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Usefulness of decentralized sequencing networks on antimicrobial resistance surveillance



Utilidad de las redes de secuenciación descentralizadas en la vigilancia de la resistencia antibiótica

The increase of bacterial antimicrobial resistance is one of the biggest challenges to modern medicine, being associated with almost five million deaths and 1.2 million attributable deaths in 2019.¹ One of the key lessons learned from SARS-CoV-2 or mpox is that highly interconnected world that we live in, allows the rapid spread of new variants and diseases to almost every continent.² Therefore, even if carbapenemase producing Enterobacteriaceae (CPE) are not prevalent at a certain location currently this may change due to the introduction of a new CPE clone rapidly.³

Moreover, in recent years health systems are getting increasingly complicated with more intricate relationship between different hospitals and health-centres. Consequently, the introduction of clonal CPE may no longer be a local problem and represent a regional problem, causing a multihospital outbreak.^{4,5}

To tackle this problem, the characterization of CPE is needed to recognize the appearance of regional outbreaks. Until now the most common strategy has been to develop hierarchical networks with core laboratories that conducted most of the molecular characterization of CPE at regional or national level. However, the appearance of new sequencing platforms such as Oxford Nanopore (ONT) sequencers coupled with the flongle flowcells⁶ represent the perfect opportunity to develop new nodal networks. In this case, instead a central laboratory performing all analysis, each laboratory of the network can produce their own sequences that can be later shared between them.

In our area the most common CPE are *Klebsiella pneumoniae* and *Escherichia coli*.⁷ However, other less common CPE such as *Citrobacter freundii* group have also been described.⁸ Therefore, the simultaneous first isolation of two carbapenem resistant *C. freundii* isolates on different hospitals in the same region raised many alarms.

The isolates were identified as *C. freundii* with the MALDI Biotyper MBT smart system (Bruker Daltonics, Germany). The antibiotic susceptibility was conducted with the Phoenix BD system (Becton Dickinson, USA) or the MicroScan Walkaway system (Beckman Coulter, USA). Both strains were carbapenem resistant and tested positive for New-Dehli-metalobetalactamase (NDM) with the CARBA-5 assay (NG Biotech, France). After swift coordination between both microbiology laboratories both were selected for a more thorough analysis by whole genome sequencing (WGS).

The DNA extraction was conducted with the MagNA Pure Bacteria 2.0 Protocol (Roche, Switzerland) and DNA concentration was quantified using the Qubit4 fluorometer (Thermo-Fisher Scientific, USA), while quality assessment was performed using the NanoDrop One spectrophotometer (Thermo-Fisher Scientific, USA). The library was prepared with 400 ng of DNA following the ONT protocol for flongles and loaded on a FLO-MIN106 flongle (ONT, United Kingdom). This approach to the library synthesis requires a hands-on time of around half an hour. Genome assembly was performed using flye⁹ and medaka.

One isolate belonged to the ST22 and harboured a bla_{NDM-5} and a IncX3 incompatibility complex on the same contig, while the other isolate was an ST18 with a bla_{NDM-1} and a IncR on the same contig, thus ruling out the hypothesis of an interhospitalary outbreak.

To the conclusion of this study no new bla_{NDM}-producing Enterobacteriaceae has been isolated in any of both hospitals besides an ST214 *Klebsiella grimonti* with a bla_{NDM-5} and a IncX3 isolated from a rectal swab of the first patient. Therefore, even if no specific plasmid analysis was conducted these findings highlight the possibility of these worrying resistant genes on transmissible elements.

All in all, we think that this is a good example of a good cooperation between nearby centres and where the current state of the sequencing technologies at clinical microbiology laboratories. Such strategies will not undermine the role of said central laboratories as they will keep a vital role in the establishment of laboratory networks capable of performing WGS, coordinating the efforts of the network and advising nodal laboratories. Meanwhile, this approach enables a swift response as performing this analysis on a local base enables the nodal centres to take the necessary measures more quickly. Moreover, in our opinion this will improve the engagement of nodal laboratories in the efforts of AMR surveillance, and we firmly believe that the WGS no longer is a technology reserved for central reference laboratories but rather an important diagnostic that should be considered in almost every clinical microbiology laboratory.

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