Rapid Outbreak Detection of Multi-Drug Resistant Organisms Using Resistance Gene Profiles

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Background

Quick and efficient strain-typing of multi-drug resistant organisms (MDROs) is crucial for the prevention of outbreaks. Currently, isolates are routinely strain-typed with MLST or core genome MLST (cgMLST), which take days to finish due to the time needed for DNA sequencing process. On the other hand, an isolate's drug resistance genes may be detected within hours using a PCR-based test. In this study we explore the plausibility of outbreak detection by clustering isolates based on their resistance gene profiles.

Material and Method

We selected 53 *P.aeruginosa* isolates with one outbreak group and 54 *E.coli* isolates with two outbreak groups. The whole genome sequences of these isolates were assembled using 2X250 bp Illumina data either generated at OpGen or downloaded from the NCBI's SRA database. The drug resistance genes in isolates were detected using OpGen's Acuitas® Whole Genome Sequencing Analysis pipeline. The isolates were then grouped by hierarchical clustering based on the resistance genes harbored in them. Each isolate was strain-typed using organism-specific cgMLST schemas created at OpGen using the Ridom® software.

Results

Out of 53 *P.aeruginosa* isolates, the cgMLST analysis identified an outbreak group of 28 isolates. Within the outbreak group (**Fig 1 A**), the isolates differ by fewer than 35 genes (out of 3694 genes tested; less than 0.95%). Total of 13 distinct drug resistance genes and three key amino acid positions in the parC and gyrase A genes that confer fluoroquinolone resistance were detected in these isolates. We then used only these 16 genes/variants to group the full set of *Pseudomonas* isolates with hierarchical clustering (**Fig. 1B**). All 28 isolates within the outbreak group were placed correctly in the same cluster with 27 isolates having the same drug resistance gene profile and the remaining isolate differing by the lack of a single drug resistance gene.

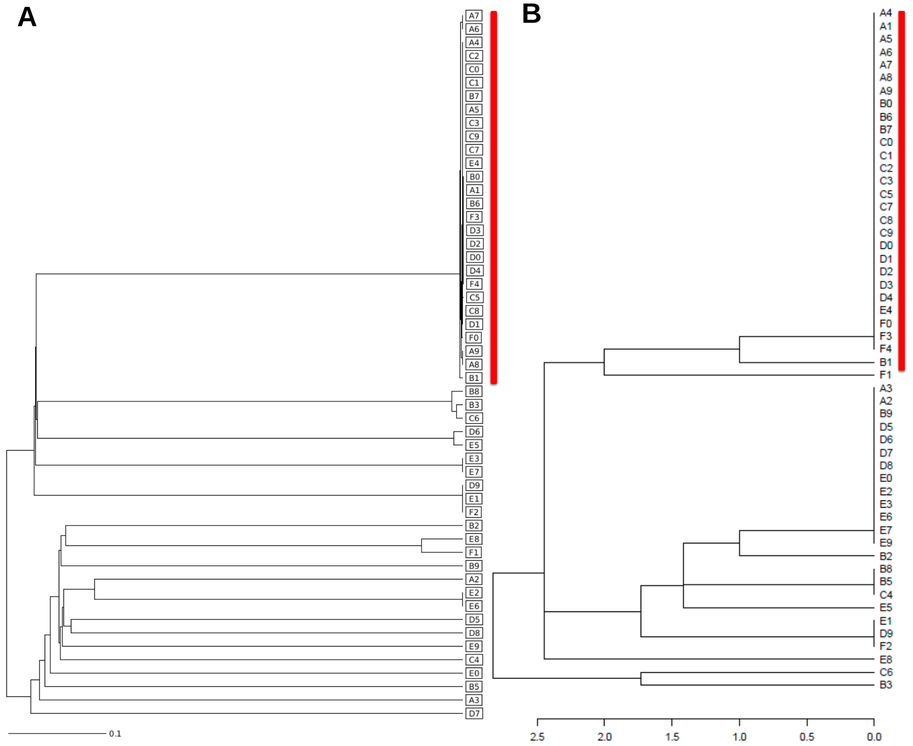

Fig. 1 Detection of *P. aeruginosa* outbreak group (highlighted by red line). (A) Using cgMLST strain-typing method; (B) Using hierarchical clustering based on the resistance genes.

Out of 54 *E. coli* isolates, the cgMLST analysis identified two outbreak groups as expected (**Fig. 2A**): one group (gray) had three isolates that differed by fewer than two genes and the other group (red) had 34 isolates that differed by fewer than 21 genes out of the 2300 genes tested, less than 0.1% in both groups. Total 69 distinct drug resistance genes and the three key amino acid positions in parC and gyrase A were found within these isolates. These 72 gene/variants were used to discriminate the *E. coli* isolates. Isolates in both groups have been correctly placed by hierarchical clustering (**Fig. 2B**), in agreement with the cgMLST analysis. For the outbreak group of 34 isolates, 32 of 34 isolates have the identical resistance gene profile. Within outbreak group of three isolates, they are grouped in the same cluster, even though they have different resistance gene profiles,

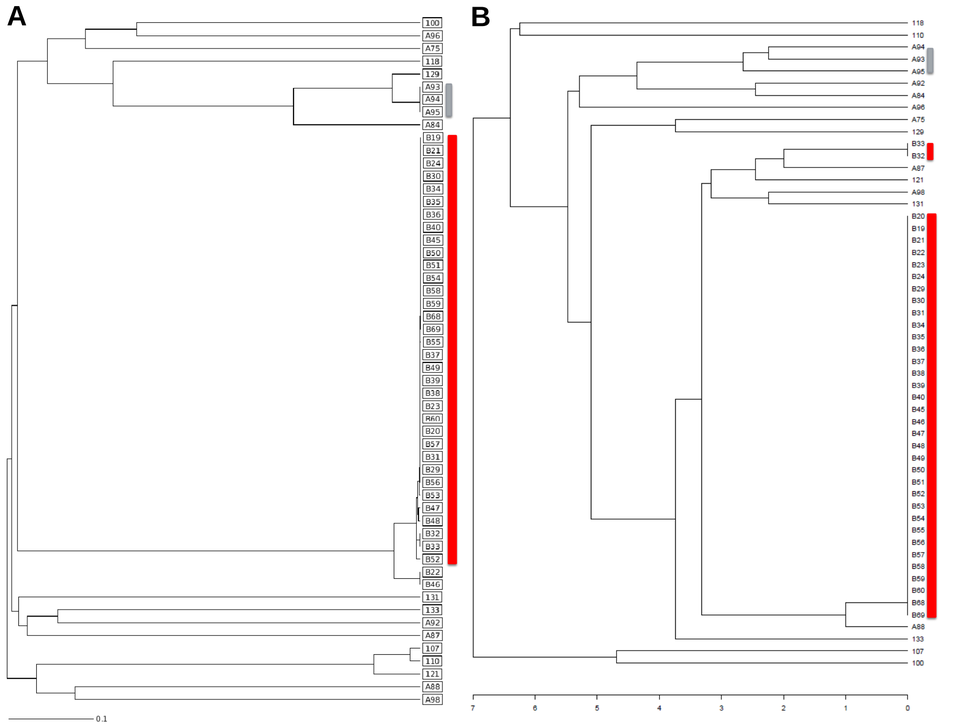

Fig. 2 Detection of two *E. coli* outbreak groups (highlighted by red and gray lines). (A) Using cgMLST strain-typing method; (B) Using hierarchical clustering based on the resistance genes.

Conclusion

Our results demonstrated that an MDRO outbreak can be accurately identified using only drug resistance gene profiles in *P. aeruginosa* and *E. coli*. Therefore, it should be possible to develop a quick and efficient surveillance system for MDRO outbreaks with a PCR-based test.