CONTROL NUMBER 5584

Assessing the Potential of Short-Read and Long-Read Sequencing to Predict Phenotypic Antimicrobial Susceptibility Testing Results in Carbapenem-Resistant *Klebsiella pneumoniae*

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INTRODUCTION

- Whole genome sequencing (WGS) is being increasingly utilized to predict phenotypic antimicrobial susceptibility testing (WGS-AST) results.
- The purpose of this study was to assess the potential to predict AST from WGS assemblies generated from both short-read and long-read sequencing technologies among carbapenemase-producing [CP] and non-CP carbapenem-resistant [CRE] *Klebsiella pneumoniae*.

MATERIALS AND METHODS

- Short-read Illumina and long-read Oxford Nanopore Technologies (ONT) WGS was performed on 78 *Klebsiella pneumoniae* (68 CP-CRE and 9 non-CP-CRE).
- Clinical specimens were isolated between 2016 and 2018 and selected by resistance to ertapenem.
- In a first step, primary SPAdes assemblies (Illumina only) and secondary Canu/Pilon assemblies (ONT corrected with Illumina short-read data) were created.
- Following, susceptibility and resistance labels were predicted from the assemblies by WGS-AST classification models trained on Ares Genetics' proprietary database, ARESdb[1].
- WGS-AST labels were compared to susceptible versus not susceptible (intermediate and resistant) AST results generated by the BD Phoenix Automated Microbiology System using Clinical and Laboratory Standards Institute interpretive guidelines [2].

RESULTS

Table 1: Summarizes the Categorical Agreement [CA] between culture-based AST and WGS-AST for CP-CRE. The decrease in CA for the smaller data set of up to 9 non-CP CRE (data not shown) is shown in the last column.

Agent	Agent Class	Assembly Type	CA [%]	TP	FP	FN	TN	Decrease in CA for non-CP CRE
Ertapenem	Carbapenem	Primary	95.5%	64	0	3	0	86.9%
		Secondary	97.0%	65	0	2	0	74.2%
Imipenem	Carbapenem	Primary	94.0%	60	2	2	3	33.5%
		Secondary	94.0%	60	2	2	3	6.9%
Meropenem	Carbapenem	Primary	89.4%	56	5	2	3	44.1%
		Secondary	89.4%	57	6	1	2	44.1%
Ceftazidime	Cephalosporin (third-generation)	Primary	94.0%	62	0	4	1	20.2%
		Secondary	95.5%	63	0	3	1	21.5%
Ceftriaxone	Cephalosporin (third-generation)	Primary	100.0%	59	0	0	1	44.4%
		Secondary	100.0%	59	0	0	1	33.3%
Cefepime	Cephalosporin (fourth-generation)	Primary	89.6%	60	3	4	0	58.1%
		Secondary	94.0%	63	3	1	0	60.1%
Aztreonam	Monobactam	Primary	97.0%	64	1	1	0	41.1%
		Secondary	95.5%	63	1	2	0	25.2%
Piperacillin-tazobactam	Penam (combination)	Primary	97.0%	65	1	1	0	48.5%
		Secondary	95.5%	64	1	2	0	47.7%
Ticarcillin-clavulanic acid	Penam (combination)	Primary	93.0%	53	0	4	0	76.1%
		Secondary	93.0%	53	0	4	0	76.1%
Ciprofloxacin	Fluoroquinolone	Primary	98.5%	63	1	0	2	23.8%
		Secondary	98.5%	63	1	0	2	36.5%
Levofloxacin	Fluoroquinolone	Primary	86.8%	57	0	9	2	61.6%
		Secondary	89.7%	59	0	7	2	62.8%
Gentamicin	Aminoglycoside	Primary	91.1%	28	4	1	23	-9.8%
		Secondary	87.5%	28	6	1	21	-14.3%
Tobramyin	Aminoglycoside	Primary	95.0%	50	3	0	7	34.2%
		Secondary	93.3%	50	4	0	6	33.0%
Overall	-	**Primary**	**93.9%**	741	20	31	42	43.7%
		Secondary	**94.1%**	747	24	25	38	39.9%

CONCLUSIONS

- WGS-AST reliably enables the detection of CP-mediated antibiotic resistance across sequencing platforms.
- Overall categorical agreement was found to be 93.9% and 94.1% for primary (SPAdes) and secondary assemblies (Canu/Pilon). The difference between both platforms was not significant.
- Both assembly types resulted in reduced sensitivity in a small set of non-CP isolates, suffering from comparatively high false negative rates, resulting in a decrease in categorical agreement. This effect was not limited to β-lactams and warrants further analysis on a larger set.

REFERENCES

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[2] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Ninth Informational Supplement. M100-S29. Wayne, PA.