Automated antimicrobial susceptibility predictions and genotypes among *Enterobacteriaceae* using Illumina and ONT sequencing data

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Background: Whole genome sequencing (WGS) enables the detailed molecular characterization of bacterial pathogens. The identification of resistance mechanisms conferring clinical phenotypes, such as carbapenemase- (CP) or extended spectrum β-lactamase-producing (ESBL), and the prediction of antimicrobial resistance (AMR), are relevant. We compare Illumina and Oxford Nanopore Technologies (ONT) sequencing platforms for the identification of genotypes mediating relevant phenotypes and predictive antimicrobial susceptibility testing (pAST) on clinical *Enterobacteriaceae*.

Methods: *Enterobacteriaceae* isolates (n: 181) including *Klebsiella* (n: 154), *Escherichia* (n: 14), and *Enterobacter* (n: 13) were categorized into carbapenem-resistant (CR) (n: 132) and susceptible (n: 49). Broth microdilution AST was performed with Sensititre GN7F and MDRGN2F (Thermo Scientific) panels following CLSI guidelines. WGS was performed on Illumina platforms and ONT (R9.4.1 flow cells; Rapid Barcoding). Genome assembly, quality control, identification, typing, AMR marker detection and pAST (26 compounds) were run on AREScloud (Ares Genetics). Molecular genotypes and pAST results were combined to predict clinical phenotypes; pAST performance and concordance of predicted phenotypes were compared between platforms. Predictive AST models for ceftriaxone/ceftazidime defined 3rd generation cephalosporin (3GC) resistance. 3GC-resistant, carbapenem-susceptible organisms were delineated by plasmid AmpCs (pAmpCs) and/or ESBLs. CR was defined with the pAST model for ertapenem. CP-CREs were defined by KPC, MBL or OXA-48.

Results: Ertapenem predictions were optimized for non-CP-CREs to 89% and 95% overall accuracy for Illumina and ONT assemblies. Accuracies for ceftriaxone/ceftazidime were 95%/91% (Illumina) and 96%/92% (ONT). CR was overpredicted on ONT with a major error (ME) of 14% (vs 2%) and underpredicted on Illumina assemblies with a very major error (VME) of 15% (vs 1%). Sensitivities of CPs, ESBLs, and pAmpCs were lower on ONT compared to Illumina with 66%, 78%, and 20%. Overall accuracy across all compounds was 93% (Illumina) and 91% (ONT) [$\chi^2 < 0.05$].

Conclusions: The performance gap for pAST between Illumina and ONT has narrowed over the last years. While significant differences still exist, especially for the detection of AMR genes, our results highlight the potential of the ONT platform for clinical microbiology if combined with robust predictive bioinformatics methods. Combining AMR genotypes and pAST using WGS approaches can help guide therapeutic management.