

Genomic investigation of a Carbapenem-resistant *Klebsiella pneumoniae* outbreak in a neonatal ICU, Gauteng, South Africa

Mahalihali A¹, Bolukaoto JY¹, Rashopole S^{1,2}, Mulaudzi SI^{1,2}, Malemela KM¹, Taukobong S³, Nchabeleng M^{1,2} and Musyoki AM¹

¹Department of Microbiological Pathology, School of Medicine, Sefako Makgatho Health Sciences University, Pretoria, South Africa

²Microbiology Department, National Health Laboratory Service, Dr George Mukhari Tertiary Laboratory, Pretoria, South Africa

³Genomics Centre, South African Medical Research Council, Tygerberg, South Africa.

Presenting author email: ankonisahomahalihali@gmail.com

Principal investigator email: andrew.musyoki@smu.ac.za

BACKGROUND AND OBJECTIVES: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) neonatal outbreaks are frequently reported in healthcare settings worldwide. These outbreaks pose a threat due to limited treatment options and the high vulnerability of neonates, which often results in rapid transmission and adverse clinical outcomes. A 2024 CRKP outbreak in a neonatal intensive care unit (NICU) at an academic hospital in South Africa necessitated genomic investigation of the circulating strains. This study characterised the genomic features of CRKP isolates from the NICU outbreak.

METHODS: The study included 20 CRKP outbreak isolates, with two non-outbreak CRKP isolates for comparative analysis. Isolates were confirmed using polymerase chain reaction (PCR), antimicrobial susceptibility profiles were determined using the VITEK®-2 system and hypermucoviscosity was assessed using the string test. Clonal relatedness was evaluated using repetitive element palindromic PCR (rep-PCR). Twelve representative outbreak isolates and all non-outbreak isolates were selected for whole genome sequencing (WGS) using MinION Mk1C platform (Oxford Nanopore Technologies). Sequence data were analysed using EPI2ME, with Flye for assembly, Medaka for polishing, Prokka for annotation, ResFinder for antimicrobial resistance gene detection, and multi-locus sequence typing (MLST) determined sequence types. Virulence genes and mobile genetic elements (plasmids) were further profiled.

RESULTS: Outbreak isolates were hypermucoviscous, multidrug-resistant, and clonally related, with a highly conserved genome predominantly assigned to sequence type ST5785. Carbapenemase genes identified were *blaNDM-5* and *blaOXA-181* which co-localised with multiple resistance determinants of other antibiotic classes, including those associated with efflux, porin modification, heavy metals, and quaternary ammonium compounds. Diverse

virulence genes and conjugative IncF replicons were detected. Non-outbreak isolates belonged to ST307 and ST437, with comparable resistance genes to those in the outbreak strains.

CONCLUSION: The outbreak was driven by a rare, highly resistant ST5785 clone with potential for persistence and dissemination, highlighting the need for continuous genomic surveillance and strengthened infection prevention control strategies.