TECHNICAL NOTE

Accurate surveillance of healthcare-acquired infections

Comprehensive isolate discrimination and characterization through microbial WGS paired with the user-friendly bioMérieux EPISEQ® CS software enables outbreak detection

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Introduction

Healthcare-acquired infections (HAIs) are a major health care concern, especially in critically ill and immunocompromised patients. The ability to prevent these infections could be facilitated by the development of standard infection control practices to identify and monitor pathogenic bacterial strains in health care environments. Laboratory methods, such as qPCR and mass spectrometry, enable rapid identification of pathogens, but are unable to trace outbreaks or perform transmission investigations.

Next-generation sequencing (NGS) allows for comprehensive characterization of bacterial genomes, including subtyping, differentiating isolates, or characterizing antimicrobial resistance (AMR) and virulence.[1] Analyzing and interpreting sequencing data quickly allows infection control personnel to respond rapidly to potential outbreaks and trace them back to the source to help prevent further transmission and infection. Whole-genome sequencing (WGS) can help manage HAIs by efficiently monitoring and properly managing pathogen spread.

To demonstrate the use of WGS in pathogen montoring, Illumina and bioMérieux collaborated to develop a 24-hour WGS workflow to characterize *Pseudomonas aeruginosa,* a multidrug-resistant pathogen recognized for its ubiquity 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.[2] Through mutation and acquisition of resistance elements, these bacteria have developed their own local populations.[3]

This application note highlights the use of the bioMérieux EPISEQ CS cloud service as part of a comprehensive WGS solution (Figure 1) that includes Illumina library preparation and sequencing to characterize isolates of *P. aeruginosa*, selected for their antimicrobial susceptibility patterns that make subtyping difficult with traditional methods.

Experimental design

The components of this study were selected to demonstrate improvements in library preparation, sequencing, and analysis in terms of performance and ease of use, making the approach accessible to laboratories without prior NGS experience. Illumina DNA Prep* is an innovative library prep method that enables a quick and easy start to the workflow from extracted DNA or directly from bacterial colonies. The small, benchtop iSeq™ 100 System can sequence up to six bacterial genomes in a single batch. This study also demonstrates how multiplexing on the iSeq 100 System enables analysis of bacteria with large genomes, like *P. aeruginosa* (genome size 5.5-7.7 Mb), with enough coverage for full characterization. bioMérieux EPISEQ CS provides automated analysis of WGS data within an hour of sequencing completion. The entire NGS workflow, from isolate to report, can be completed in under 24 hours.

* Formerly available as Nextera™ DNA Flex Library Preparation Kit

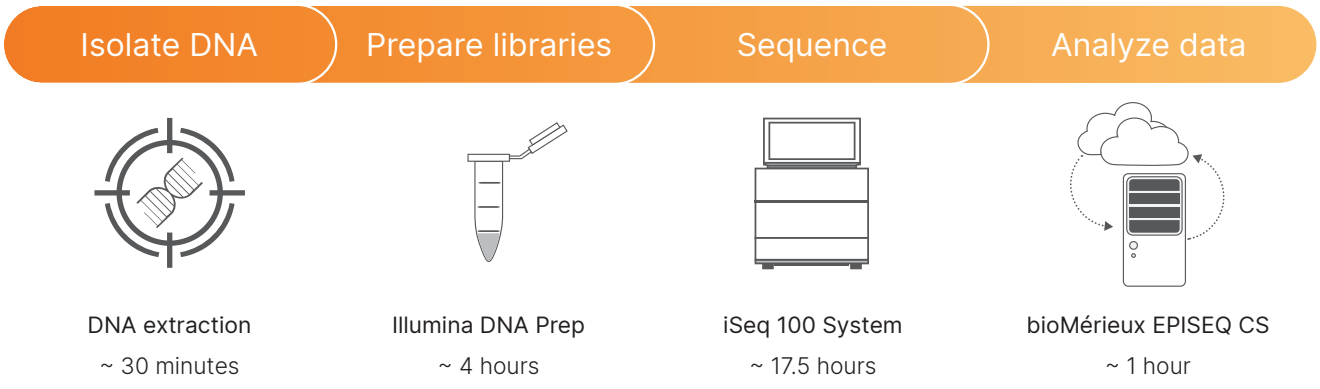

Figure 1: Bacterial WGS workflow—In a streamlined, comprehensive workflow, bacterial genomes can be sequenced and analyzed within 24 hours.

Library preparation and sequencing

P. aeruginosa isolates exhibiting multiple drug resistance (MDR) were obtained from a university hospital in France between February 2004 and August 2005 from four different specimen sites (sputum, stool, mouth, or catheter). After DNA extraction, libraries were prepared using Illumina DNA Prep and sequencing was performed on the iSeq 100 System using 2 × 150 bp reads.

Data analysis

From the raw sequence data, bioMérieux EPISEQ CS automatically performed quality control (QC) analysis, *de novo* assembly, multilocus sequence typing (MLST), whole-genome MLST (wgMLST), resistome characterization, virulome characterization (for relevant organisms), and phylogenetic analysis. This whole-genome level of bacterial strain typing and characterization allowed users to identify the infectious pathogen source and define transmission pathways quickly.

Learn about the analysis functions in EPISEQ CS, www.biomerieux-usa.com/product/episeq-cs

Accurate strain identification by MLST analysis

For determining bacterial relatedness, MLST is a common procedure that characterizes isolates of bacterial species using the sequences of internal fragments (450-500 bp) of a limited number (7-10) of housekeeping genes. The bioMérieux EPISEQ CS software includes a curated collection of 30,000+ reference genomes belonging to a menu of 13 bacterial HAI-related species (Table 1). Using MLST data, it was observed that the allelic profile of the four analyzed hospital samples was the same and corresponded to the *P. aeruginosa* ST235 strain from the collection in the database (Figure 2).

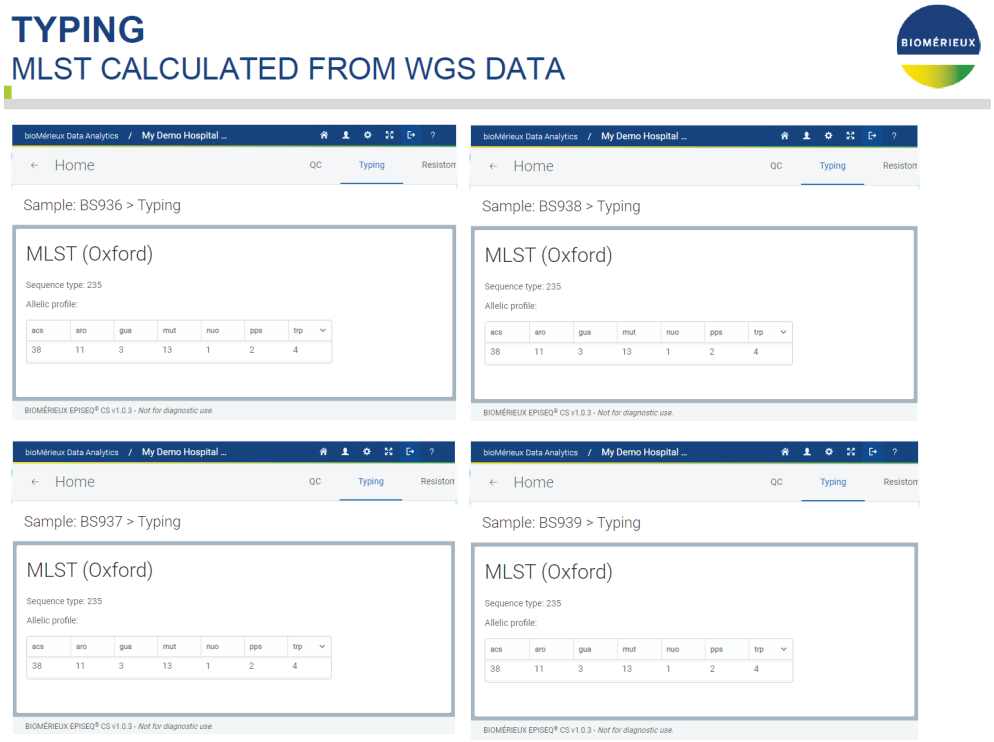

Figure 2: Report of MLST typing calculated from WGS—WGS data easily characterizes the four *P. aeruginosa* isolates as ST235 subtype, but at the level of resolution (MLST) used for analysis, it is unable to determine if they form a cluster.

Table 1: Thirteen bacterial species that cover most HAI-related organisms

Acinetobacter baumannii	*Klebsiella aerogenes*
Burkholderia cepacia complex	*Klebsiella oxytoca*
Clostridioides difficile	*Klebsiella pneumoniae*
Enterobacter cloacae complex	*Pseudomonas aeruginosa*
Enterococcus faecalis	*Serratia marcescens*
Enterococcus faecium	*Staphylococcus aureus*
Escherichia coli/Shigella	

Bacterial species identified by bioMérieux and analyzed using EPISEQ CS

High-resolution epidemiological analysis of isolates from wgMLST data

The bioMérieux EPISEQ CS phylogenetic analysis is based on wgMLST, a typing approach based on genome-wide gene-by-gene comparisons that include several thousand loci (~1500-4000) not limited to core genes. For hospital outbreak investigations, wgMLST can identify subtle differences often overlooked by other genotyping methods such as MLST or pulsed-field gel electrophoresis (PFGE).

The wgMLST-based dendrogram of the four samples in this study demonstrated that one of the isolates was slightly more distant (99.74% similarity) from the other three (99.93% similar). The three isolates similar enough to be classified as a cluster were grouped in the dendrogram and highlighted in the similarity table (Figure 3). WGS data revealed that the distinct isolate had a slightly smaller (6.66 Mb) genome than the other three samples (6.77 Mb); this 110 kb difference in genome size was enough to explain the separation in the clustering pattern (data not shown).

Resistome prediction

To further characterize isolates, bioMérieux EPISEQ CS provides resistome and virulome information. For genes with known mutations conferring AMR, the software showed no differences between the four strains, in agreement with their identical antibiotic resistance pattern determined by traditional phenotypic methods, such as antibiotic susceptibility testing (AST). WGS data also showed that the *SoxR* gene was present in three of the four strains, but was missing in the isolate with a smaller genome (Figure 3). In *P. aeruginosa*, *SoxR* can directly up-regulate the expression of the MexGHI-OpmD (multidrug) efflux pump. This result suggested that the *SoxR*-deficient isolate may exhibit a different antimicrobial susceptibility under certain conditions. All other detected antibiotic resistance genetic markers found in the four strains confirmed that they were MDR organisms.

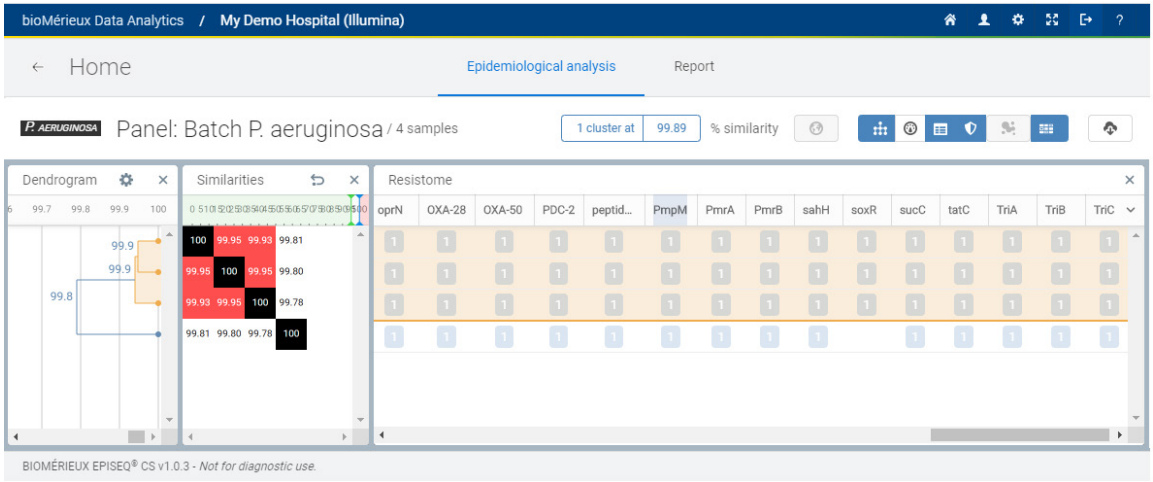

Figure 3: wgMLST dendrogram showcases sample differences—WGS data easily highlights subtle differences between isolates, providing visual clues of which isolates are similar and which are distinct. bioMérieux EPISEQ CS automatically sets thresholds for wgMLST allelic similarity percentage used to consider isolates as part of a cluster (red). Resistome markers help in differentiating between related organisms; in the resistome panel the number of copies is indicated for each detected marker.

Summary

HAIs are a major health care concern, attributing to high patient morbidity and mortality and increased healthcare costs. Using an Illumina WGS workflow with bioMérieux EPISEQ CS analysis, it is possible to identify and distinguish between closely related bacterial strains. With NGS innovations such as the fast Illumina DNA Prep and the compact iSeq 100 System, bacterial genomes can be fully sequenced and analyzed within 24 hours.

bioMérieux EPISEQ CS 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 is automated and requires no bioinformatics expertise.

P. aeruginosa, a bacterial pathogen commonly found in hospital infections, was chosen to demonstrate the utility of an NGS workflow because of the abundance of closely related multidrug-resistant strains that are difficult to distinguish by traditional microbiological methods. The bioMérieux EPISEQ CS software rapidly confirmed previously known AST results, identified each of the four isolated strains as ST235, and further characterized one isolate as different from the three others that formed a cluster. Additional information was provided by automated features of bioMérieux EPISEQ CS, such as differences in genome size and a potential difference in the AMR profile harbored by a specific isolate.

References

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