

# Dissemination of *Pseudomonas aeruginosa* bla<sub>NDM-1</sub>-positive ST308 clone in Singapore

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## Introduction

*Pseudomonas aeruginosa* (*P. aeruginosa*), a Gram-negative opportunistic pathogen ubiquitously found in the environment, is a major cause of nosocomial infections [1].

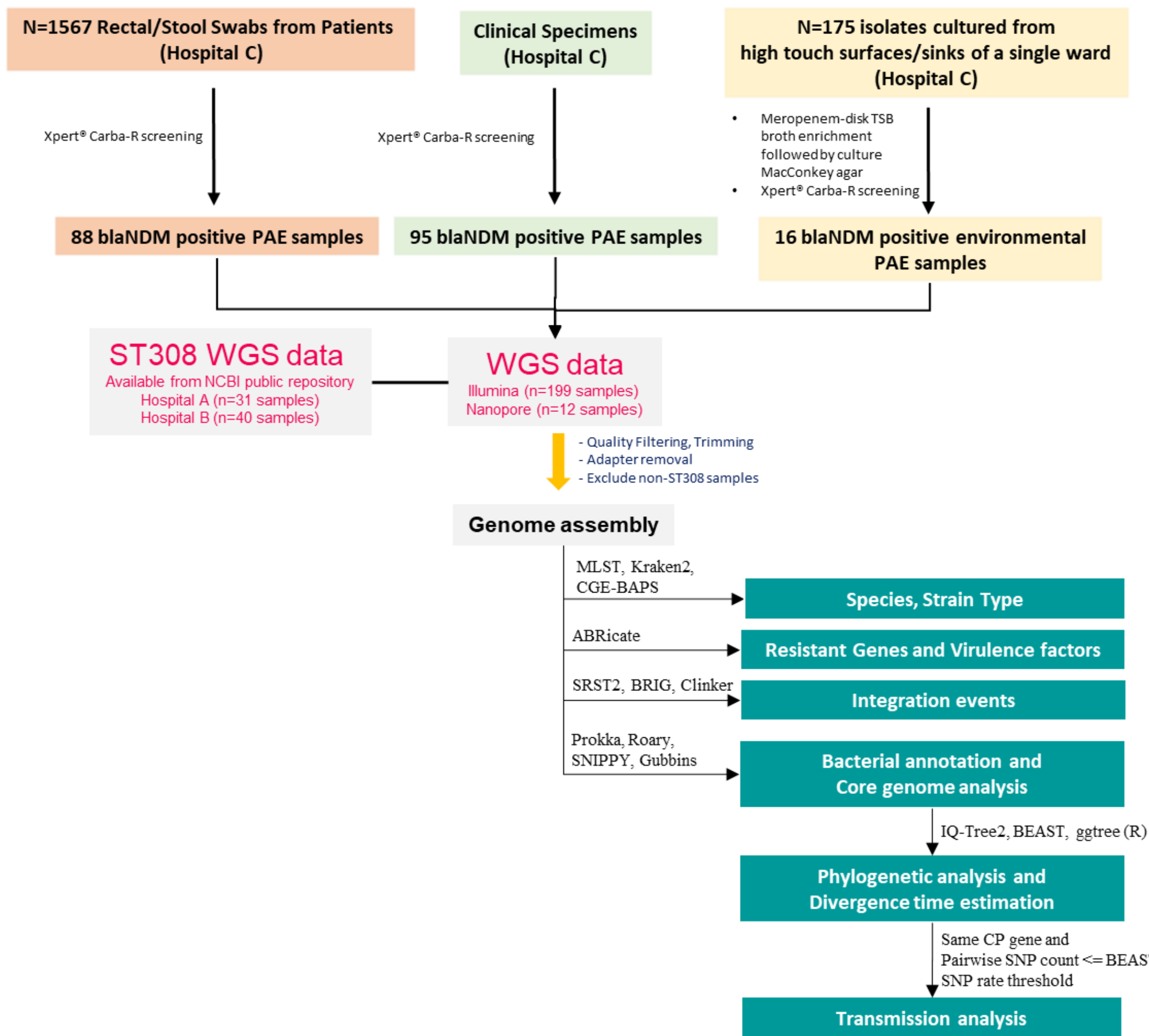
Although the population structure of *P. aeruginosa* has generally been conceptualized as panmictic, recent reports have increasingly shown that nosocomial multi-drug resistant and extensively drug-resistant (MDR/XDR) *P. aeruginosa* are not panmictic but largely characterized by 10 main high-risk clones (ST235, ST111, ST233, ST244, ST357, ST308, ST175, ST277, ST654 and ST298) [2].

Globally, ST308 has been known to bear bla<sub>IMP</sub> and bla<sub>VIM</sub>, and the only published report of bla<sub>NDM-1</sub>-positive *P. aeruginosa* ST308 to our knowledge was from the neighboring country of Malaysia, where a single isolate was identified [3].

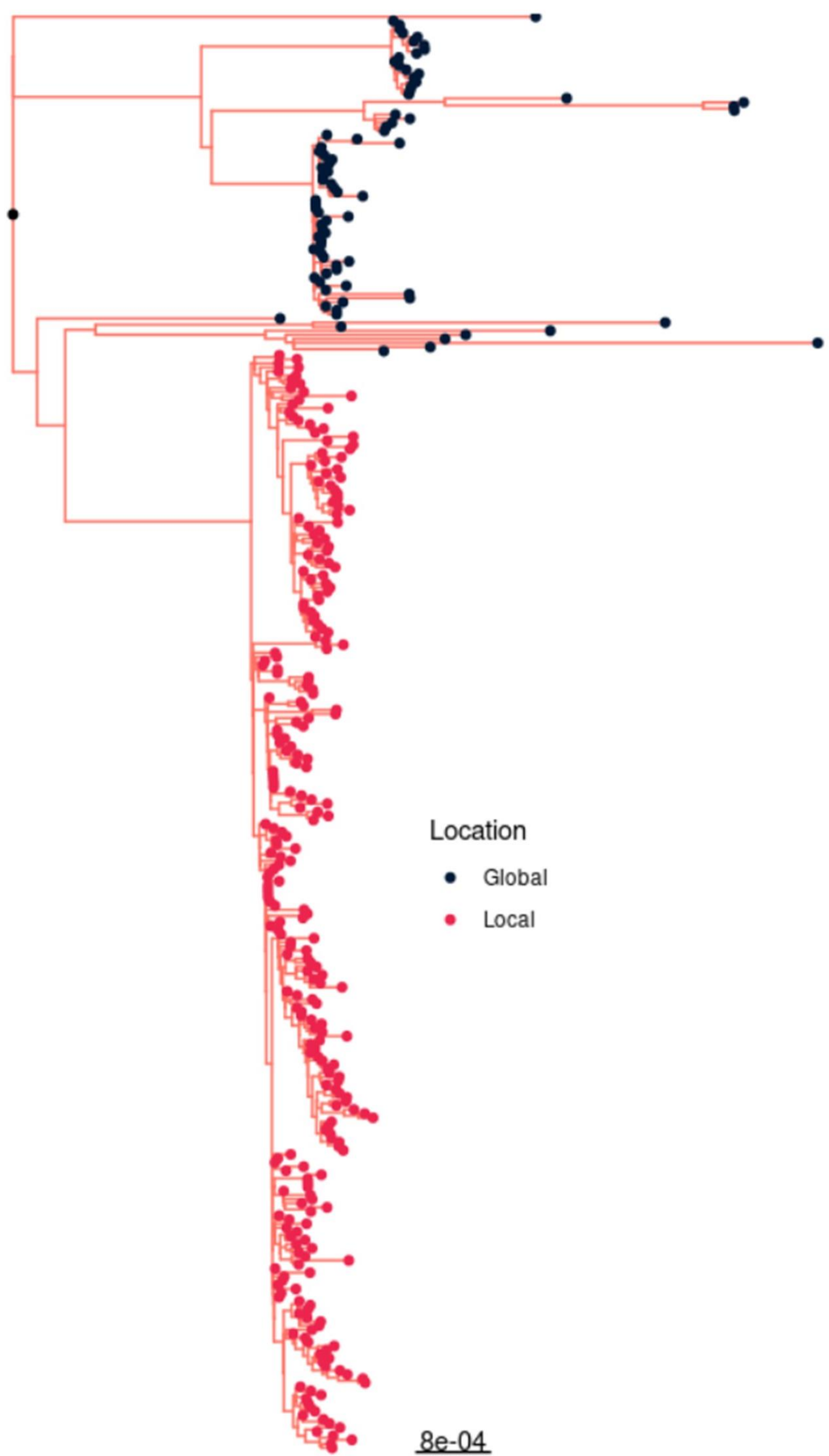
In this work, we aimed to determine the phylodynamics and evolution of bla<sub>NDM-1</sub>-positive *P. aeruginosa* ST308 in Singapore.

We combined our genomic data with the publicly available genome sequences and meta-data from two other local hospitals and examined the resistome and virulome of bla<sub>NDM-1</sub>-positive *P. aeruginosa* ST308 and the relationship of clinical and environmental isolates.

## Methods



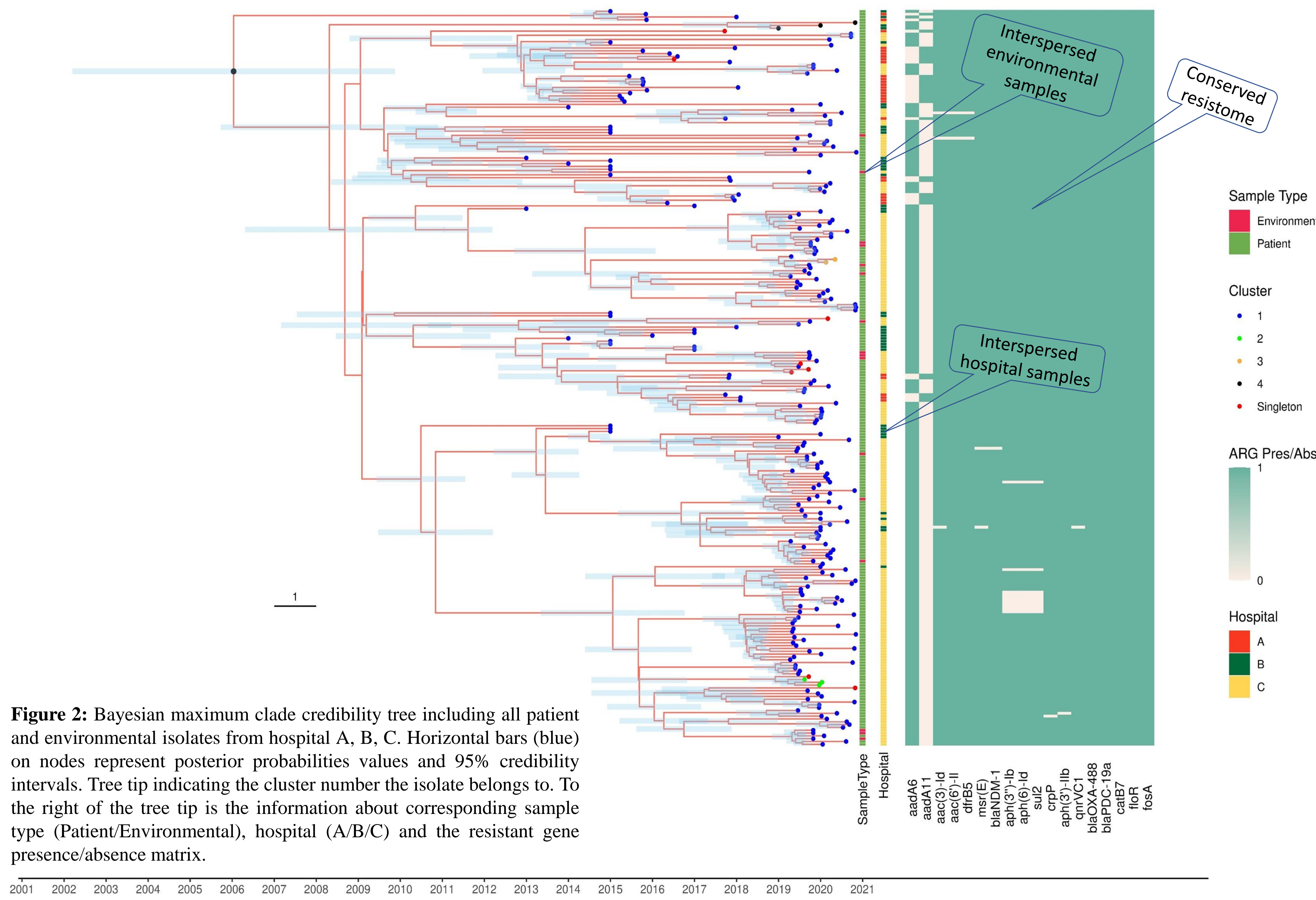
## Phylogenetic analysis



**Figure 1:** A maximum-likelihood phylogenetic tree constructed between the 266 local bla<sub>NDM-1</sub>-positive (31 from hospital A, 40 from hospital B and 195 from hospital C) and 83 global bla<sub>NDM-1</sub>-negative ST308 genomes originating from Germany, France and Spain.

The results revealed that all local bla<sub>NDM-1</sub>-positive ST308 isolates from Singapore form a unique and distinct clade from that of global isolates.

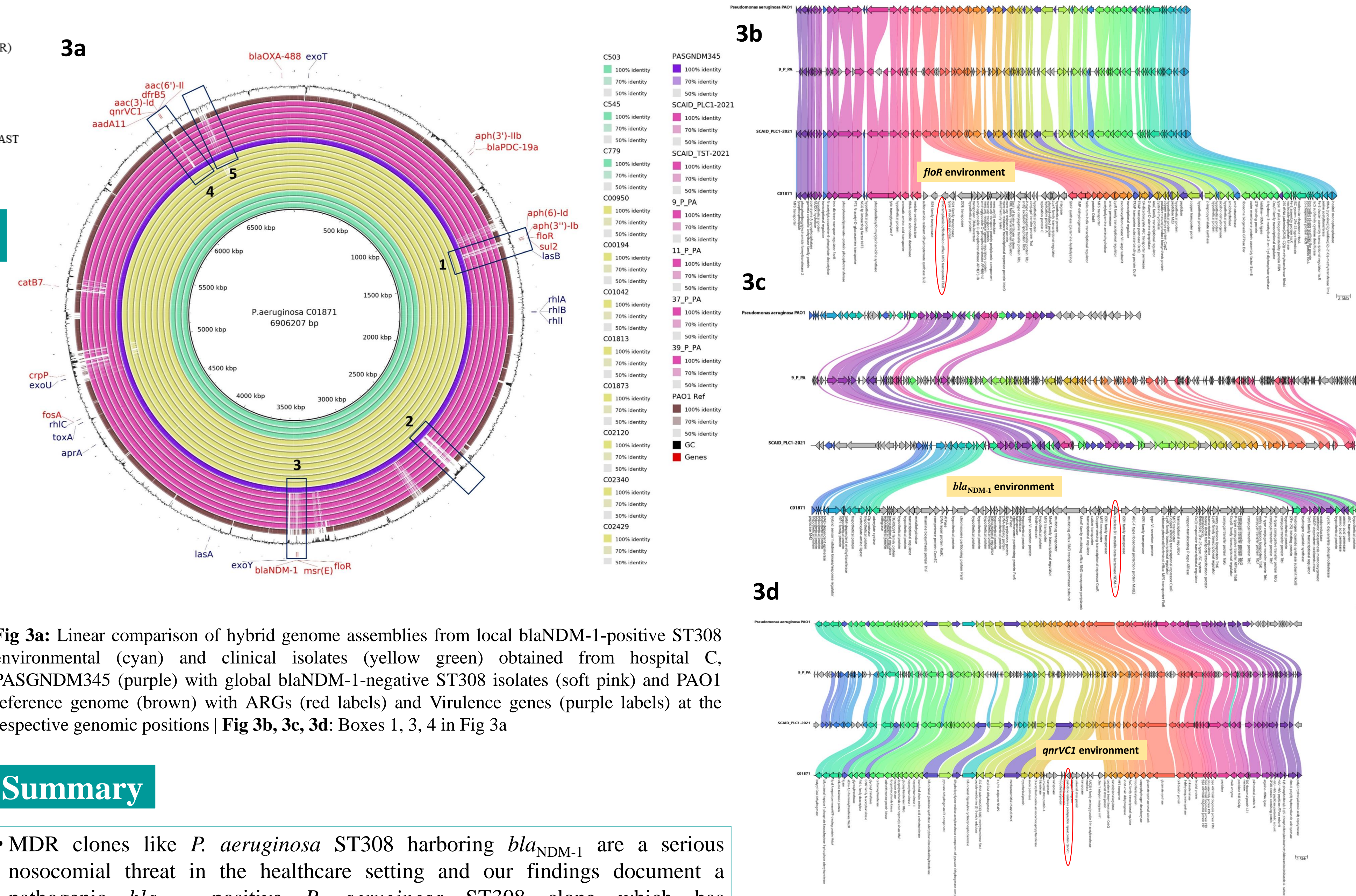
## Divergence time estimation



**Figure 2:** Bayesian maximum clade credibility tree including all patient and environmental isolates from hospital A, B, C. Horizontal bars (blue) on nodes represent posterior probabilities values and 95% credibility intervals. Tree tip indicating the cluster number the isolate belongs to. To the right of the tree tip is the information about corresponding sample type (Patient/Environmental), hospital (A/B/C) and the resistant gene presence/absence matrix.

- Analysis of patient and environmental samples suggest transmission between humans and environment.
- Majority of isolates from three different tertiary care hospitals formed a single large clonal transmission cluster, strongly supporting cross transmission between the three healthcare institutions.
- The analysis estimated that the initial divergence of local bla<sub>NDM-1</sub>-positive ST308 clone in Singapore occurred in the year 2005 (with a substitution rate  $2.421 \times 10^{-7}$  (95% HPD interval  $2.0522 - 2.7958 \times 10^{-7}$ ) substitution/site/year, equivalent to 1.75 SNPs/genome/year)

## Chromosomal integrations in local ST308 clone



**Fig 3a:** Linear comparison of hybrid genome assemblies from local bla<sub>NDM-1</sub>-positive ST308 environmental (cyan) and clinical isolates (yellow green) obtained from hospital C, PASGNDM345 (purple) with global bla<sub>NDM-1</sub>-negative ST308 isolates (soft pink) and PAO1 reference genome (brown) with ARGs (red labels) and Virulence genes (purple labels) at the respective genomic positions | **Fig 3b, 3c, 3d:** Boxes 1, 3, 4 in Fig 3a

## Summary

- MDR clones like *P. aeruginosa* ST308 harboring bla<sub>NDM-1</sub> are a serious nosocomial threat in the healthcare setting and our findings document a pathogenic bla<sub>NDM-1</sub>-positive *P. aeruginosa* ST308 clone which has disseminated across the three sampled institutions with possible environmental reservoirs contributing to transmission.
- Similar to prior research, we observed that chromosomal integration of ARGs play a large role in ARG dissemination in *P. aeruginosa* ST308. This is unlike *Enterobacteriales* where plasmid-mediated transmission is a major mode of ARG dissemination [4].
- Surveillance in Singapore and beyond for dissemination is essential to determine if existing measures are sufficient to control spread of this ST308 clone.

## Acknowledgements

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