Prediction of antimicrobial susceptibility of pneumococci based on whole genome sequencing data: a direct comparison of two online predictive tools to conventional antimicrobial susceptibility testing

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BACKGROUND

- At the **Belgian Reference Centre** for invasive pneumococcal disease: **~20%** of all isolates are characterized by **reduced beta-lactam susceptibility**
- Tools are available to predict antimicrobial susceptibility based on genomic data.
- **Our study assessed the performance** of two predictive tools for antimicrobial susceptibility testing (AST) making use of phenotypic AST as golden standard.

METHODS

- **Bacterial isolates:** 538 unique clinical *S. pneumoniae* isolates with a wide range of MICs for different antibiotics, collected from the Belgian Reference Centre and a quality control set including ATCC-49619 and ten CCUG strains.
- **Phenotypic AST:** disk diffusion and broth microdilution (Sensititre, Thermo Scientific) following EUCAST guidelines.
- **Whole genome sequencing (WGS):** DNA isolation with DSP Mini Kit (QIAGEN), Illumina Nextera XT DNA library preparation and sequencing with MiSeq or Hiseq2500 instruments.
- **Predictive tools - Pathogenwatch and AREScloud:** Pathogenwatch predicts MICs for beta-lactam antibiotics based on a machine learning model trained with penicillin-binding protein profiles and for non-beta-lactam the prediction is based on the presence or absence of antibiotic resistance genes. AREScloud used machine learning models trained on genomic features to predict MICs for beta-lactam and non-beta-lactam antibiotics.
- **Evaluated antibiotics**: benzyl-penicillin, amoxicillin, cefotaxime, erythromycin, trimethoprim-sulfametoxazole and tetracycline were included.
- **Performance evaluation:** Categorical agreement (CA), essential agreement (EA), major error (ME), and very major error (VME) rates were calculated for the two predictive tools taking phenotypic AST as the reference (following Clinical and Laboratory Standards Institute (CLSI) guidelines).
- **Processing time**: a subset of 20 sequences (8.08Gb) out of the final dataset of 517 sequences was tested at 3 different times of the day for each prediction tool.

RESULTS

	Phenotypic characterisation		
Antibiotics	**S % (*n*)**	**I % (*n*)**	**R % (*n*)**
(Benzyl)penicillin	79.1 (*409*)	16.8 (*87*)	4.1 (*21*)
Amoxicillin	93.6 (*484*)	1.5 (*8*)	4.8 (*25*)
Cefotaxime/ceftriaxone	94.8 (*490*)	4.3 (*22*)	1.0 (*5*)
Erythromycin	78.3 (*405*)	1.7 (*9*)	19.9 (*103*)
Trimethoprim-sulfomethoxazole	77.4 (*400*)	7.4 (*38*)	15.3 (*79*)
Tetracycline	77.8 (*402*)	3.7 (*19*)	18.6 (*96*)

Table 1 Results of phenotypical AST testing of 517 *Streptococcus pneumoniae* isolates

	AREScloud				Pathogenwatch			
Antibiotics	CA (%)	ME (%)	VME (%)	EA (%)	CA (%)	ME (%)	VME (%)	EA (%)
(Benzyl)penicillin	94.2	0.0	0.0	86.2	94.4	0.0	0.0	90.8
Amoxicillin	97.3	0.2	0.0	94.5	96.5	0.0	0.0	95.4
Cefotaxime/ceftriaxone	98.5	0.0	0.0	97.2	98.8	0.0	0.0	98.2
Erythromycin*	98.3	0.5	6.8	NA	89.9	0.2	49.5	NA
Trimethoprim-sulfomethoxazole*	92.8	8.0	2.5	NA	98.1	1.1	1.3	NA
Tetracycline*	97.3	1.9	6.3	NA	91.9	1.43	37.5	NA

Table 2 Categorical agreement (CA), major error (ME), very major error (VME) and essential agreement (EA) rates for AREScloud and Pathogenwatch WGS-AST compared to phenotypic AST. CA was calculated for antibiotics with complete data for phenotypical methods and predictive tools. EA was calculated only for strains with MICs for beta-lactam antibiotics. Cells highlighted in green passed the CLSI (M52 guideline) acceptance criteria for antimicrobial susceptibility testing systems: CA ≥90%, ME and VME <3%. * For this antibiotic, the tool reported only R and non-R, for which the I category from phenotypic AST was taken together with the S category as 'non-R' for analysis.

Out of 549 total isolates, 32 isolates were excluded for further analysis due to incomplete results obtained with the AST prediction tools. A dataset of 517 isolates was used for the final analysis, (Table 1).

Beta-lactam antibiotics

- The CA, ME and VME rates for all three **beta-lactam** antibiotics met the CLSI criteria (CA>90%, ME and VME <3.0%). For the EA rate, the CLSI criteria were met (>90%) for all, except for the benzyl-penicillin prediction by AREScloud (86.2%) (Table 2).

Non-beta-lactam antibiotics

- The CA agreement for **erythromycin** met the CLSI criteria with AREScloud (98.3%) and almost with Pathogenwatch (89.9%). The ME rate of both tools met the CLSI criteria. Nevertheless, high VME rates of 6.8% for AREScloud and 49.5% for Pathogenwatch were identified
  - High VME of Pathogenwatch: 44 isolates had a non-R prediction from Pathogenwatch but were phenotypically resistant and resistant with AREScloud. In 36 of these 44 isolates, we were able to identify the presence of the *ermB* gene. In the remaining 8, the *ermB* was absent.
- **Trimethoprim-sulfamethoxazole** predictions showed high CA rates (>90%) for both predictive tools.
- The CA rate for **tetracycline** for AREScloud was 97.3% and for Pathogenwatch was 91.1%. The ME rate also met the CLSI criteria but not for VME (6.3% and 37.5% for AREScloud and Pathogenwatch respectively).
  - High VME for Pathogenwatch: 30 isolates were predicted as non-R by Pathogenwatch but were phenotypically resistant and resistant by AREScloud. We were able to identify the *tetM* gene in all strains, although for 23 isolates, a deletion of +/- 174 bp was present in the *tetM* gene.

Processing Time Comparison

- Both tools had comparable upload speed (Pathogenwatch: 33.71Mb/s; AREScloud: 29.15Mb/s), AREScloud was slightly faster for providing AST results, averaging 02:31 hours compared to 03:06 hours for Pathogenwatch.

CONCLUSIONS

- Both predictive tools exhibit very good performance for beta-lactam antibiotic predictions.
- For the non-beta-lactam antibiotics also high categorical agreement was observed, but (very) major error rates were higher than acceptable for erythromycin and tetracycline. The error rates were higher for Pathogenwatch compared to ARESCloud.
- In the context of more and more pneumococcal genomes that become available, these tools may help to study large datasets to better understand (evolutions in) antimicrobial susceptibility.
- These genomic tools offer an alternative for phenotypic AST which is important in a clinical context but also in a context of National Reference Centres that are moving from phenotypic and PCR-based testing to WGS of pneumococci. Despite promising results, continuous validation and improvements are essential.

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