Rigshospitalet
Diagnostisk Center

Poster number 5187

APPLYING LONG READ SEQUENCING TO ELUCIDATE GENOMIC ADAPTION DURING HOSPITAL OUTBREAKS

REGION H

Session AAR01 Surveillance of Antimicrobial Resistance: Molecular Typing, Clinical and Molecular Epidemiology 6/11/2022 10.00 AM

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INTRODUCTION

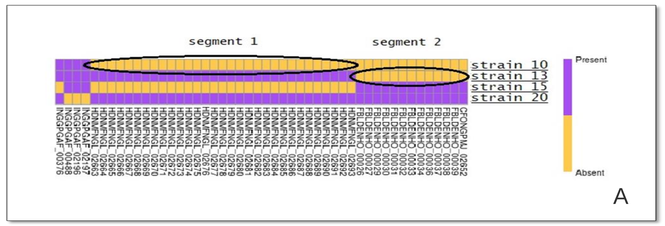
The study of the genetic adaptation of bacteria is important to understanding clonal adaptation during a hospital outbreak.
Illumina DNA sequencing identifies outbreaks of bacterial clones by analyzing single nucleotide polymorphisms. Although Illumina provides accurate sequencing reads, the use of its short reads alone results in fragmented genome assemblies.
Oxford Nanopore Technologies (ONT) platforms generate long DNA fragments, but with more error-prone reads compared to Illumina sequencing.
Unicycler (1) is a hybrid assembly tool for bacterial genomes, that produces accurate assemblies from combined Nanopore and Illumina sequencing data.
Here, we studied two outbreak populations with the aim of evaluating gene content changes in a hospital setting with respect to horizontal gene transfer.

MATERIALS AND METHODS

Isolates were selected from two outbreaks with methicillin resistant *Staphylococcus aureus* (MRSA) and linezolid resistant *Staphylococcus epidermidis* (LRSE), respectively. Obtained from clinical samples at Rigshospitalet, they were selected for Nanopore and Illumina sequencing.
Hybrid assembly of long and short reads was conducted using Unicycler..
Heatmaps were created for analyses of gene absence/presence, using **GenAPI** (2).

RESULTS

The hybrid assembly data showed that the MRSA outbreak consisted of two main genomic events, while the LRSE outbreak had three main genomic events.
In the **MRSA** outbreak, a plasmid and a transposon located on the chromosome carried two resistance genes - erythromycin resistance (*Erm*C) and aminoglycoside phosphotransferase (*aph(3)-Illa*) that refers to kanamycin resistance - were gained during the outbreak.
In the **LRSE** outbreak, a resistance island - with fusidic acid resistance gene (*fusB*) as well as a transposon - was lost in four isolates, while a plasmid carrying two resistance genes - erythromycin resistance (*msrA*) and Beta-lactamase (*blaZ 2*) - were gained in three isolates.



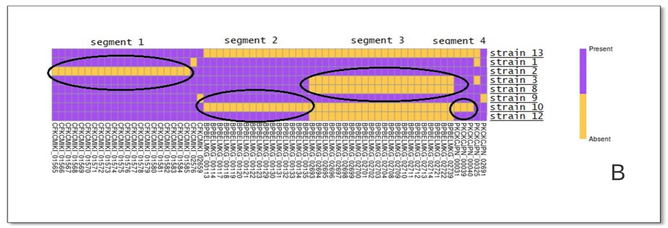


Fig. 1:
Heatmap from hybrid assembly data were created for analyses of gene absence/presence, using GenAPI https://github.com/MigleSur/GenAPI.
The map illustrates location (locus tags) with variation across the isolate collection, illustrating genes that were either present (purple) or absent (orange), revealing lost or acquired genes.
A: MRSA Heatmap. Segments 1 – 2 illustrate the genomic events in the MRSA strain collection. Segment 1; plasmid gained in strain 11 to 14 and 16 to 20. Segment 2 Transposon gained in strain 7, 15 and 20.
B: LRSE Heatmap. Segments 1 – 4 illustrate the genomic events in LRSE strain collection. Segment 1 Resistance Island lost in strain 2. Segment 2; Transposon gained in strain 7, 15 and 20.Segment 3 Plasmid gained by strain 1, 2 and 9. Segment 4 where genes included in segment 3.

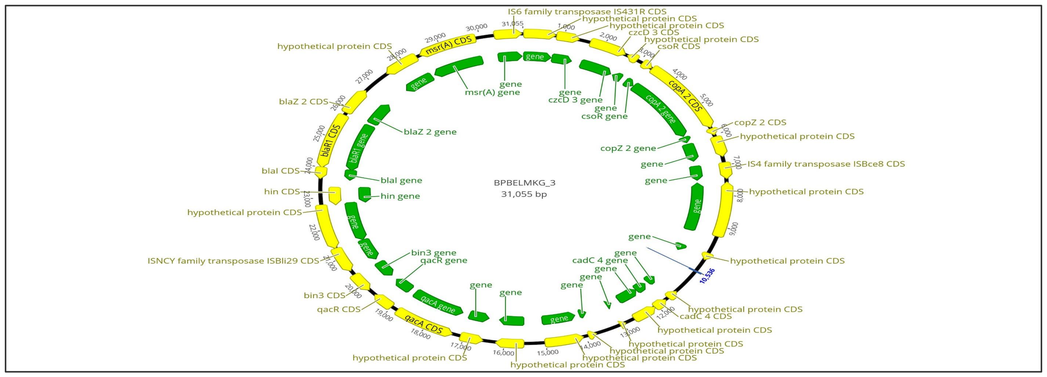


Fig. 2:
LRSE isolate 1, contig 3, illustrating a plasmid found in strain 1, 2 and 9. Contig 3 includes genes for antiseptic resistance protein (*qacA*),erythromycin resistance gene (*msrA*), and Beta-lactamase (*blaZ 2*).
Geneious version 2021.2 created by Biomatters. Available from https://www.geneious.com.

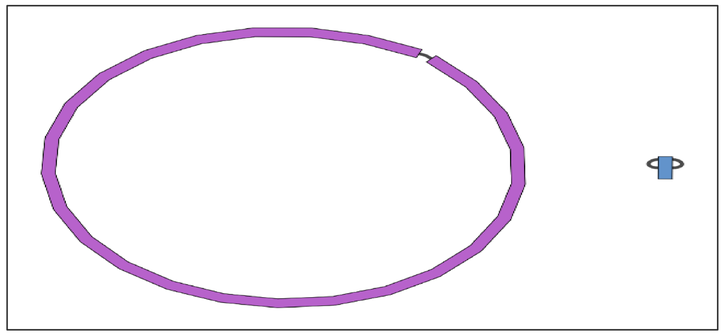


Fig. 3:
Genomic graph structure of a hybrid assembly graph. Bandage, a bioinformatic tool for creating interactive visualizations of assembly graphs, was used for visualization, using default parameters https://github.com/rrwick/Bandage
The graph shows a closed chromosome and one circularized plasmid.in strain 7 in the LRSE outbreak.

CONCLUSION

Creating hybrid assemblies using Unicycler, enables the obtainment of closed genomes and circularized plasmids. The data in this study showed that hybrid assembly data provided an accurate depiction of genomic events. In both outbreaks we were able to determine the location of genes on chromosomes or plasmids and detect transmission events of horizontal gene transfer between bacteria in outbreak situations. Both collections showed genetic variation in resistance gene content during the outbreak.

References
1. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol. 2017;13(6):1–22.
2. Gabrielaite M, Marvig RL. GenAPI: A tool for gene absence-presence identification in fragmented bacterial genome sequences. bioRxiv. 2019;5:1–8.