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Letter to the Editor

# "Contribution of the Whole Genome Sequencing to surveillance programs of carbapenemase-producing *Enterobacteriaceae* (CPE) strains"

*Dear Editor,*

We recently identified colonization and infection by KPC-producing *Klebsiella pneumoniae* strains in two patients from our Transplant Unit. Whole Genome Sequencing (WGS) performed with Illumina MiSeq revealed that strains from each patient were the same and belonged to ST11. Our findings emphasize the value of supporting effective antibiotic stewardship programs based on surveillance cultures for detecting colonised patients. In addition, WGS helped to demonstrate the nosocomial plasticity of *K. pneumoniae* ST11, with observed loss and acquisition of antibiotic resistance genes during hospitalisation, contributing to the understanding of the dynamics of antimicrobial resistance strains within the hospital.

Mortality rates by carbapenemase producing *Enterobacteriaceae* (CPE) infections remains high at 30%, with no significant differences noted among antibiotic regimens. While recent studies focusing on early detection and treatment of KPC have helped to mitigate its impact [1], the use of surveillance cultures to identify CPE colonisation continues to be controversial [2]. Culture-based methods (including Centers for Disease Control and Prevention protocols) with chromogenic media or specialized agars and double-disk synergy tests for detecting CPE, are convenient due to their availability and low cost. However, their limited sensitivity and long turnaround time may not always be optimal for infection control [3]. Whereas most studies examine clonal relationships of nosocomial CPE infections [4], few investigate the clonal status of colonising strains concurrently, limiting accurate surveillance. This is particularly the situation in low-income countries that have many limitations for the detection of CPE with WGS techniques due to its high cost.

Our CPE surveillance program involves regular screening for CPE and other carbapenem-resistant Gram-negative bacteria using the chromogenic medium CHROMagar™ KPC. This screening takes place on the first day of admission for patients transferred from other healthcare facilities, including tertiary care hospitals, nursing homes, and rehabilitation centers, including bedridden patients that are admitted to our hospital, as well as for those on hemodialysis and bone marrow or solid organ transplant recipients. Additionally, screening is conducted when a patient has had a positive culture for CPE within the last three months or when they are moved from the Intensive Care Unit (ICU) to a general ward.

In 2019, two patients from our Transplant Unit were colonized and infected with multidrug-resistant (MDR) KPC-producing *K. pneumoniae* (KPC-Kp) at the Hospital Alemán of Buenos Aires City, Argentina. *K. pneumoniae* HA39pKpn and HA40Kpn strains were isolated from a 38-year-old kidney transplant patient. The HA39pKpn strain was found in a rectal swab taken seven days after a positive urine culture for HA40Kpn, following routine screening to identify colonisations after CPE infections in hospitalised patients at our institution. *K. pneumoniae* HA48pKpn and HA49Kpn strains were isolated from a 51-year-old liver transplant patient; HA48pKpn was identified from a rectal swab on day 59 of hospitalisation performed due to an inter-ward transfer of patient HA48, while HA49Kpn was isolated from peritoneal fluid on day 73.

After the positive CPE colonisation result, contact precautions were implemented to prevent further horizontal transmission. To investigate the dynamics of colonising and infecting strains, it was necessary to determine the clonality of the strains and perform genomic studies. To analyze the phylogenetic relationship between strains, bioinformatic analysis based on WGS, Average Nucleotide Identity (ANI), and the Snippy program were performed. We found that the four strains of KPC-Kp from our study belonged to *K. pneumoniae* ST11, an international high-risk clone uncommon in Argentina but dominant in China [5]. Strains colonising and infecting each patient were closely related, as shown by both the ANI values (HA39pKpn/HA40Kpn: 100%, and HA48pKpn/HA49Kpn: 100%) and the number of SNP in the core genome (18 SNP between HA39pKpn and HA40Kpn, and 0 SNP between HA48pKpn and HA49Kpn). The low number of SNP found between strains suggests that each patient was colonised and infected by the same strain.

Additionally, comparing HA39pKpn with HA48pKpn and HA49Kpn revealed 3658 and 3428 SNP, respectively, indicating that each patient was colonised and infected by different sublineages within *K. pneumoniae* ST11. For patient HA39, the strain isolated on the third day of hospitalization corresponded to HA40Kpn, a nosocomial infection. In patient HA48, colonisation with HA48pKpn was also due to a strain from our institution, as a rectal swab on the first day of hospitalization was negative for CPE. The detection of a colonising MDR KPC-Kp strain guided the empirical antibiotic treatment decision, contributing to patient's discharge without infectious sequelae. No infection was observed 30 days post-discharge.

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The antibiotic susceptibility testing revealed that all four strains were MDR. HA39pKpn was susceptible to amikacin and resistant to trimethoprim-sulfamethoxazole, unlike HA40Kpn. HA39pKpn harbored a novel allele of the *dfrA22* gene cassette with 5 mutations compared to previously described (FM957884), while acquisition of *rmtD* in HA40Kpn strain could explain these differences.

Interestingly, HA39pKpn harbored two carbapenemases, $bla_{KPC-2}$ and $bla_{OXA-163}$, the latter being absent in HA40Kp strain. On the other hand, HA48pKpn and HA49Kpn from patient HA48 showed an identical profile of susceptibility to the antibiotics tested and they shared the same antimicrobial resistance genes (Data not shown) as well as having PmrA with R256G mutation conferring resistance to colistin [6].

Our findings highlight the role of CPE surveillance supported by WGS in nosocomial surveillance. Also, horizontal genetic transfer processes were shown to contribute to MDR spread within the hospital, challenging antibiotic stewardship programs.

The use of WGS was crucial for precise identification and characterization of resistant bacterial strains, enabling tailored treatment plans and improving patient outcomes by ensuring the most effective therapies. Further studies will help decipher the behavior of MDR and extremely drug-resistant strains within inpatients, providing a clearer picture of the dynamics of resistance in the hospital environment. This knowledge will contribute to developing infection control guidelines.

## Author contributions

DC obtained funding and managed the project. DC and NGA conceived and designed the study. VEA, NM, AA performed the bioinformatics analysis. AGM, MP, EC, CM, JC, BF and LFC executed the experiments. NGA, VEA and MPQ analyzed and curated the data. DC wrote the manuscript. All authors read and critically revised the manuscript and approved the final manuscript.

## Conflict of interest statement

All authors declare no competing interests.

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## Data availability statement

This Whole Genome Shotgun project has been deposited at GenBank under the accession numbers JAODTN000000000, JAODTO000000000, JAODTP000000000 and JAODTQ000000000. The versions described in this paper are version JAODTN010000000, JAODTO010000000, JAODTP010000000 and JAODTQ010000000.

## Ethics statement

Patient's confidentiality is maintained throughout the study, and the study did not carry any additional risk to the patients. Participation in the study did not interfere with patient's management. Ethical approval was given by the Ethics Committee of the Hospital Alemán of Buenos Aires.

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