HYBRID ASSEMBLY AS A USEFUL TOOL FOR TRACING OF ANTIMICROBIAL RESISTANCE SPREAD

Aleksandra Giza MSc*, Ewelina Kamińska MSc, Ewelina Iwan PhD,
Dariusz Wasyl DVM, PhD, DSc
National Veterinary Research Institute, 57 Partyzantow Avenue, 24-100 Pulawy, Poland
*aleksandra.giza@piwet.pulawy.pl

Antimicrobial resistance (AMR) has become an increasing problem for public health and food production. Food production chain need to be monitored, due to risk of outbreaks and endangering the public. Bacteria addressed by the monitoring are often carriers of multiple resistance genes, that frequently are located on plasmids. Those mobile elements are one of the main routes for resistance spread. Scientific literature commonly describes cases related to a single plasmid, that was carried by several bacteria.

Whole Genome Sequencing (WGS) is currently the new “golden standard” used in investigation of foodborne bacteria outbreaks. It enables hazard identification, transmission pathways of the microorganisms, source attribution analyses, and thereby more targeted risk assessment. This application can be carried out using second or third generation sequencing platforms. Illumina platform (2nd generation) provides short, highly accurate results. Oxford Nanopore (3rd generation) platform generates less accurate, but longer reads, that are useful for reconstruction and characterisation of genomic structures e.g. chromosomes, plasmids. Data usage from each of the platforms alone does not allow for either perfect identification of AMR genes or proper reconstruction of specific genomic structure – closed plasmid or chromosome. However, hybrid assembly is a powerful bioinformatic approach, that enables both. The principle of this method is simultaneous assembly of consensus sequence from Illumina and Oxford Nanopore reads.

Tab. 1. Comparison of Illumina platform (MiSeq) and Oxford Nanopore platform (MinION) [3,4]

Sequencing	Illumina (MiSeq)	Oxford Nanopore (MinION)
Generation	Second	Third
Max read length	Short (up to 300bp)	Long (up to 100 kb)
Error rate (%)	0,1	5-15
Applications	- species identification - investigation of genomic content of bacterial isolates (e,g. resistance gene prevalence) - variant detection and assessing genetic relatedness (e.g. for outbreak investigation or population genetic studies)	- superior performance in the analysis of long, repeated regions - frequently used to finish previous short-read assemblies (rising contiguity, solving fragmented regions, closing gaps)
Advantages	- high fidelity reads - low input requirement	- no upper limit to read length - sequencer is mobile (can be used in the field) - no need for spatialized laboratory infrastructure
Disadvantages	- complete genomes are impossible to obtained - requires advanced laboratory infrastructure	- high error rate - high input requirement (1µg)

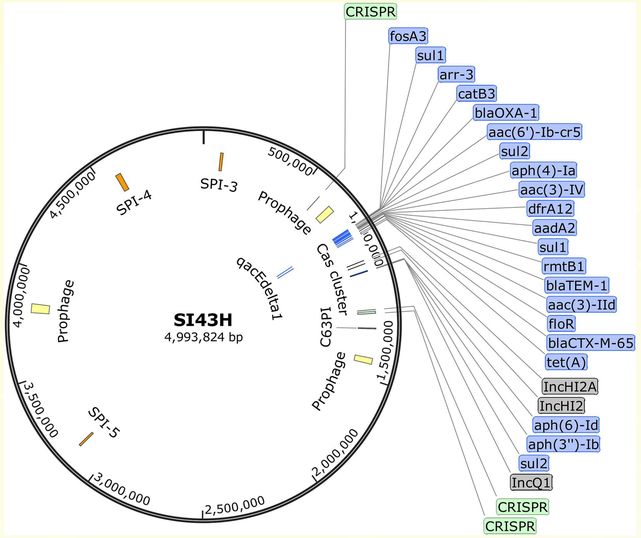

Fig. 1. Circular chromosome map of S. Indiana isolate 43 (SI43H) based on its hybrid assembly. In the genome were detected three plasmids (IncHI2, IncHI2A, and IncQ1) integrated into chromosome [1].

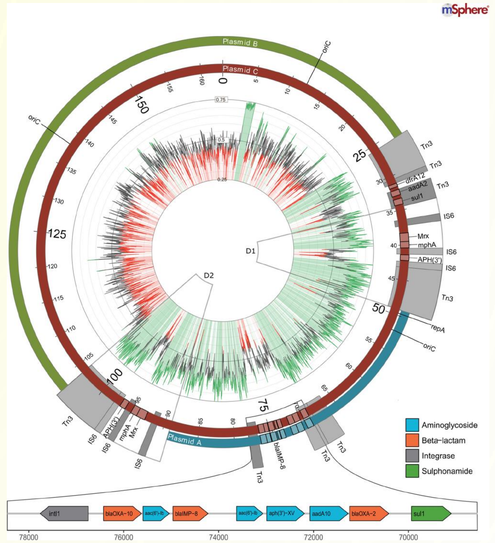

Fig. 3. Example of detailed hybrid alignment of 3 plasmids (A, B, and C) retrived from different bacterial *species* (*Pseudomonas aeruginosa*, *Citrobacter freundii*, *Citrobacter cronae*) from a Hospital Setting. These plasmids are carrier for AMR genes (resistance to aminoglycosides, beta-lactams, and sulfonamides [5].

Summary

Hybrid assembly can be expensive and requires specialized knowledge to interpret the results, but it adds a new quality to genomic data. It allows for reconstruction and characterization of AMR carrying plasmids and chromosomes, localization of AMR genes and determination of their genomic environment. Those allows for in-depth and focused studies on the spread of resistance mechanisms in different environments and ecological niches.

References

1. Chen Z., Kuang D., Xu X. et al.: Genomic analyses of multidrug-resistant Salmonella Indiana, Typhimurium, and Enteritidis isolates using MinION and MiSeq sequencing technologies. PLOS ONE July 2, 2020
2. Molina-Mora JA, Campos-Sánchez R, Rodríguez C et al.: High quality 3C de novo assembly and annotation of a multidrug resistant ST-111 Pseudomonas aeruginosa genome: enchmark of hybrid and non-hybrid assemblers. Scientific Reports 2020, 10:1392
3. van Dijk EL., Jaszczyszyn Y., Naquin D., Thermes C.: The Third Revolution in Sequencing Technology. Trends in Genetics, September 2018, Vol. 34 (9)
4. Peter S., Bosio M., Gross C. et al.:Tracking of Antibiotic Resistance Transfer and Rapid Plasmid Evolution in a Hospital Setting by Nanopore Sequencing. Clinical Science and Epidemiology, July/August 2020 Vol. 5 (4)