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Assessing the *P. aeruginosa* epidemic with Nextera™ DNA Flex, the iSeq™ 100 System, and bioMérieux EPISEQ® CS software

Access a higher level of characterization and strain discrimination through whole-genome microbial sequencing paired with user-friendly software and a comprehensive report.

Introduction

Hospital-acquired infections are a major healthcare concern, especially in critically ill and immuno-compromised patients. The ability to prevent such infections could be facilitated by development of standard practices to identify and monitor pathogenic bacterial strains in the environment. *Pseudomonas aeruginosa* is a multidrug-resistant pathogen recognized for its ubiquity, antibiotic resistance mechanisms, and association with serious illnesses. Drug-resistant strains have been found in ventilator-associated pneumonia and various sepsis syndromes, leading to increased mortality in hospitalized patients.[1]

The ability to detect and track antibiotic-resistant clones efficiently is important to inform timely treatment decisions and prevent further transmission of infection. Within a single assay, next-generation sequencing (NGS) provides the ability to identify pathogenic strains and characterize the resistance genes that they carry.[2] With a comprehensive workflow that can be completed in two days, the status of identified strains can be efficiently monitored in a hospital environment (Figure 1). Powerful and user-friendly software tools enable this goal by reducing the amount of time required to interpret sequence data.

This application notes describes detection of *P. aeruginosa* in a comprehensive workflow that uses whole-genome sequencing (WGS) of four closely related bacterial isolates coupled with the bioMérieux EPISEQ® CS cloud service. With a capacity for developing resistance, the ST235 strain of *P. aeruginosa* is one of the most widespread causes of hospital-acquired infections across Europe. Through mutation and acquisition of resistance elements, these bacteria have developed their own local populations. From different environmental sources, four strains were selected based on their close genetic relations with similar antimicrobial susceptibility patterns that make typing difficult with traditional methods.

The components of this study were selected to demonstrate improvements in library prep, sequencing, and analysis for performance, efficiency, and ease of use (Figure 1). The Nextera™ DNA Flex Library Preparation Kit is an innovative library prep method that supports quick and easy library preparation directly from bacterial colonies. The smallest of the Illumina sequencing instruments, the iSeq™ 100 System, can sequence four bacterial genomes in 17.5 hours. Because *P. aeruginosa* has a large genome size (5.5-7.7 Mb), this study also demonstrates how multiplexing on the iSeq 100 System enables analysis of bacteria with large genomes with greater than 48× coverage. The bioMérieux EPISEQ® CS web-based software enables analysis of WGS of four samples in about one hour. The entire NGS workflow can be completed in two days (Figure 1).

Methods

Library preparation and sequencing

P. aeruginosa isolates exhibiting multiple drug resistance (MDR) were collected in University Hospital (France) between February 2004 and August 2005 from four different specimen sites (sputum, stool, mouth, or catheter). Extracted genomic DNA was the direct input to the Nextera DNA Flex Library Preparation Kit.[3] Sequencing was performed on the iSeq 100 System (Figure 1).

Data analysis

From WGS raw data, the bioMérieux EPISEQ® CS cloud service automatically performs multilocus sequence typing (MLST), whole-genome MLST (wgMLST), resistome characterization, virulome characterization, and phylogenetic analysis. This whole-genome cutting-edge level of bacterial strain typing and characterization allows users to identify the infectious pathogen source and define transmission pathways quickly

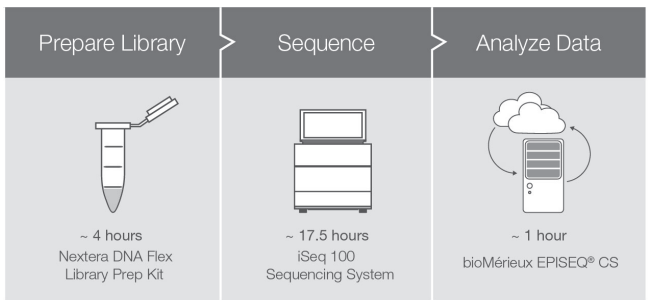

Figure 1: Bacterial whole-genome sequencing workflow—In a streamlined, comprehensive workflow, large bacterial genomes can be sequenced and analyzed within two days. The three stages include Nextera DNA Flex Library Preparation, sequencing on the iSeq 100 System, and data analysis with the bioMérieux EPISEQ® CS software.

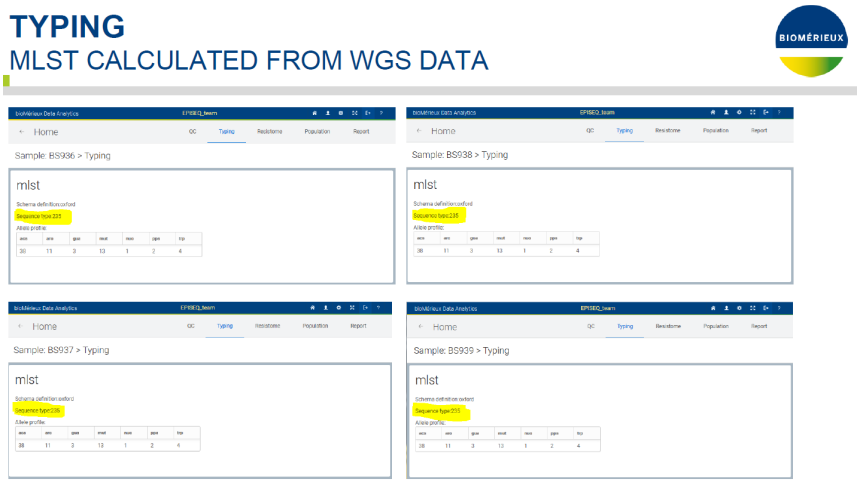

Figure 2: Report of MLST typing calculated from WGS of the four ST235 *P. aeruginosa* isolates.

Strain identification

For determining bacterial relatedness, WGS offers the highest possible resolution of closely related microbial genomes. MLST is a common procedure for characterizing isolates of bacterial species using the sequences of internal fragments (450-500 bp) of usually seven house-keeping genes. For hospital outbreak investigations, wgMLST can identify subtle differences often overlooked by other genotypic methods such as MLST or pulsed-field gel electrophoresis (PFGE).

The bioMérieux EPISEQ® CS phylogenetic analysis is based on wgMLST, a typing approach using genome-wide gene-by-gene comparison, including several thousand loci (typically 1500 – 4000) which are not limited to core genes. The bioMérieux EPISEQ® CS software also includes a curated collection of 30,000+ reference genomes belonging to a menu of 13 bacterial HAI-related species. As expected, the nearest neighbor of the four analyzed samples is the genome of a ST235 *P. aeruginosa* strain from the collection in the database. Thus, the identity of the ST235 strain was further confirmed (Figure 2).

Epidemiological analysis

The bioMérieux EPISEQ® CS automated bioinformatics workflow includes de novo assembly from WGS data and QC steps. For further characterization of isolates, the bioMérieux EPISEQ® CS can generate a wgMLST-based dendrogram. The dendogram of the four samples in this study (Figure 3) demonstrates that one of the isolates (BS938) is slightly more distant (99.74% similarity) from the other three (99.93%). BS938 has a slightly smaller (6.66 Mb) genome than the other three samples (6.77 Mb), and this 110 kb difference in genome size is most likely related to the observed clustering pattern.

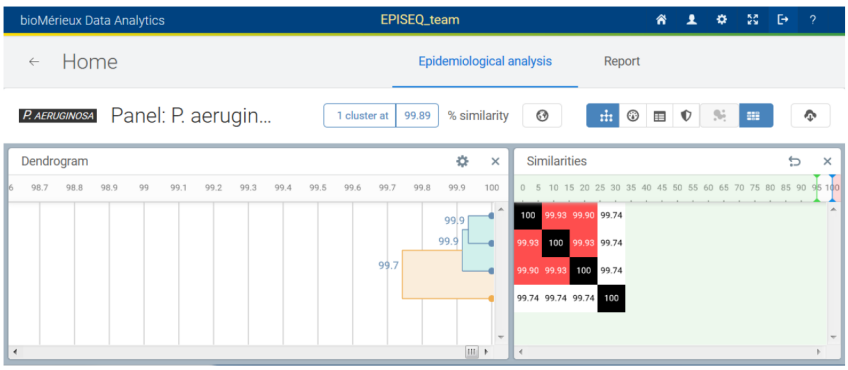

Figure 3: Dendrogram created by the bioMérieux EPISEQ® CS software shows similarities and differences between all four genomes.

Resistome and virulome prediction

To go further in studies, bioMérieux EPISEQ® CS also provides isolate resistome and virulome information. For genes with known mutations conferring antibiotic resistance, the EPISEQ CS software demonstrated the lack of differences for detected mutations in any of the four strains. However, the *SoxR* gene was present in three of the four strains, but was missing in the isolate with a smaller genome, BS938. In *P. aeruginosa*, *SoxR* is able to directly upregulate the expression of the MexGHI-OpmD (multidrug) efflux pump. All other detected antibiotic resistance genetic markers found in the four strains confirmed that they are MDR organisms. For virulome, no virulence features were found in any of the isolates.

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Conclusion

Using an NGS workflow with bioMérieux EPISEQ® CS software, it is possible to identify and distinguish between closely related bacterial strains. With recent NGS innovations such as the fast Nextera DNA Flex Library Prep and small iSeq 100 System, large bacterial genomes can be sequenced at 48× coverage and analyzed within two days. bioMérieux EPISEQ® CS software expands upon traditional MLST-based methods to integrate significantly more input data. Using information available from WGS, this cutting-edge level of bacterial strain typing and characterization enables rapid identification of the source of infectious pathogens and elucidation of transmission pathways. The whole genome data analysis process takes around one hour for all four samples.

P. aeruginosa, a bacterial pathogen commonly found in hospital infections, was chosen to demonstrate the utility of a NGS workflow because of the abundance of closely related multidrug resistant strains that are difficult to distinguish my traditional microbiological methods. The bioMérieux EPISEQ® CS software rapidly confirmed previous results, identifying each of four isolated strains as ST235, and further characterized as a multidrug resistant organism. Additional information was provided by automated features of bioMérieux EPISEQ® CS, such as phylogenetic differences, differences in genome size, and differences in the resistance genes harbored by each isolate.

References

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