



## Letter to the Editor

### New NDM-producing and *bla*<sub>KPC-2</sub> harbouring *Klebsiella pneumoniae* ST6668 (CC147) clone in Milan, Northern Italy



Editor: Xi Li

Dear Editor,

*Klebsiella pneumoniae* (*Kp*) is a major nosocomial pathogen known for its high genetic diversity. In the last decade *Kp* strains producing *Klebsiella pneumoniae* carbapenemase (*Kp*-KPC) have been the most frequent carbapenem resistant strains in Europe and in Italy [1]. In Northern Italy, carbapenem resistance is mainly driven by plasmid-borne KPC-2/3, linked to outbreak-prone clones like ST258/512, ST307, and ST101 [1].

The emergence of New Delhi metallo-beta-lactamase (NDM)-producing Enterobacterales is a major global concern. The *bla*<sub>NDM</sub> gene not only confers resistance to most  $\beta$ -lactams, but is often accompanied by several resistance determinants in multidrug-resistant (MDR) pathogens, and the gene is often associated with hospital- and community-acquired infections [2].

Starting from 2019 in Italy a large diffusion of NDM-1-producing *Kp* Sequence Type (ST) 147 occurred [3], including a large outbreak in the Tuscany region [4]. In 2023, Mileto and colleagues (2023) reported the spreading of the ST147-related *Kp* lineage ST6668 in Pavia and Voghera cities, in Northern Italy [5].

Here we describe the isolation, during a nosocomial outbreak, of a new clone of NDM-producing *Kp* ST6668 characterized by a diverging gene resistance profile, including the plasmid *IncFII* and *bla*<sub>KPC-2</sub> carbapenemase (Fig. 1 and Table S1).

The figure provides a graphical overview of key information related to the outbreak and the isolated clone. On the left, the representative phylogenetic tree shows that strain Sacco-ST6668-2 is genetically divergent (indicated by the dashed line) from the other strains isolated during the outbreak. In the centre, the timeline of isolation dates and the main genetic characteristics of the strains are presented. All strains harbour the *bla*<sub>NDM</sub> gene, and all strains except Sacco-ST6668-2 carry an *IncFII* plasmid harbouring the *bla*<sub>KPC-2</sub> gene. Moreover, only Sacco-ST6668-2 carries the *bla*<sub>OXA-1</sub> gene.

In the Medical Ward of Presidio Sacco Hospital (ASST Fatebenefratelli Sacco, Milan), weekly microbiological surveillance for MDR organisms is conducted based on individual patient risk factors. The surveillance protocol includes rectal, cutaneous, and nasal swabs to guide targeted infection prevention strategies. Between January 20 and February 2, 2025, five patients tested positive for carbapenem-resistant *Kp*, identified via MALDI-TOF MS (BioMérieux, France), with isolates producing NDM and KPC enzymes. As these cases involved patients in adjacent beds cared for

by the same medical and nursing staff, the Hospital Infection Control Committee (CIO) suspected a potential outbreak, and a molecular investigation was carried out.

Antibiotic resistance profiling of the strains was performed using a combination of the VITEK® 2 system (bioMérieux, France; interpreted according to EUCAST 2025 guidelines), the Xpert Carba-R assay (Cepheid, Sunnyvale, CA), and whole-genome sequencing (WGS) conducted on the Ion GeneStudio™ S5 System (Ion Torrent, Thermo Fisher Scientific, Waltham, MA), followed by comprehensive bioinformatic analyses, including *in silico* Multi-Locus Sequencing Typing (MLST), core SNP phylogenetic analysis and *in silico* determination of resistance and virulence gene presence.

All strains were identified as *Kp* belonging to sequence type ST6668 and presented a low-virulent pattern of virulence genes comparable to that observed in the *Kp* strain isolated in Pavia and Voghera in 2023.

Core SNP-based phylogenetic analysis revealed that four out of the five strains formed a distinct sub-lineage within ST6668, separated from the remaining strain by at least 175 SNPs. This sub-lineage was also characterised by a unique plasmid profile and distinct resistance determinant pattern (Fig. S1).

Moreover, the *in silico* antibiotic resistance profiling revealed that these four divergent strains harbour the *bla*<sub>KPC-2</sub> carbapenemase gene, localised on a *IncFII* plasmid (Fig. 1). Indeed, all four WGS assemblies co-localized the *bla*<sub>KPC-2</sub> gene and the *IncFII* incompatibility group on the same contigs, which ranged in size from 71,669 to 83,225 nucleotides and shared a 71,669-nt overlapping region containing a total of 11 single nucleotide polymorphisms (SNPs).

This *bla*<sub>KPC-2</sub>/*IncFII* combination has been frequently associated with epidemic lineages ST258, ST512, and ST307, suggesting that plasmid exchange events have occurred. To identify a possible source of plasmid transmission, the *IncFII*-containing contigs were then Blastn searched against the plasmid collection PLSDB 2025. The analysis returned four matches. Three out of the four plasmids (NZ\_CP022920.1, NZ\_CP022923.1, NZ\_CP022917.1) were obtained from *Kp* ST307 strains isolated during a 2014–2015 outbreak in Korea, while the fourth (NZ\_MW650887.1) originated from a university hospital in Rome between 2019 and 2020.

Overall, our findings illustrate the dynamic nature of *Kp* epidemiology, where emerging sub-lineages can rapidly gain clinically significant resistance traits through horizontal gene transfer. The detection of a novel ST6668 clone co-producing NDM and KPC in Milan underscores the need for integrated genomic surveillance, strict infection control, and continued research into the evolutionary mechanisms underlying resistance convergence.

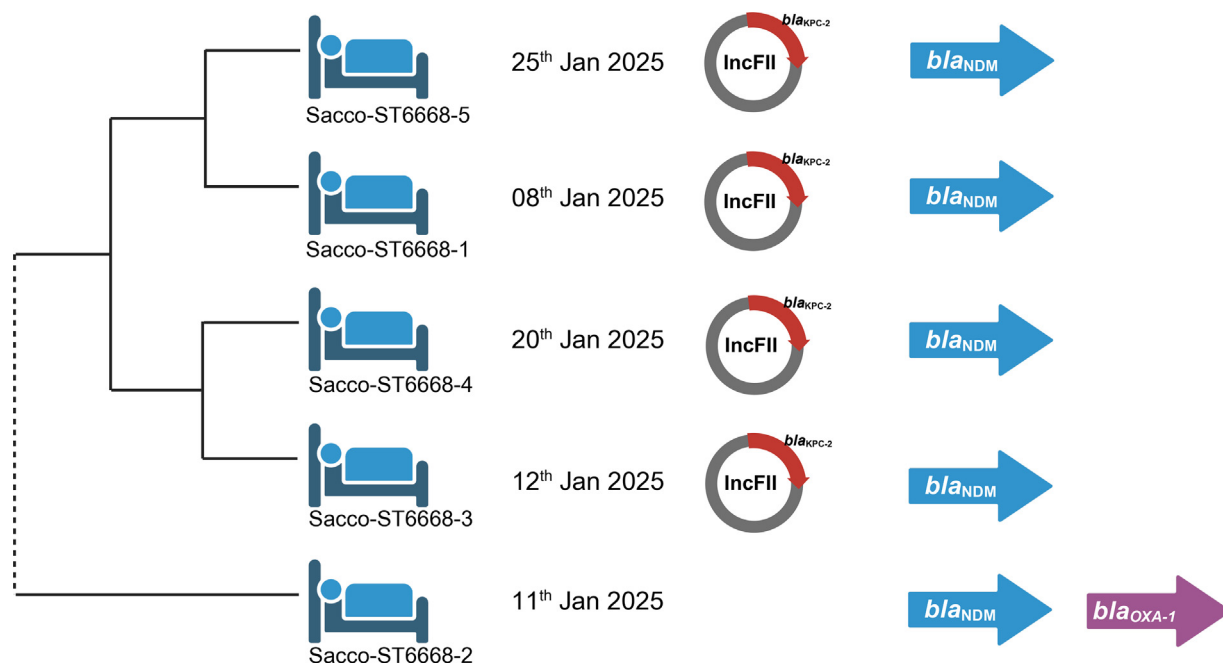


Figure 1. Graphical summary.

#### Data availability

Sequencing data are available on NCBI under BioProject PRJNA1266604.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jgar.2025.11.016](https://doi.org/10.1016/j.jgar.2025.11.016).

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