6], Yuki Matsumoto[7], Daisuke Motooka[7], Warawut Leolerd[8], Pitak Santanirand[8], Masato Suzuki[5], Keigo Shibayama[9], Kazunori Tomono[3], Tetsuya Iida[1,2,8,10] and Shigeyuki Hamada[1]

[1]*Japan-Thailand Research Collaboration Center on Emerging and Re-emerging Infections, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan;* [2]*Department of Bacterial Infections, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan;* [3]*Department of Infection Control and Prevention, Graduate School of Medicine, Osaka University, Osaka, Japan;* [4]*Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan;* [5]*Antimicrobial Resistance Research Center, National Institute of Infectious Diseases, Tokyo, Japan;* [6]*Faculty of Public Health, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon, Thailand;* [7]*Department of Infection Metagenomics, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan;* [8]*Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand;* [9]*Department of Bacteriology II, National Institute of Infectious Diseases, Tokyo, Japan;* [10]*Center for Infectious Disease Education and Research, Osaka University, Osaka, Japan*

*Corresponding author. E-mail: akeda@biken.osaka-u.ac.jp

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Background: *Klebsiella pneumoniae* ST258 and ST11 carrying bla_{KPC} are among the most widespread carbapenem-resistant *K. pneumoniae* strains worldwide. Our carbapenem-resistant Enterobacteriaceae surveillance in Thailand revealed a nationwide dissemination of *K. pneumoniae* ST16 isolates carrying bla_{NDM-1} and $bla_{OXA-232}$.

Objectives: To analyse the genomic details of this nationwide dissemination by focusing on plasmids and virulence factors.

Methods: Using WGS data of 119 *K. pneumoniae* ST16 isolates carrying bla_{NDM-1} obtained in our previous surveillance study, clonality of chromosomes and plasmids of the isolates with carriage of virulence factors was evaluated.

Results: Of the 119 isolates, 111 carried plasmid pKP151_NDM1, and all 104 isolates harbouring $bla_{OXA-232}$ carried plasmid pKP151_OXA232. These 104 K. pneumoniae ST16 isolates showing chromosomal clonality possessed both pKP151_NDM1 and pKP151_OXA232, demonstrating clonal dissemination of K. pneumoniae ST16 with these plasmids. The isolates had essentially similar virulence factors as those of K. pneumoniae ST16 clones carrying bla_{KPC}, which were recently reported as highly invasive clones in Brazil.

Conclusions: The potential global dissemination of these invasive clones with resistance to several antibiotics highlights the importance of appropriate monitoring and strict standard precautions.

Introduction

Among MDR Enterobacteriaceae isolates, which are being disseminated worldwide, carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is of major concern because alternative treatment options, even for common bacterial infections, are limited. Carbapenem resistance is primarily conferred by carbapenemases such as KPC, OXA-48 and NDM, which hydrolyse carbapenems. *K. pneumoniae* of the clonal group (CG) 258, including ST258 and ST11, carrying bla_{KPC}, is the most frequently reported CRKP worldwide.[1] Outbreaks of *K. pneumoniae* ST11 and ST258 carrying bla_{NDM-1}[2] and bla_{NDM-5},[3] respectively, have recently been sporadically reported, implying a worldwide clonal spread of *K. pneumoniae* CG258 acquiring various carbapenemase genes. Meanwhile, Andrey *et al.*[4] reported an outbreak of *K. pneumoniae* ST16 producing KPC-2, associated with a higher mortality rate than *K. pneumoniae* ST258 clones. Global dissemination of *K. pneumoniae* ST16 carrying both bla_{NDM-1} and $bla_{OXA-232}$ has been reported,[5,6] calling for careful monitoring of these highly virulent clones. During our nationwide surveillance of

(a)

Isolate	Carbapenemase	Coverage (%) of pKP151-NDM1	Coverage (%) of pKP151-OXA232	Isolate	Carbapenemase	Coverage (%) of pKP151-NDM1	Coverage (%) of pKP151-OXA232
C229	NDM-1 OXA-232	100	100	C600	NDM-1 OXA-232	92.7452	100
C233	NDM-1 OXA-232	100	100	C795	NDM-1 OXA-232	93.3478	100
C267	NDM-1 OXA-232	100	100	C635	NDM-1 OXA-232	92.4811	100
C250	NDM-1 OXA-232	100	100	C628	NDM-1 OXA-232	92.9743	100
KP353	NDM-1 OXA-232	90.9319	100	C632	NDM-1 OXA-232	92.4699	100
C239	NDM-1 OXA-232	100	100	C633	NDM-1 OXA-232	92.6335	100
C244	NDM-1	100	-	C790	NDM-1 OXA-232	92.774	100
C246	NDM-1 OXA-232	100	100	C791	NDM-1	93.4196	-
C225	NDM-1 OXA-232	100	100	C567	NDM-1 OXA-232	93.3183	100
C228	NDM-1 OXA-232	95.4947	100	C570	NDM-1 OXA-232	93.501	100
C234	NDM-1 OXA-232	96.8108	100	C833	NDM-1 OXA-232	92.6566	100
C251	NDM-1 OXA-232	96.8579	100	C792	NDM-1 OXA-232	92.667	100
KP181	NDM-1	99.98	-	C559	NDM-1 OXA-232	93.2313	100
C247	NDM-1 OXA-232	100	100	KP146	NDM-1 OXA-232	100	100
C263	NDM-1 OXA-232	100	100	KP155	NDM-1 OXA-232	100	100
C242	NDM-1	100	-	KP109	NDM-1 OXA-232	100	100
C262	NDM-1 OXA-232	100	100	KP193	NDM-1 OXA-232	100	100
C222	NDM-1 OXA-232	100	100	KP104	NDM-1 OXA-232	98.5139	100
C230	NDM-1 OXA-232	100	100	KP150	NDM-1 OXA-232	98.1452	100
C236	NDM-1 OXA-232	100	100	KP129	NDM-1 OXA-232	96.217	100
C252	NDM-1 OXA-232	100	100	KP111	NDM-1 OXA-232	100	100
C238R	NDM-1 OXA-232	100	100	KP225	NDM-1 OXA-232	100	100
C210	NDM-1 OXA-232	100	100	KP156	NDM-1 OXA-232	100	100
KP314	NDM-1 OXA-232	89.0061	100	KP124	NDM-1 OXA-232	100	100
KP343	NDM-1 OXA-232	88.7388	100	KP228	NDM-1 OXA-232	98.4046	100
KP307	NDM-1 OXA-232	100	100	KP147	NDM-1 OXA-232	100	100
KP317	NDM-1 OXA-232	100	100	KP148	NDM-1 OXA-232	99.842	100
C126	NDM-1 OXA-232	100	100	KP195	NDM-1 OXA-232	99.8859	100
KP256	NDM-1 OXA-232	100	100	KP107	NDM-1 OXA-232	99.7685	100
KP227	NDM-1	100	-	KP226	NDM-1 OXA-232	99.7989	100
KP378	NDM-1	100	-	KP108	NDM-1 OXA-232	99.9409	100
KP210	NDM-1 OXA-232	100	100	KP105	NDM-1 OXA-232	99.7334	100
KP217	NDM-1 OXA-232	100	100	KP190	NDM-1 OXA-232	99.9912	100
KP209	NDM-1 OXA-232	100	100	KP130	NDM-1 OXA-232	99.9665	100
KP342	NDM-1 OXA-232	100	100	KP102	NDM-1 OXA-232	99.9258	100
KP212	NDM-1	100	-	KP112	NDM-1 OXA-232	99.6648	100
KP204	NDM-1 OXA-232	100	100	KP230	NDM-1 OXA-232	100	100
KP211	NDM-1	100	-	KP247	NDM-1 OXA-232	100	100
KP202	NDM-1 OXA-232	100	100	C413	NDM-1 OXA-232	100	100
C073	NDM-1 OXA-232	100	100	KP280	NDM-1 OXA-232	100	100
KP384	NDM-1 OXA-232	93.8793	100	KP237	NDM-1 OXA-232	92.8953	100
KP44	NDM-1	12.5358	-	KP361	NDM-1 OXA-232	93.8107	100
C175R	NDM-1	29.1467	-	C138	NDM-1 OXA-232	97.32	100
C166	NDM-1	27.7261	-	KP164	NDM-1 OXA-232	97.66	100
C164	NDM-1	30.2258	-	KP236	NDM-1 OXA-232	100	100
C165R	NDM-1	30.9122	-	KP229	NDM-1 OXA-232	100	100
C411	NDM-1 OXA-232	94.2353	100	KP241	NDM-1 OXA-232	100	100
C415	NDM-1 OXA-232	93.9081	100	KP223	NDM-1 OXA-232	100	100
C831	NDM-1 OXA-232	89.4802	100	KP232	NDM-1 OXA-232	100	100
C268	NDM-1 OXA-232	100	100	KP110	NDM-1 OXA-232	100	100
C520	NDM-1 OXA-232	93.2792	100	KP240	NDM-1	100	-
C796	NDM-1 OXA-232	92.932	100	KP122	NDM-1 OXA-232	100	100
C343	NDM-1 OXA-232	93.3566	100	**KP151**	NDM-1 OXA-232	100	100
C634	NDM-1 OXA-232	93.3247	100	KP152	NDM-1 OXA-232	100	100
C794	NDM-1 OXA-232	92.4372	100	KP233	NDM-1 OXA-232	100	100
C793	NDM-1 OXA-232	93.0238	100	KP239	NDM-1 OXA-232	100	100
C629	NDM-1	93.1547	-	KP238	NDM-1 OXA-232	100	100
C627	NDM-1 OXA-232	92.9256	100	KP123	NDM-1 OXA-232	100	100
C626	NDM-1 OXA-232	93.2736	100	KP125	NDM-1 OXA-232	100	100
				KP188	NDM-1 OXA-232	100	100

(b)

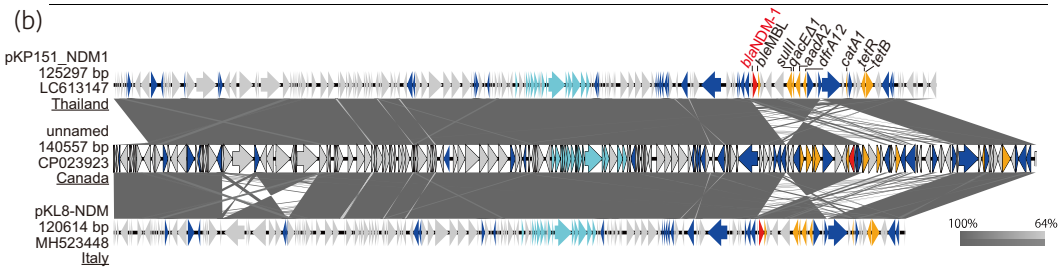

Figure 1. Similarity of the plasmids of study isolates and comparison with previously reported plasmids from other countries. (a) Similarity of the plasmids compared with the plasmids of KP151. Following mapping raw sequence reads obtained from HiSeq 3000 or PacBio RS II to the sequences of plasmids pKP151_NDM1 and pKP151_OXA232, coverages of the reference plasmids were calculated. (b) The structure of pKP151_NDM1 compared with that of previously reported plasmids. Block arrows indicate confirmed or putative ORFs and their orientations. Arrow size is proportional to the predicted ORF length. The colour code is as follows: red, carbapenem resistance gene; yellow, other antimicrobial resistance gene; light blue, conjugative transfer gene; blue, mobile element. Putative, hypothetical, or unknown genes are represented as grey arrows. The grey-shaded area indicates regions with high identity between sequences. Accession numbers of the plasmids are indicated below the plasmid size.

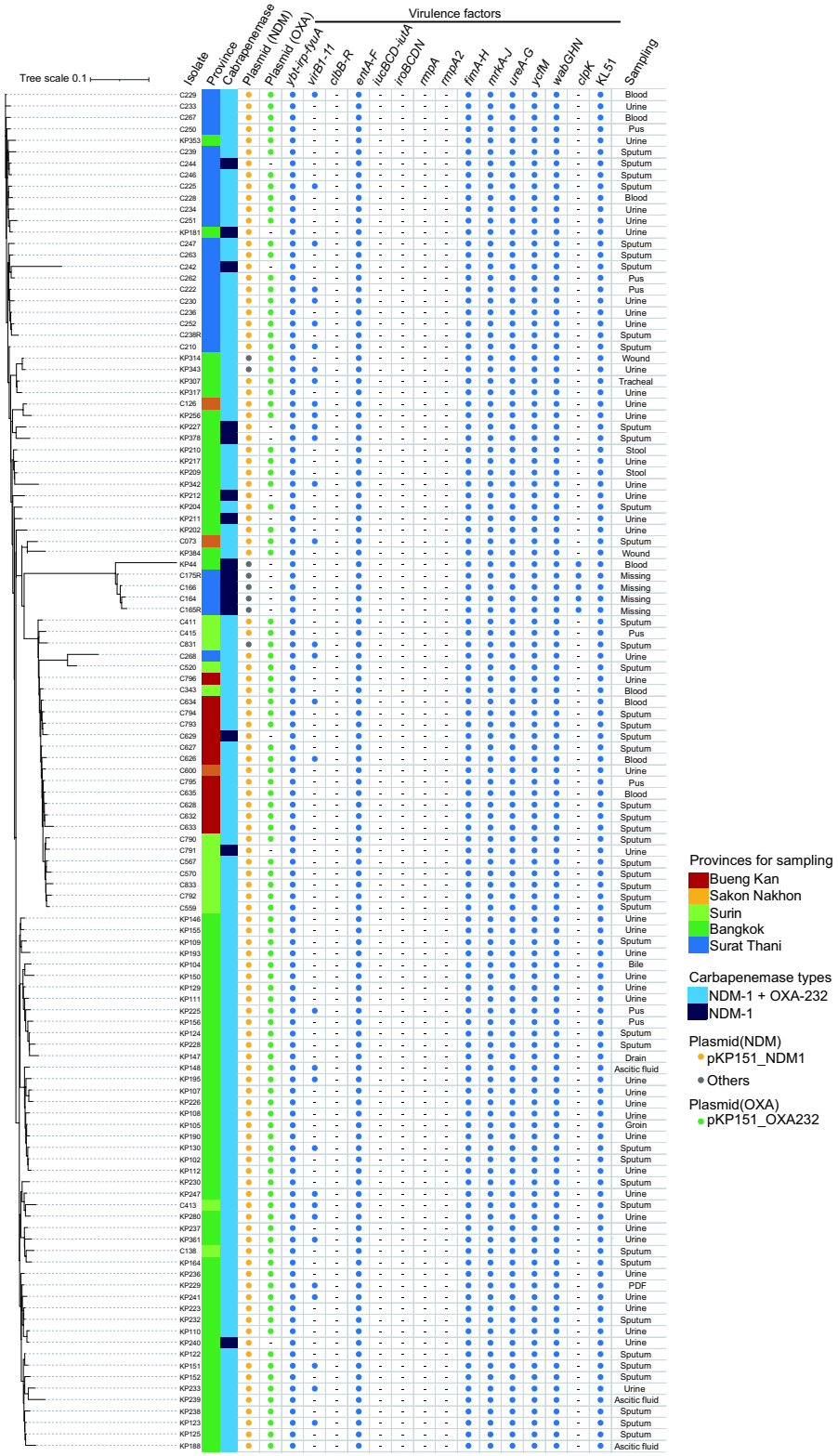

Figure 2. Chromosomal phylogeny, plasmidome and virulence factors of *K. pneumoniae* ST16 isolates carrying bla_{NDM-1}. Chromosomal phylogeny was constructed using CSI Phylogeny. Samples are colour-coded according to their collection provinces (as shown in the figure). pKP151_NDM1 and pKP151_OXA232 were used as reference plasmids for sequence mapping; plasmids carrying bla_{NDM} distinct from pKP151_NDM1 were classified as 'others'. Carriage of virulence genes was confirmed by aligning the contigs of sequenced genomes of each isolate to our virulence factor data set (Table S1).

carbapenem-resistant Enterobacteriaceae (CRE) isolates in Thailand, we identified 119 *K. pneumoniae* ST16 isolates carrying bla_{NDM-1}.[7] Here, we analysed the clonality of chromosomes and plasmids of these isolates and evaluated virulence factors shared among them.

Materials and methods

Bacterial isolates and antimicrobial susceptibility testing

Previously, we conducted nationwide surveillance of CRE isolates in Thailand, covering 11 representative hospitals in six provinces, between 2012 and 2017, and collected 747 CRE isolates from various clinical specimens of 736 patients (Figure S1, available as Supplementary data at *JAC-AMR* Online).[7] Among 493 CRE isolates carrying bla_{NDM} from 489 patients (confirmed using PCR and sequencing), 119 *K. pneumoniae* ST16 isolates carrying bla_{NDM-1} obtained from 119 patients were analysed in this study. Antimicrobial drug susceptibility was determined as previously reported.[7]

WGS analysis

WGS of all isolates was conducted using the Illumina HiSeq 3000 (Illumina, San Diego, CA, USA) or PacBio RS II (Pacific Biosciences, Menlo Park, CA, USA) platform.[8] Using a combination of GridION (Oxford Nanopore Technologies, Oxford, UK) and Illumina HiSeq 3000 on the representative isolate KP151 carrying both bla_{NDM-1} and $bla_{OXA-232}$, the complete sequence of plasmids pKP151_NDM1 and pKP151_OXA232 was determined, and plasmid clonalities were investigated by mapping the raw HiSeq 3000 and PacBio RS II reads of all isolates to the sequences of pKP151_NDM1 and pKP151_OXA232, using the Burrows–Wheeler aligner.[9] Coverages of the reference plasmids by sequence reads were calculated using SAMtools,[10] with 90% identity and coverage cut-offs. Following the identification of antimicrobial resistance genes and annotation using ResFinder 2.1[11] and RASTtk,[12] the genomic structures of the plasmids were compared with those of plasmids identified with BLAST using Easyfig.[13] Following sequence alignments using CLC Genomics Workbench 11.0.1 (CLC Bio, Aarhus, Denmark) with default settings, SNP distance analysis was performed with CSI Phylogeny 1.4,[14] using *K. pneumoniae* strain KPNIH1 (NZ_CP008827.1) as a reference. iTOL (https://itol.embl.de/) was used to illustrate the phylogenetic trees. The virulence factors were analysed as previously reported,[4] using 95% identity and coverage cut-offs. The virulence factors analysed are shown in Table S1.

Results and discussion

We performed comprehensive genomic analysis of 119 *K. pneumoniae* ST16 isolates carrying bla_{NDM-1} obtained from hospitals in five provinces in Thailand (Figure S2). First, plasmids carrying bla_{NDM-1} or $bla_{OXA-232}$ harboured by the representative isolate KP151 were determined. The IncColKP3 plasmids carrying $bla_{OXA-232}$ identified in the United States and the Netherlands were identical to pKP151_OXA232 (Figure S3), whereas the IncF1A/F1B plasmids carrying bla_{NDM-1}, which have high similarity to pKP151_NDM1, were identified in Italy and Canada (Figure 1). This implies that both plasmids (pKP151_NDM1 and pKP151_OXA232) are disseminated widely, potentially globally. Notably, pKL8-NDM was identified from a *K. pneumoniae* ST16 isolate carrying bla_{NDM-1} and $bla_{OXA-232}$ isolated in Italy.[5] Plasmid clonalities of the study isolates were investigated by mapping sequence reads to the reference plasmids pKP151_NDM1 and pKP151_OXA232 (Figure 1). Of the 119 isolates, 111 carried pKP151_NDM1, and all 104 isolates harbouring $bla_{OXA-232}$ carried pKP151_OXA232. SNP analysis of the core genomes revealed the chromosomal clonality of *K. pneumoniae* ST16 isolates carrying bla_{NDM-1}, with most differences being <60 SNPs (maximum 87 SNPs, except in eight isolates), whereas six of the eight isolates that were distinct from the predominant clones did not carry $bla_{OXA-232}$ (Figure S4). The majority of *K. pneumoniae* ST16 isolates showing chromosomal clonality possessed both pKP151_NDM1 and pKP151_OXA232, demonstrating clonal dissemination of *K. pneumoniae* ST16 with these plasmids.

The study isolates were obtained from various patient specimens, including urinary, lung, abdominal, abscess and venous blood specimens, implying that there is no infection-site specificity (Figure 2). All isolates were highly resistant to various antibiotics, including aztreonam and levofloxacin (Figure S5). Colistin and aminoglycosides are potential candidates for combination therapy, with colistin, gentamicin and amikacin susceptibility of 76.4% (91/119), 99.1% (118/119) and 100% (119/119), respectively. Furthermore, we analysed the virulence factors of these highly invasive clones (Figure 2). These isolates shared various virulence-determinant genes (*ybt-irp-fyuA*, *entA-F*, *fimA-H*, *mrkA-J*, *urea-G*, *wabGHN* and capsular genes of KL51) corresponding to highly invasive *K. pneumoniae* ST16 clones producing KPC, which was recently reported in Brazil.[4] Hypervirulence genes (*iucBCD-iutA*, *iroBCDN*, *rmpA* and *rmpA2*) were not identified in the ST16 isolates in the current and previous studies, implying the involvement of unknown key factors in the ST16 virulence profile.

We report nationwide clonal dissemination of *K. pneumoniae* ST16 isolates carrying bla_{NDM-1} and $bla_{OXA-232}$ in Thailand. These clones possess the same virulence genes as highly invasive *K. pneumoniae* ST16 clones, causing various infections. Appropriate monitoring of the global dissemination of this invasive clone with broad spectrum antimicrobial resistance and prevention of its dissemination are urgently required.

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Transparency declarations

None to declare. The manuscript was edited by Editage, a language-editing company.

Author contributions

R.A., Y.A. and S.H. conceptualized the study; N.S. and Y.S. performed the experiments; R.A., D.T., Y.A., Y.M. and D.M. analysed the data; R.A. drafted the manuscript. All authors have revised and approved the final manuscript.

Data availability

WGS data are available from the DNA Data Bank of Japan under the accession numbers listed in Table S2.

Supplementary data

Figures S1 to S5 and Tables S1 and S2 are available as Supplementary data at *JAC-AMR* Online.

References

1 David S, Cohen V, Reuter S *et al*. Integrated chromosomal and plasmid sequence analyses reveal diverse modes of carbapenemase gene spread among *Klebsiella pneumoniae*. *Proc Natl Acad Sci U S A* 2020; **117**: 25043–54. https://doi.org/10.1073/pnas.2003407117

2 Yu F, Hu L, Zhong Q *et al*. Dissemination of *Klebsiella pneumoniae* ST11 isolates with carbapenem resistance in integrated and emergency intensive care units in a Chinese tertiary hospital. *J Med Microbiol* 2019; **68**: 882–9. https://doi.org/10.1099/jmm.0.000981

3 Zhang X, Wang W, Yu H *et al*. New Delhi metallo-β-lactamase 5-producing *Klebsiella pneumoniae* sequence type 258, Southwest China, 2017. *Emerg Infect Dis* 2019; **25**: 1209–13. https://doi.org/10.3201/eid2506.181939

4 Andrey DO, Dantas PP, Martins WBS *et al*. An emerging clone, *Klebsiellapneumoniae* carbapenemase 2-producing *K. pneumoniae* sequence type 16, associated with high mortality rates in a CC258-endemic setting. *Clin Infect Dis* 2020; **71**: e141–50. https://doi.org/10.1093/cid/ciz1095

5 Espinal P, Nucleo E, Caltagirone M *et al*. Genomics of *Klebsiella pneumoniae* ST16 producing NDM-1, CTX-M-15, and OXA-232. *Clin Microbiol Infect* 2019; **25**: 385.e1––e5. https://doi.org/10.1016/j.cmi.2018.11.004

6 Boonyasiri A, Jauneikaite E, Brinkac LM *et al*. Genomic and clinical characterisation of multidrug-resistant carbapenemase-producing ST231 and ST16 *Klebsiella pneumoniae* isolates colonising patients at Siriraj Hospital, Bangkok, Thailand from 2015 to 2017. *BMC Infect Dis* 2021; **21**: 142. https://doi.org/10.1186/s12879-021-05790-9

7 Takeuchi D, Kerdsin A, Akeda Y *et al*. Nationwide surveillance in Thailand revealed genotype-dependent dissemination of carbapenem-resistant Enterobacterales. *Microb Genom* 2022; **8**. https://doi.org/10.1099/mgen.0.000797

8 Sugawara Y, Akeda Y, Hagiya H *et al*. Spreading patterns of NDM-producing Enterobacteriaceae in clinical and environmental settings in Yangon, Myanmar. *Antimicrob Agents Chemother* 2019; **63**: e01924-18. https://doi.org/10.1128/AAC.01924-18

9 Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 2009; **25**: 1754–60. https://doi.org/10.1093/bioinformatics/btp324

10 Li H, Handsaker B, Wysoker A *et al*. The sequence alignment/map format and SAMtools. *Bioinformatics* 2009; **25**: 2078–9. https://doi.org/10.1093/bioinformatics/btp352

11 Zankari E, Hasman H, Cosentino S *et al*. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 2012; **67**: 2640–4. https://doi.org/10.1093/jac/dks261

12 Brettin T, Davis JJ, Disz T *et al*. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci Rep* 2015; **5**: 8365. https://doi.org/10.1038/srep08365

13 Sullivan MJ, Petty NK, Beatson SA. Easyfig: a genome comparison visualizer. *Bioinformatics* 2011; **27**: 1009–10. https://doi.org/10.1093/bioinformatics/btr039

14 Kaas RS, Leekitcharoenphon P, Aarestrup FM *et al*. Solving the problem of comparing whole bacterial genomes across different sequencing platforms. *PLoS One* 2014; **9**: e104984. https://doi.org/10.1371/journal.pone.0104984