

# Prospective surveillance and rapid whole genome sequencing detects an unsuspected outbreak of carbapenemase-producing *Klebsiella pneumoniae* in a UK hospital

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## Introduction

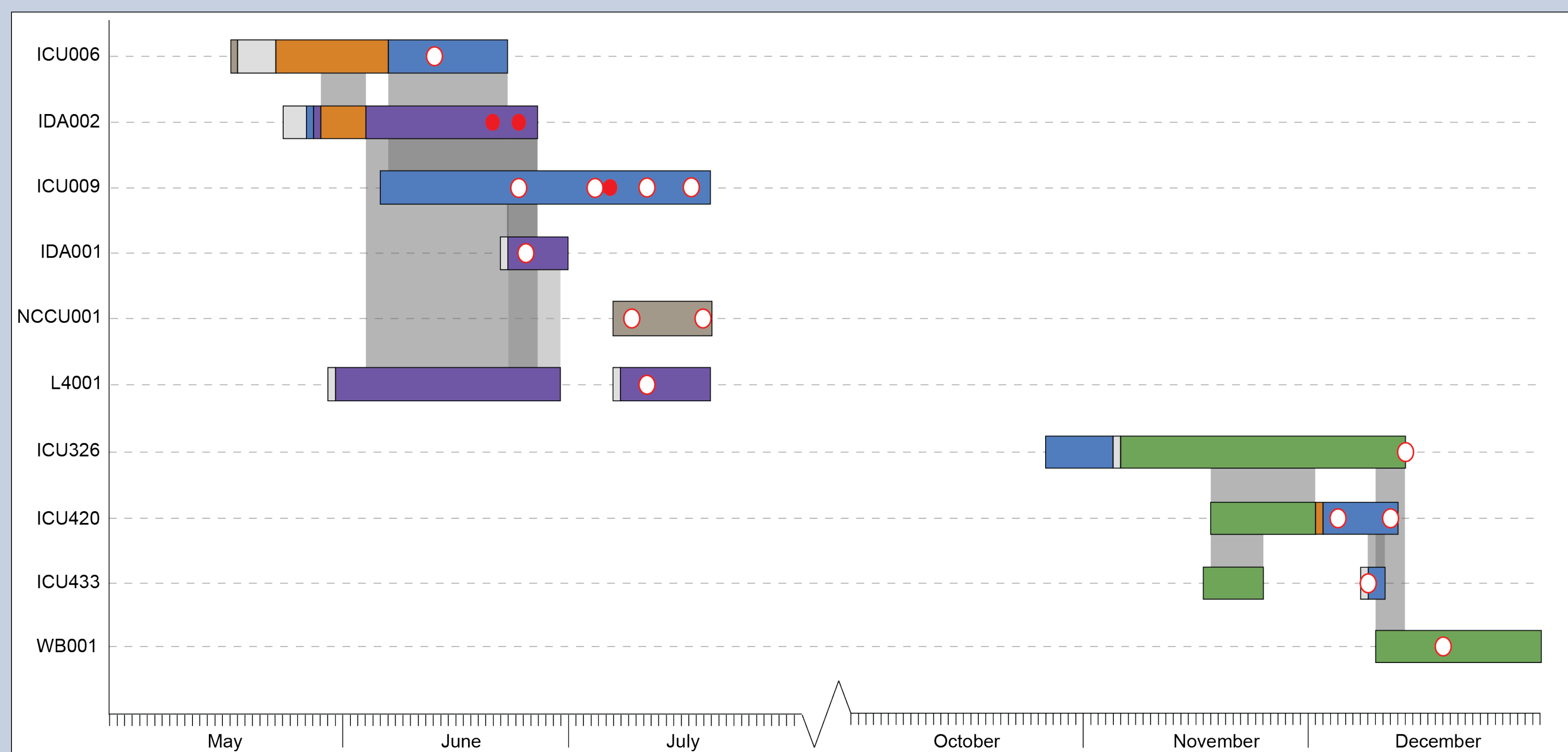
Enterobacteriaceae such as *Klebsiella pneumoniae*, are a major cause of healthcare-associated infections. Antimicrobial resistance caused by carbapenemase-producing Enterobacteriaceae (CPE) is increasing globally, and treatment options are very limited. The prevalence of CPE in UK intensive care units is unknown, as national screening guidelines only recommend screening in patients considered to be at high risk of CPE. Therefore, we conducted a surveillance study for multidrug-resistant (MDR) organisms in the intensive care unit (ICU) using whole-genome sequencing.

## Methodology

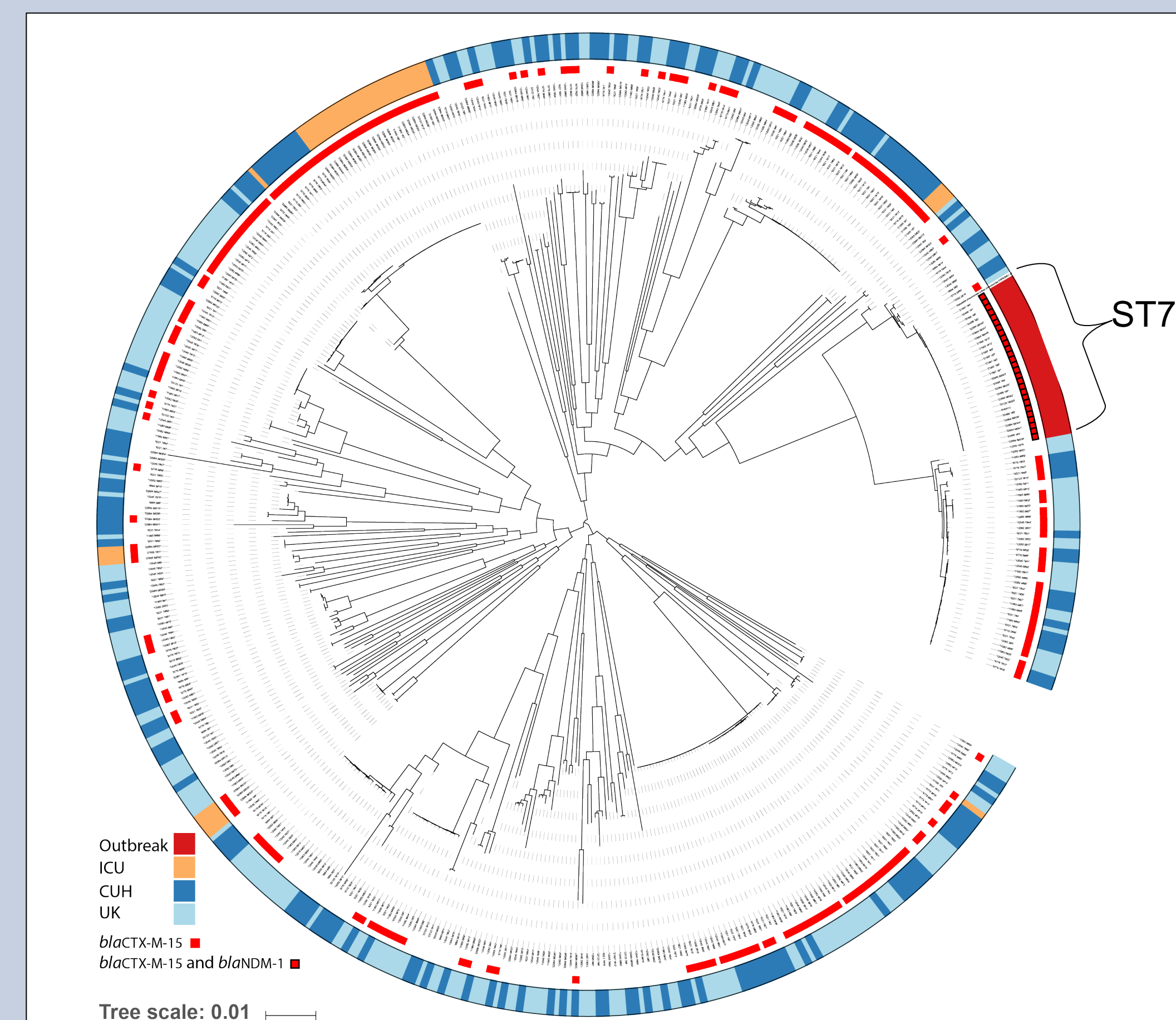
We conducted a surveillance study to determine the prevalence of MDR organisms in an adult ICU. All patients admitted to the ICU during the study period (June – December 2016) were enrolled in the study and screened for MDRO on admission, on discharge, and weekly during the ICU stay. Surveillance samples included rectal swabs or stool, urine, sputum or tracheal aspirates and wound swabs. Isolates were characterised taxonomically and for their antibiotic susceptibility profiles, before undergoing whole-genome sequencing, to determine the relatedness of the isolates. Environmental samples were collected monthly from the ICU and ward B (involved in the second outbreak), from patient bed bays and fixed and mobile computer stations.

## Results

- During the first month of the study one participant (ICU006) had a rectal swab positive for an MDR *K. pneumoniae*, which was highly resistant to carbapenem antibiotics but susceptible to colistin
- Screening and epidemiological investigation identified a further five patients, with epidemiological links to the index case, who were carrying or infected with a multidrug-resistant *K. pneumoniae* strain (Figure 1).
- Five months after the last case, two additional patients on the ICU tested positive for multi-drug resistant *K. pneumoniae*
- Contact tracing indicated involvement of an additional ward (ward B) and screening of these patients identified two further carriers of the MDR *K. pneumoniae* suggesting reappearance of the outbreak strain



**Figure 1:** Epidemiological timeline of patients carrying or infected with NDM-1 positive *K. pneumoniae*. Patients are detailed on the vertical axis and time along the horizontal axis. Horizontal coloured blocks show stays on different wards by each patient. Vertical lighter boxes indicate possible contacts between patients on wards. Filled red circles indicate positive infection samples. White circles with red outlines indicate positive carriage samples.



**Figure 2:** Phylogenetic tree of core genome single nucleotide polymorphisms (SNPs) in outbreak and contextual *K. pneumoniae* isolates. The outer coloured ring indicates the location type of each isolate. Red boxes show the presence of the ESBL *bla*<sub>CTX-M-15</sub> gene. Red boxes with black borders indicate the presence of *bla*<sub>CTX-M-15</sub> and *bla*<sub>NDM-1</sub> genes

- Pairwise SNP comparison of suspected outbreak isolates revealed highly related isolates suggesting recent transmission (Figure 3, grey shaded areas)
- Isolates from both outbreaks differed from each other by up to 9 SNPs
- The isolate from the 'index' patient was identical to that of the second patient involved in the outbreak
- Three environmental isolates from ward B were identical to 2 isolates from patients in the December outbreak
- Contemporary non-outbreak isolates differed to outbreak isolates by a minimum of 21000 SNPs
- Outbreak isolates carried more antimicrobial resistance genes (20-24) than non-outbreak ESBL isolates (2-19)
- Other closely related isolates indicated by dark blue areas in Figure 3 were longitudinal isolates collected from the same patients
- In particular, one patient carried the same lineage in their stool, urine and sputum.

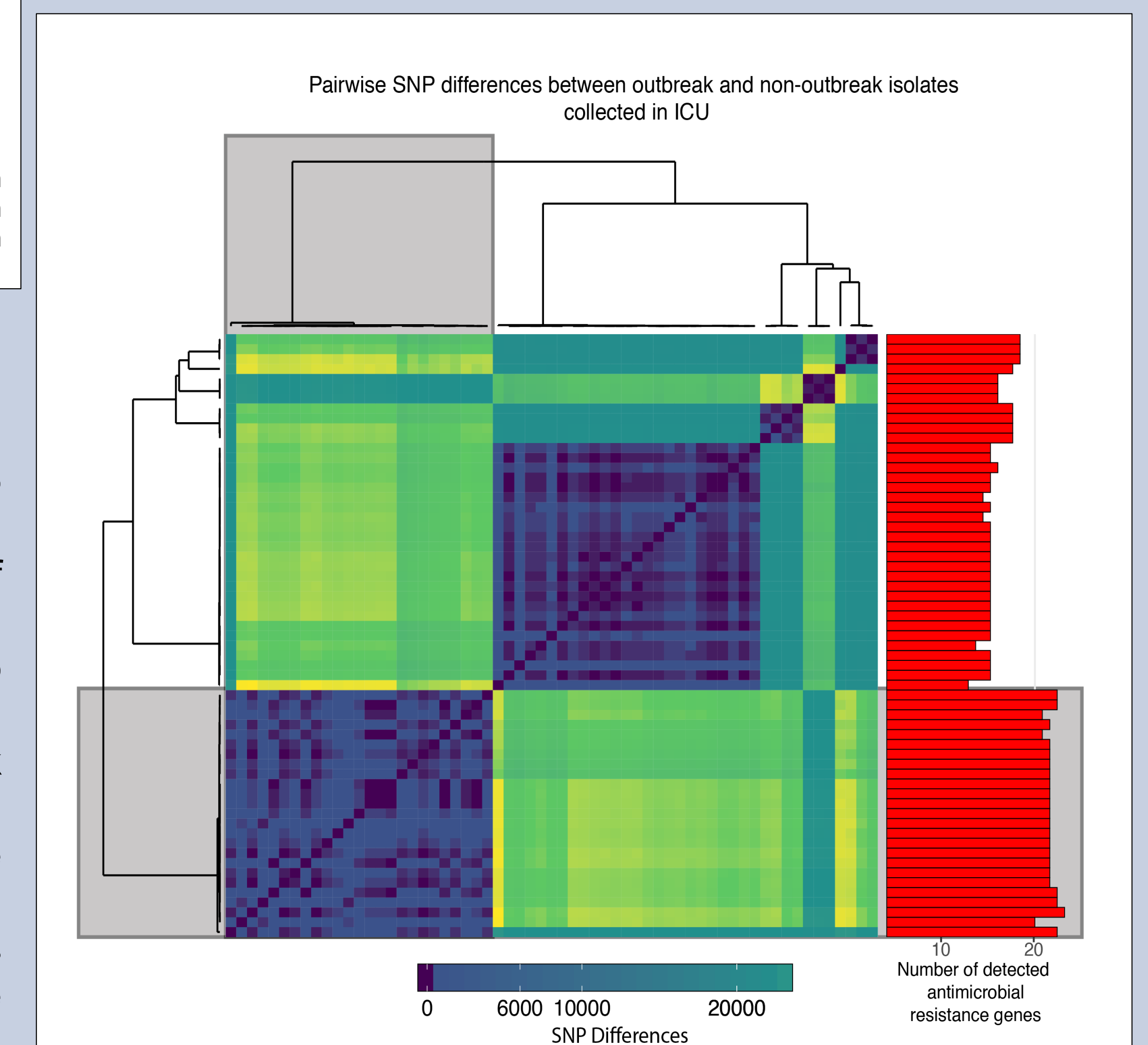
## Conclusions

- Prospective surveillance of ICU patients identified two unsuspected outbreaks *K. pneumoniae* carrying *bla*<sub>NDM-1</sub>, in patients with no risk factors for CPE.
- The outbreak isolates were not closely related to other hospital isolates, suggesting a recent introduction of this lineage
- We suggest that surveillance for CPE should be extended to all patients in critical care settings.

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## Results continued

- Whole genome sequencing of contextual UK<sup>1</sup> and local *K. pneumoniae* isolates showed that the two outbreaks were caused by the same lineage (Figure 2)
- *K. pneumoniae* collections demonstrated high levels of diversity across the species
- *bla*<sub>NDM-1</sub> negative *K. pneumoniae* isolates collected from ICU during the outbreak were genetically distinct
- Each positive patient carried their own lineage with the exception of those involved in the outbreak
- Outbreak isolates were sequence type (ST)78, a novel single-locus variants of ST14
- *bla*<sub>NDM-1</sub> was identified in all outbreak isolates and 2 unrelated isolates from the midlands of the UK; a third midlands isolate carried *bla*<sub>NDM-5</sub>
- The closest related, non-outbreak isolate was an ST14 isolate, 67 SNPs different to the closest outbreak isolate



**Figure 3:** Heat map showing pairwise SNP differences between outbreak and non-outbreak isolates. Grey shaded areas indicate pairwise comparisons between outbreak isolates. The red bar chart on the right hand side details the number of antimicrobial resistance genes identified in each isolate.