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Letter to the Editor

Emergence of IMP-producing hypervirulent *Klebsiella pneumoniae* carrying a pLVPK-like virulence plasmid

Sir,

Whilst classic *Klebsiella pneumoniae* isolates cause infections mainly in hospitalised or immunocompromised patients, invasive infections due to hypervirulent *K. pneumoniae* (hvKp) isolates often involve otherwise healthy persons living in the community. Among the major clones of hvKp identified thus far, isolates of capsular genotype K1 belonging to sequence type 23 (ST23) are the most common [1]. Although the genetic background of hvKp isolates is diverse, these isolates carry in common pLVPK-like virulence plasmids harbouring major virulence genes such as *rmpA* (regulator of mucoid phenotype A) and those encoding siderophores (e.g. *iroBCDN, iucABCD* and *iutA*) [1]. Whilst hvKp isolates are usually susceptible to commonly used antimicrobial agents, recent studies have described the emergence of carbapenemase-producing hvKp strains. These strains have been described mainly from mainland China and appear to be spreading in the country [2,3]. Genetic analysis of carbapenemase-producing hvKp strains suggests that these strains emerged by two pathways, through acquisition of resistance genes by hvKp strains (e.g. K1-ST23) and through acquisition of virulence genes by multidrug-resistant strains (e.g. K47-ST11) [2,3].

Here we report the identification of an IMP-type carbapenemase-producing hvKp strain. *Klebsiella pneumoniae* THC11 is a meropenem-resistant isolate collected in a molecular epidemiological study of carbapenemase-producing Enterobacteriaceae conducted in a community hospital in Nara, Japan [4]. THC11 was isolated from a urine sample of an 82-year-old woman on Day 150 of hospitalisation and the infection was treated with levofloxacin. Although the patient eventually died during hospitalisation, the contribution of the infection to this outcome could not be determined. THC11 belongs to ST23 and carries the $bla_{\text{IMP-6}}$ gene, suggesting that the isolate is a carbapenemase-producing hvKp strain. THC11 was positive for the string test, showing a viscous string of >5 mm in length when a colony was stretched with an inoculation loop. Whole-genome sequencing of THC11 was performed using Illumina MiSeq (Illumina Inc., San Diego, CA) and MinION (Oxford Nanopore Technologies, Oxford, UK). Library preparation for Illumina MiSeq sequencing was performed with a QIAseq FX DNA Library Kit (QIAGEN, Tokyo, Japan). DNA extraction, library preparation and two-dimensional sequencing for MinION sequencing were performed using a NucleoBond® AXG 20 column (Takara Bio, Shiga, Japan) combined with a NucleoBond® Buffer Set III (Takara Bio), Rapid Barcoding Kit SQK-RBK004 (Oxford Nanopore Technologies) and R9.4 flow cell (Oxford Nanopore Technologies), respectively. Hybrid de novo assembly both using MiSeq and MinION reads was conducted with SPAdes v.3.12.0 after initial reads created by MiSeq were quality trimmed with Trimmomatic v.0.38.

The capsular genotype was identified as K1 using Kaptive (http://kaptive.holtlab.net/). THC11 carried a pLVPK-like plasmid (pTHC11-1) harbouring the virulence genes *rmpA, rmpA2* (with frameshift), *iroBCDN, iucABCD* and *iutA* (Fig. 1A). In addition, THC11 carried an IncN plasmid (pTHC11-2) harbouring *aacA4*, $bla_{\text{IMP-6}}$, *aadA2* and *sul1* in a class 1 integron (In*722*). The genetic structure of pTHC11-2 resembled that of pKPI-6, an IncN plasmid harbouring $bla_{\text{IMP-6}}$ and $bla_{\text{CTX-M-2}}$ carried by a *K. pneumoniae* strain isolated in Hiroshima, Japan (Fig. 1B). Whilst $bla_{\text{IMP-6}}$ was successfully transferred to a rifampicin-resistant *Escherichia coli* K12 mutant by conjugation, *rmpA* was not identified by PCR in the transconjugant, further substantiating the location of $bla_{\text{IMP-6}}$ and virulence genes on different plasmids. Since K1-ST23 isolates have been associated with carriage of a pLVPK-like plasmid, it can be speculated that THC11 originally carried the pTHC11-1 virulence plasmid and acquired the pTHC11-2 resistance plasmid thereafter [1]. *Escherichia coli* ($n=4$), *K. pneumoniae* ($n=4$, in addition to THC11), *Enterobacter cloacae* ($n=1$) and *Proteus mirabilis* ($n=1$) isolates carrying IncN plasmids and $bla_{\text{IMP-6}}$ and $bla_{\text{CTX-M-2}}$ genes were isolated in the same year from patients hospitalised in the same ward where the patient from whom THC11 was isolated was hospitalised [4]. Therefore, it is possible that THC11 acquired pTHC11-2 from other IMP-6-producing Enterobacteriaceae inhabiting hospitalised patients or the hospital environment.

Whilst hvKp isolates producing KPC, NDM and OXA-48-like enzymes have been identified, to the best of our knowledge this is the first report of identification of IMP-producing hvKp [2,3]. A recent molecular epidemiological study showed that ca. 5% of *K. pneumoniae* clinical isolates in Japan were K1-ST23 [5]. Although IMP enzymes are the most common carbapenemases in Japan, these isolates represent <1% of *K. pneumoniae* clinical strains isolated in 2016 according to the nationwide surveillance of antimicrobial resistance conducted by the National Institute of Infectious Diseases and Ministry of Health, Labour and Welfare of Japan (https://janis.mhlw.go.jp/english/report/index.html). The fact that an hvKp strain has acquired a resistance plasmid carrying bla_{IMP} despite the low prevalence of IMP-producing isolates in Japan may pose a new public-health concern.

Accession no

All genome sequences have been deposited in the NCBI database under BioProject accession no. PRJDB8050.

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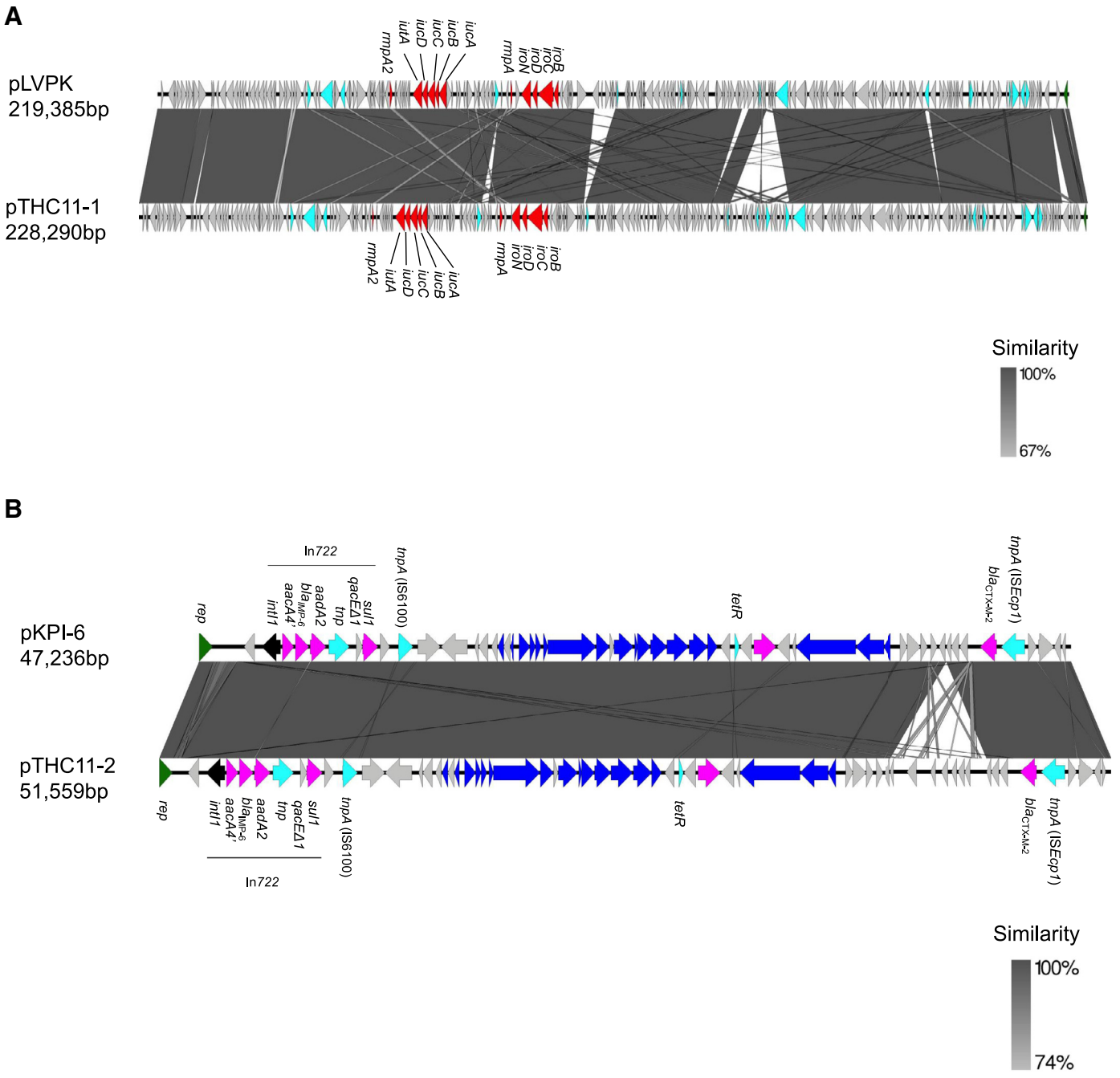

Fig. 1. Schematic representation of the genomic structure of (A) plasmid pTHC11-1 (GenBank accession no. AP019549) compared with pLVPK (AY378100) and (B) plasmid pTHC11-2 (AP019550) compared with pKPI-6 (AB616660) drawn using EasyFig v.2.1. Solid arrows indicate confirmed or putative open reading frames (ORFs) and their orientations; arrow size is proportional to the predicted ORF length. The colour code is as follows: green, replication initiation protein genes; blue, conjugal transfer genes; cyan, transposase genes; black, integrase genes; magenta, antimicrobial resistance genes; red, virulence genes; grey, other.

Competing interests

None declared.

Ethical approval

Not required.

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Sohei Harada*
Department of Infectious Diseases, Fujita Health University School of Medicine, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake, Aichi 470-1192, Japan

Kotaro Aoki, Yoshikazu Ishii
Department of Microbiology and Infectious Diseases, Toho University School of Medicine, Tokyo 143-8540, Japan

Yuki Ohno
Department of Clinical Bacteriology, Clinical Laboratory Medicine, Tenri Hospital, Tenri, Nara 632-8552, Japan

Akihiro Nakamura, Masaru Komatsu
Department of Clinical Laboratory Science, Faculty of Health Care, Tenri Health Care University, Tenri, Nara 632-0018, Japan

Kazuhiro Tateda
Department of Microbiology and Infectious Diseases, Toho University School of Medicine, Tokyo 143-8540, Japan

*Corresponding author. Tel.: +81 562 93 2800; fax: +81 562 93 4003.
E-mail address: haradas-tky@umin.ac.jp (S. Harada)

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