Benchmarking of genotypic detection of antimicrobial resistance (AMR) genes

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Antimicrobial resistance (AMR) of foodborne pathogens is important for guiding treatment and surveillance of the antimicrobial resistance prevalence. Phenotypic susceptibility testing such as disk diffusion and Minimal Inhibitory Concentration (MIC) determination is a time-consuming and laborious process. Whole genome sequencing (WGS) offers an alternative to the phenotypic testing for determining the susceptibility to a range of antibiotics in a single test.

Purpose of the benchmarking exercise

The purpose of this study was to benchmark several of the currently available bioinformatics software tools for identification of AMR genes. A well-characterized set of food pathogen isolates (*Salmonella* and *E. coli*) that have been phenotypically tested for their susceptibility to several antimicrobials were compared to the genotypic profiles based on whole genome sequence data.

Benchmarked tools

The following tools with default parameters were assessed in the benchmarking exercise:

1) ResFinder 1.2 from DTU (available as command line and online tool)
   - BLAST-based detection of horizontally acquired genes and chromosomal point mutations (command line version)
2) KmerResistance 2.1 from DTU (available as command line and online tool)
   - Kmer-based detection of horizontally acquired genes (command line version used)
3) SRST2 v0.1.7 from http://katholt.github.io/srst2/ (available as command line only)
   - Mapping based detection of resistance genes
4) PHE Genefinder from PHE (available as command line only)
   - Mapping based detection of horizontally acquired genes and point mutations

Species/genomes included

Two datasets were collected for the purpose of this study.

The Animal and Plant Health Agency (APHA) collected 125 *Salmonella* isolates. Bacterial DNA was extracted using the MagNA Pure LC DNA Isolation Kit III (Roche) according to manufacturer's instructions and sequencing libraries were prepared using the NexteraXT sample preparation method for sequencing on the Illumina HiSeq

platform with paired-end 2x125bp reads (http://www.illumina.com).

The National Food Institute at DTU collected 164 *E.coli* isolates. Genomic DNA was extracted using an Invitrogen Easy-DNA™ Kit (Invitrogen, Carlsbad, CA, USA) and DNA concentrations were determined using the Qubit dsDNA BR assay kit (Invitrogen). The genomic DNA was prepared for Illumina pair-end sequencing using the Illumina (Illumina, Inc., San Diego, CA) NexteraXT® Guide 150319425031942 following the protocol revision C (http://support.illumina.com/downloads/nextera_xt_sample_preparation_guide_15031942.html). A sample of the pooled NexteraXT Libraries was loaded onto a Illumina HiSeq reagent cartridge using HiSeq Reagent Kit v2. The libraries were sequenced using an Illumina Hiseq platform.

The final datasets consisted of 289 isolates. Raw reads were trimmed using bbduk2 (part of the suit bbtools version 36.49) with the following cut-off; 1) length of read >= 50 bp, 2) Phred score per base >= 20. De novo assembly was performed using SPAdes with minimum Kmer coverage at 2 and minimum contig size at 500 bp.

For dataset from APHA, the antimicrobial susceptibility testing of the 125 *Salmonella* isolates was performed and interpreted using the Kirby-Bauer disk diffusion method, on Isosensitest Agar (Oxoid) as described by the British Society for Antimicrobial Chemotherapy (BSAC). The following antimicrobials were included in the testing with the listed disc concentrations (µg per ml): nalidixic acid (30); tetracycline (10); neomycin (10); ampicillin (10); furazolidone (15); ceftazidime (30); sulphamethoxazole/trimethoprim (25); chloramphenicol (30); amikacin (30); amoxicillin/clavulanic acid (30); gentamicin (10); streptomycin (10); sulphonamide compounds (300); cefotaxime (30); apramycin (15); ciprofloxacin (1).

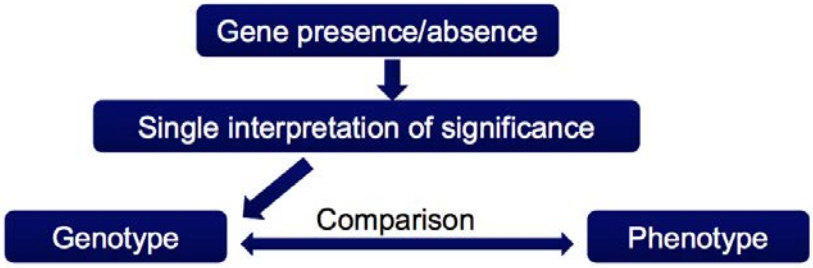
Minimum inhibitory concentration (MIC) determination was performed at DTU on the 164 *E.coli* isolates using commercially prepared dehydrated panels, EUVSEC and EUVSEC2 (Sensititre; TREK Diagnostic Systems Ltd., East Grinstead, England). EUCAST epidemiological cut-off values were used as interpretative criteria to determine the phenotypic resistance (http://www.eucast.org). Quality control was performed by using reference strain *E. coli* ATCC 25922 according to CLSI guidelines.

Method

Four tools were benchmarked in this study: ResFinder [1], KmerResistance [2], SRST2 [3], PHE Genefinder.

Availability of tools:
ResFinder: https://cge.cbs.dtu.dk/services/ResFinder/
KmerResistance: https://cge.cbs.dtu.dk/services/KmerResistance/
SRST2: https://katholt.github.io/srst2/
PHE Genefinder (in-house software, not publicly available)

The genotypic testing was performed independently by the different collaborating partners and results were afterwards compared. Thus, DTU tested the ResFinder tool, PHE tested the KmerResistance tool and the Genefinder, and APHA tested the SRST2 tool. The phenotypic susceptibility data were used as proxy for the true result. The genotypic results were compared to the phenotypic susceptibility data and the performance of the tools was assessed by calculating the specificity, sensitivity, accuracy and the Matthew's Correlation Coefficient (MCC). Additional statistical tests of agreement were also applied based on learning from the serotype benchmarking exercise. Given that resistance to different classes of antibiotics is conferred by different genes, it was decided to break down the results by antibiotics since some of the software tools perform better at calling a profile for different classes.



Overall results

The results for specificity, sensitivity, accuracy and MCC for all antibiotic classes are presented in Table 1 and Table 2 and the accuracy in predicting different classes of antibiotic in Figure 1, Figure 2 and Supplementary Table 5 (Annex E).

All tools tested provided an approximate accuracy of around 90% when testing the *Salmonella* genomes (Table 1). All tested tools achieved an overall lower accuracy, between 80-82%, when testing the *E. coli* dataset (Table 2). The accuracy in predicting resistance in *E. coli* for β-lactams and fluoroquinolones using all tools was low, ranging between 55% - 58% and 82% - 84%, respectively (Figure 2 and Supplementary Table 5 (Annex E)).

Table 1. Results from Salmonella dataset for all antibiotic classes

Software	Specificity	Sensitivity	Accuracy	MCC
KmerResistance	0.95	0.74	0.86	0.72
ResFinder	0.95	0.83	0.90	0.79
SRST2	0.93	0.80	0.87	0.74
PHE GeneFinder	0.97	0.83	0.90	0.81

Table 2. Results from E. coli dataset for all antibiotic classes

Software	Specificity	Sensitivity	Accuracy	MCC
KmerResistance	0.91	0.46	0.80	0.41
ResFinder	0.89	0.60	0.82	0.51
SRST2	0.89	0.57	0.81	0.48
PHE GeneFinder	0.90	0.53	0.81	0.47

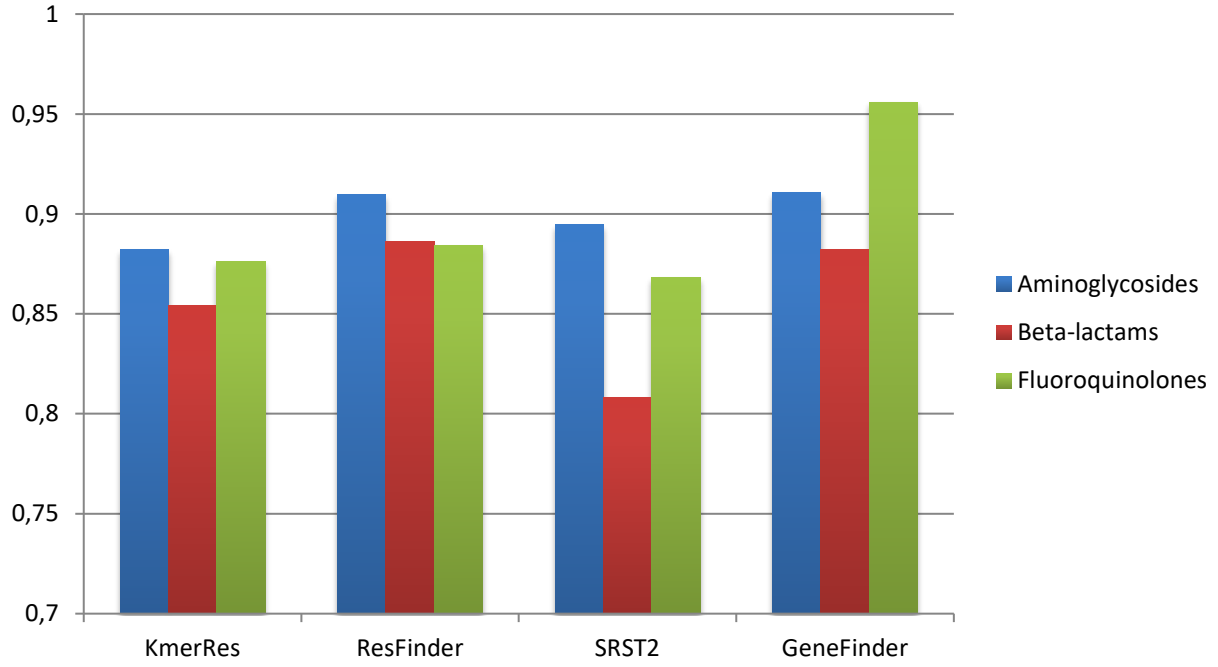


***Figure 1**. Accuracy obtained by the benchmarked tools for three antimicrobial classes for the tested Salmonella dataset. Y-axis represents accuracy ratio expressed as a fraction of 1.*

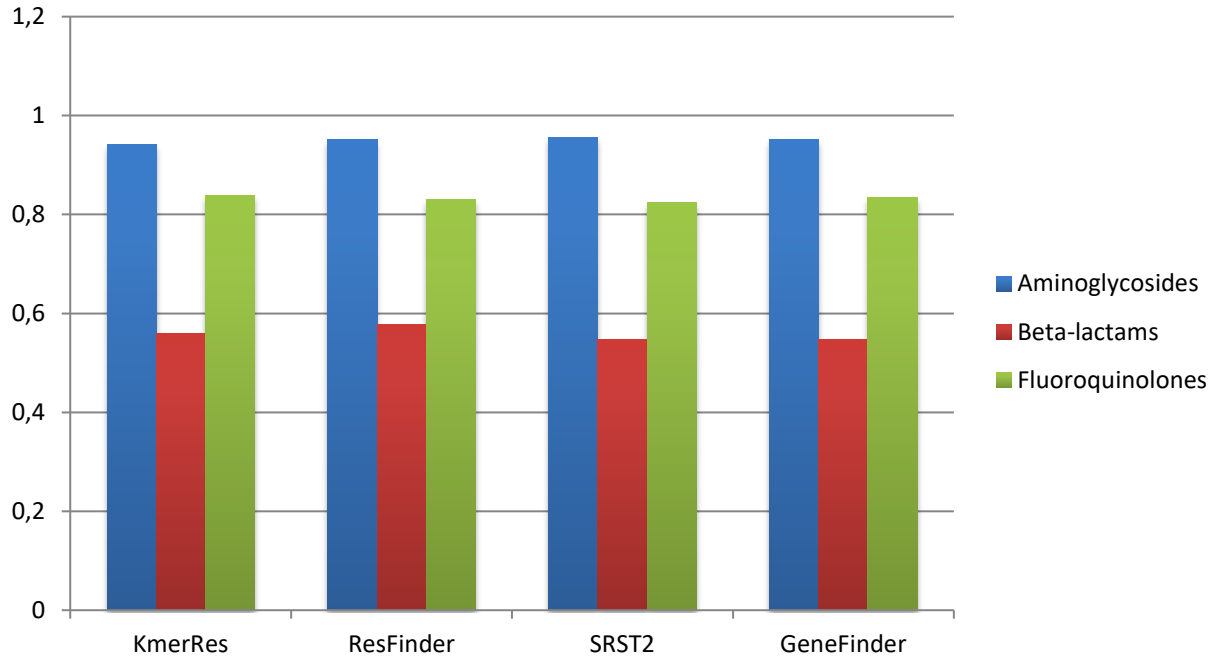


***Figure 2.** . Accuracy obtained by the benchmarked tools for three antimicrobial classes for the tested E.coli dataset. Y-axis represents accuracy ratio expressed as a fraction of 1.*

Conclusion

The tools providing the highest degrees of specificity, sensitivity, MCC and accuracy in *Salmonella* data were the ResFinder 1.2 and PHE GeneFinder tools (no version available; tests performed on 01.02.2017). ResFinder also provided the highest accuracy and MCC in predicting resistance in the *E. coli* genomes, while GeneFinder provided the highest MCC in predicting resistance in the *Salmonella* genomes.

All tools revealed an approximate 90% correlation with the phenotypic susceptibility testing for *Salmonella*. Only the PHE Genefinder predicted resistance to fluoroquinolones based on chromosomal point mutation and hereby performed with a higher accuracy than other tools for fluoroquinolone resistance (Figure 1).

All tools performed with a lower accuracy when testing *E. coli*. A very low accuracy was achieved in profiling β-lactam (Figure 2). This could be due to the possible bias in the dataset that included a number of *E. coli* containing upregulated chromosomal *ampC* mutations (mediating β-lactam resistance) which none of the tools could predict. By including the methods to detect *ampC* mutations and other chromosomal point mutations, the concordance for β-lactam and fluoroquinolone resistance can be increased. Therefore, *ampC* mutations and other chromosomal point mutations will soon be included in a new version of ResFinder.

The results of this benchmarking study showed that predicting antimicrobial resistance using WGS is a feasible and realistic alternative to phenotypic susceptibility testing. In addition, for the *Salmonella* and the *E.coli* datasets, different criteria were applied for the definition of phenotypic resistance, as this can also influence the results. The comparability of the phenotypic results should be taken into account because phenotypic criteria for defining resistance and susceptible were different. This might affect the results on the correlations between phenotypes and genotypes.

The miscorrelation rate (cases where the tools predicted a different antimicrobial profile than the expected) were 10-14% in the *Salmonella* dataset. These miscorrelations are suspected to be caused by mistakes in the phenotypic susceptibility testing.

Additional notes

It is recommended to retest the phenotypic susceptibility for the isolates showing discordant results with the genotypic prediction tools and the expected genotypic resistance profile derived from phenotypic susceptibility testing. This is especially important for the isolates where all tools showed identical miscorrelations. Additionally, the sequencing quality also influence the performance of the tools.

References

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