Accuracy of different bioinformatics methods in detecting antibiotic resistance and virulence factors from *Staphylococcus aureus* whole genome sequences.

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Running Head: *S. aureus* Whole-genome Sequence Method Comparison

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Abstract (249 words, limit 250 words)

Background: In principle, whole genome sequencing (WGS) can predict phenotypic resistance directly from genotype, replacing laboratory-based tests. However, the contribution of different bioinformatics methods to genotype-phenotype discrepancies has not been systematically explored to date.

Methods: We compared three WGS-based bioinformatics methods (Genefinder (read-based), Mykrobe (de Bruijn graph-based) and Typewriter (BLAST-based)) for predicting presence/absence of 83 different resistance determinants and virulence genes, and overall antimicrobial susceptibility, in 1379 *Staphylococcus aureus* isolates previously characterised by standard laboratory methods (disc diffusion, broth and/or agar dilution and PCR).

Results: 99.5% (113830/114457) of individual resistance-determinant/virulence gene predictions were identical between all three methods, with only 627 (0.5%) discordant predictions, demonstrating high overall agreement (Fliess-Kappa=0.98, p<0.0001). Discrepancies when identified were in only one of the three methods for all genes except the cassette recombinase, *ccrC(b)*. Genotypic antimicrobial susceptibility prediction matched laboratory phenotype in 98.3% (14224/14464) cases (2720 (18.8%) resistant, 11504 (79.5%) susceptible). There was greater disagreement between the laboratory phenotypes and the combined genotypic predictions (97 (0.7%) phenotypically-susceptible but all bioinformatic methods reported resistance; 89 (0.6%) phenotypically-resistant, but all bioinformatics methods reported susceptible) than within the three bioinformatics methods (54 (0.4%) cases, 16 phenotypically-resistant, 38 phenotypically-susceptible). However, in 36/54 (67%), the consensus genotype matched the laboratory phenotype.

Conclusions: In this study, the choice between these three specific bioinformatic methods to identify resistance-determinants or other genes in *S. aureus* did not prove critical, with all demonstrating high concordance with each other and phenotypic/molecular methods. However, each has some limitations and therefore consensus methods provide some assurance.

Introduction

Staphylococcus aureus causes both superficial infections (such as boils) and life-threatening disease including septicaemia (1). There were 11,405 *S. aureus* bacteraemias in England in 2015/2016 (2); 7.2% were meticillin resistant *S. aureus* (MRSA) which has increased costs and poorer patient outcomes (3). Fast accurate resistance prediction is key to managing *S. aureus* infections. Molecular-based methods directed at detecting specific genes, e.g. through rapid multiplex PCR and microarrays, can reduce time to identify resistance determinants and time on broad-spectrum antibiotics (4-6). However, they require specific primers that impact sensitivity and specificity.

In principle, whole genome sequencing (WGS) has the potential to predict phenotypic resistance directly from genotype, replacing laboratory-based phenotypic tests (7). Several studies report high concordance between genotypic predictions based on known or novel resistant determinants and phenotypic methods (8-13). However, these studies used varying sequence processing pipelines and bioinformatics methods to identify *in silico* resistance determinants. Without formal comparisons between the various methods, it is unclear whether the underlying differences affect results, or whether differences in methodology could cause some of the observed discrepancies between genotypic predictions and phenotype.

Here, we therefore compare three WGS-based bioinformatics methods (Genefinder (read-based), Mykrobe (de Bruijn graph-based) and Typewriter (BLAST-based)) in terms of predictions of presence/absence of different resistance determinants, and overall prediction of antimicrobial susceptibility and presence/absence of virulence genes, from short-read Illumina WGS.

Results

Short-read Illumina WGS were available from 1,389 samples, 992 from a collection held in Oxford (previously described by Gordon *et al* (9, 10)) and 397 from Public Health England (PHE) Staphylococcus Reference Service, Colindale. Ten samples were excluded due to mixed/contaminated WGS results, leaving 1,379 for analysis. Samples were analysed by Genefinder and Typewriter (Table 1) after sequence mapping and variant calling and by Mykrobe from raw fastq reads.

84 genes were included: 46 acquired resistance genes, five sets of chromosomal variants within genes associated with resistance, three cassette chromosome recombinases *ccrA*, *ccrB* and *ccrC* including three variants of *ccrC* (*ccrCa, ccrCb, ccrCc*) and 28 virulence genes (Supplementary Table 1). 99.5% (113830/114457) of the individual resistance-determinant/virulence gene predictions were identical between all three methods (Supplementary Table 1, Figure 1), with only 627 (0.5%) discordant predictions, demonstrating high overall agreement (Fliess-Kappa=0.98, p<0.0001). Overall, one method disagreed with both other methods in 0.23% for Typewriter (263/114457 predictions), 0.16% Mykrobe (183/114457) and 0.16% Genefinder (181/114457). The three most common discrepancies for Typewriter were the non-detection of virulence genes identified by other methods (*seu* 57 samples, *chp* 46 samples, *sei* 33 samples). Similarly, for Genefinder the three most common discrepancies were non-detection of resistance genes (*qacB* 44 samples, *dfrC* 34 samples) or other genes (*ccBb* 22 samples) identified by other methods. Genefinder reported the presence of *dfrA*, *qacA* or *ccrC*(b)

genes in these samples. In contrast, Typewriter and Mykrobe reported the presence of two *dfr*, two *qac* and three *ccrC* genes, where the detected variants for each of these three genes shared more than 90% nucleotide identity. The most common discrepancies for Mykrobe were identifying resistance/other genes as present when the other two methods called them absent (*aadE/ant(6)-Ia* 28 samples, *blaZ* 19 samples, *ccrCB* 22 samples). No gene was ever identified as present by Typewriter alone. 14 of the 84 genes had >1% discrepancies (maximum 4.3% for *seu*), but the majority of discrepancies were in only one method for all genes except *ccrC(b)*.

Discrepancies were similar in acquired resistance genes (0.3%, 221/63434) and chromosomal resistance genes (0.1%, 8/5516), but slightly larger for *ccr* genes (1.8%, 123/6895) and virulence genes (0.7%, 275/38612) (Supplementary Table 2). Percentage discrepancies varied modestly across the different sample sets, being higher for the PHE set (1.1%, 349/32,928; particularly for *ccr* genes with 4.2% (83/1,960) discrepancies), intermediate for the Oxford derivation set (0.6%, 233/42084) and lowest for the Oxford validation set (0.1%, 45/40,824) (Supplementary Table 2).

Genotypic predictions of antimicrobial susceptibility were also identical in 99.6% of cases (16,477/16,548 predictions, Table 2). Of the 71 discrepancies in susceptibility prediction between the methods, 42% (30/71) occurred with Typewriter reporting susceptible when Genefinder and Mykrobe reported resistant, and 49% (35/71) occurred with Mykrobe reporting resistant where Genefinder and Typewriter reported susceptible.

Comparing genetic predictions to laboratory phenotypes (restricted to samples either phenotypically resistant or susceptible), in 98.3% (14224/14464) cases all three bioinformatics methods and the gold standard laboratory results agreed completely (2720 (18.8%) resistant, 11504 (79.5%) susceptible) (Table 3a, Figure 2). There was greater disagreement between the laboratory phenotypic results and the combined genotypic predictions than within the three bioinformatics methods. In 97 (0.7%) instances, the laboratory phenotype was susceptible but all bioinformatic methods reported resistance. Of these, 33% (32/97) were for penicillin, 23% (22/97) clindamycin and 11% (11/97) erythromycin, with smaller numbers for fusidic acid (7), tetracycline (6), mupirocin (6), methicillin (5), ciprofloxacin (4), gentamicin (3) and rifampicin (1), and none for trimethoprim. In 89 (0.6%) instances, the laboratory phenotype was resistant, but all three bioinformatics methods reported susceptible, most commonly to gentamicin (21%, 15/89), ciprofloxacin (17%, 15/89) and fusidic acid (15%, 13/89). The remaining 54 (0.4%) cases (16 phenotypically-resistant, 38 phenotypically-susceptible) had different genotypic predictions made from the different methods. However, in 36/54 (67%), the consensus genotype (predicted by two of the three methods) matched the laboratory phenotype.

PCR/array results were available for some virulence genes (14) and *mecA*/*mecC* for all 397 PHE isolates. Compared with genetic predictions, in 96.8% (3983/4115) cases all three bioinformatics methods and the PCR/array results agreed completely (3364 (81.7%) absent, 619 (15.0%) present) (Table 3b, Supplementary Figure 1). As for antimicrobial resistance, there was greater disagreement between the laboratory PCR/array results and the combined genotypic predictions than within the three bioinformatics methods, with 81 (2.0%) cases where all three methods called a gene present that had not been detected by PCR/array and 12 (0.3%) where no method called a gene present that had been detected by PCR/array, in comparison with 39 (0.9%) discrepant predictions between the methods. In 20/39 (51%), the consensus genotype matched the PCR/array result.

The sensitivity and specificity of all three bioinformatics methods compared to laboratory phenotypic methods in predicting antimicrobial susceptibility was very similar. Across the 14464 genotypic predictions, Typewriter had the lowest overall sensitivity (0.964 (95% CI 0.956-0.970), but the highest specificity (0.992 (0.990-0.993)), while Mykrobe had higher sensitivity (0.967 (0.960-0.974)) and lowest specificity (0.989 (0.987-0.990)). Genefinder's performance fell between Mykrobe and Typewriter for specificity (0.990 (0.988-0.992)) with a sensitivity equal to Mykrobe (0.967 (0.960-0.973)). Specificity and sensitivity varied across the different antibiotics (Figure 3), but were broadly similar between the three methods, overall and within each dataset (Supplementary Table 3). There were no vancomycin resistant isolates identified by either phenotyping or bioinformatics methods. Similarly, specificity and sensitivity to identify PCR-detected virulence and other genes varied across the different genes, but were broadly similar between the three methods (Supplementary Figure 2).

Discussion

Whilst WGS is increasingly used to detect antibiotic resistance and virulence determinants, to our knowledge this is the first study that compares three methods for predicting genotype on large numbers of isolates. As discussed in the recent European Committee on Antimicrobial Susceptibility Testing (EUCAST) report (15), discordance can occur between phenotypic and genotypic resistance due to inadequate limits of detection for WGS methods, incomplete understanding of the genotypic basis of phenotypic resistance, flaws with the phenotypic or molecular (e.g. PCR) methods currently used to detect resistance, and/or WGS failures including lack of assembly caused by multiple operons or similar sequences, incomplete gene coverage, non-functional genes (e.g., due to presence of stop codons/indels) or cropped contigs.

Here we found that three different approaches to identifying genetic determinants of resistance and virulence (Genefinder, Mykrobe and Typewriter) agreed in 99.5% predictions. Genefinder and Mykrobe were fast, taking under five minutes whereas Typewriter, while also taking a few minutes per sample, required initial genome assembly that increased turnaround time by up to three hours. Mykrobe and Typewriter are freely available (https://github.com/iqbal-lab/Mykrobe-predictor and https://github.com/tgolubch/typewriter respectively); Genefinder is not but the underpinning methods are relatively straightforward, and the freely available SRST2 (https://github.com/katholt/srst2) follows an analogous mapping approach (16) which would likely provide very similar results with the same catalogue. Previous comparisons of bioinformatics methods relevant to the microbiology community are limited. Bradley et al (9) found good concordance between Mykrobe and SeqSphere (17), an allele-based method that detects presence/absence of a limited number of resistance and virulence markers. SeqSphere took longer than Mykrobe as, like Typewriter, it uses Velvet assemblies. Other previous studies have shown 100% concordance between resistome and toxome in 14 MRSA isolates (18), 98.6% concordance across 5288 susceptibility predictions in 308 *S. aureus* isolates (both MRSA and MSSA) (19), 100% concordance for selected resistance and toxin gene presence/absence in 18 MRSA strains (17), and 97%/97% sensitivity/specificity for Typewriter and 99.1%/99.6%

sensitivity/specificity for Mykrobe for predicting phenotypic resistance in the Oxford validation samples used here (9, 10). A comparison between microarray and WGS in 154 isolates reported 1.7% discordancy in detecting resistance and virulence genes (20), mainly due to failure of WGS to detect enterotoxins and super antigens (similar to Typewriter in this study).

Individually, the three programs demonstrated high concordance, but interestingly, in almost all genes only one of the three bioinformatics methods did not identify a determinant that the other two methods did identify, or vice versa. The most common discrepancy with Typewriter was failing to identify virulence genes identified by Mykrobe and Genefinder (namely, *seu*, *chp* and *sei*). Two of these genes, *sei* and *seu*, are located on the enterotoxin gene cluster (*egc*) (21, 22), referred to as an enterotoxin gene nursery (23), and the other, *chp*, on a prophage (24). Such regions may be particularly susceptible to recombination (25, 26) and paralogs. As Typewriter uses BLAST, it may have a higher chance of detecting one of multiple closely related genes than the other two methods.

Similarly to Typewriter, the most common discrepancy with Genefinder was failing to identify genes reported by Typewriter or Mykrobe, particularly *ccrB, qacB* (*quaternary ammonium compound B*, conferring resistance to chlorexidine (27) via an efflux drug pump, but differing from another gene, *qacA*, by only seven nucleotides (28)), and *dfrC* (a dihydrofolate conferring resistance to trimethoprim believed to be the origin of the more common transposon-associated *drfA* gene). The fact that Genefinder identified only one variant of acquired *dfr* and *qac* may indicate that the other two methods were misidentifying paralogs (29). Alternatively, as Genefinder detects pre-determined alleles, recombination of partial genes or differences in flanking sites or genomic variation alone may reduce its ability to detect some genes. One advantage of Genefinder is its ability to detect variations in multicopy genes such as the ribosomal RNA encoding genes associated with linezolid resistance in staphylococci.

In contrast, Mykrobe most commonly identified a determinant that other methods did not, particularly *aadE(ant6')-Ia,* an adenyltransferase encoding resistance to aminoglycosides. This gene is associated with small plasmids flanked by direct repeats of staphylococcal insertion sequence IS257 (30). Although Mykrobe is kmer-based, it requires a high match across the whole gene, not just flanking sequences, so the reason for this is unclear. Mykrobe also had a higher false-positive rate in *blaZ*, as reported previously (9). Although this was previously attributed to phenotypic errors, the fact that neither Genefinder nor Typewriter identified *blaZ* in these isolates suggests the algorithm/threshold may need adjusting for this gene. Mykrobe also had a high false-positive rate for the *ccrCB* gene, which is part of the cassette chromosome recombinase (*ccr*) associated with SSC*mec* (31). As all *ccrC* genes share >87% similarity, and were not included in the original Mykrobe implementation, further investigation and modification of sequence identity thresholds may be required to accurately classify this gene, whose different alleles can have 60-82% sequence identity.

Overall, the comparison highlights key challenges inherent in all methods. First is the trade-off between specificity and sensitivity to detect specific genes/variants, and the need for adjustment based on specific features, such as proximity to repetitive elements or similarity with other alleles. Specific genes may also require different approaches, e.g. the *ccr* genes were the most discordant overall in the study. These genes were more often present in the Staphylococcal reference laboratory isolates, increasing overall error rates for this sample set. Reference libraries of genes/variants also require frequent updating with new alleles, and appropriate thresholds must be set to allow separate copies of closely related genes (e.g. *qacA* and *qacB*) to be detected if genuinely present. Taking the consensus prediction across the three different bioinformatics methods is one strategy for balancing these different trade-offs. As error rates were low overall, this only improved genetic predictions slightly, but in samples where the susceptibility is unknown it could be valuable, particularly if the two fast implementations (GeneFinder, Mykrobe) are used, followed by the slower assembly-based method only if they disagree.

Our main findings were that the largest discordance occurred between phenotype and genotype regardless of the method used to predict genotype, and that the "consensus" genotypic prediction agreed with the phenotype in two-thirds of the small number of cases where bioinformatics methods made different predictions. Where bioinformatics methods are concordant, but disagree with phenotype, the unresolved question is which is "correct", in terms of a drug achieving clinical cure in a patient infected with this strain. Penicillin and clindamycin/erythromycin were most likely to be called resistant by all methods but susceptible by phenotyping. Previous studies of erythromycin and clindamycin resistance have reported positive *ermC* PCR results from non-detectable resistance phenotypes (32) and have suggested that plasmids conferring resistance to these antibiotics may be lost in subculture (9, 33). Sensitivity to penicillin by phenotypic methods where genotype methods predict resistance has been reported previously (34, 35) and the evidence suggests that phenotyping underreports resistance. The EUCAST guidelines illustrate the challenges in distinguishing between penicillin-resistant and -

susceptible isolates based on fuzzy versus sharp zones (36). Overall therefore it is plausible that genetic detection of resistance may reflect more closely the impact of the strain on a patient.

Interpretation where phenotyping reports resistance but WGS methods predict susceptibility is more difficult. One possibility is small colony variants (SCV) being present phenotypically but overgrown in WGS culture and thus not represented in the sequence. Resistance associated with gentamicin, fusidic acid and ciprofloxin, the main antibiotics where this phenomenon was observed, is observed with SCV phenotypes (37, 38). An alternative explanation is novel resistance mechanisms, for example, ciprofloxacin (39), leading to false-negative WGS predictions. The need for a continuously updated curated database is a key challenge for WGS methods. As more sequencing occurs, novel mutations will be identified in resistance genes that may or may not confer phenotypic resistance, but these can at least be identified and tested; identifying entirely new resistance-conferring genes is more complex and prediction software that can recognize new, clinically important genes a priori would be a valuable addition to an analysis pipeline. However, we observed similar differences between concordant genotypic predictions and both phenotypic antimicrobial susceptibilities and single gene PCR results, suggesting that the underlying causes may not necessarily be related to resistance. As previously noted, agreement between WGS and phenotyping is higher (98.6%) than between phenotyping undertaken by two separate laboratories (97.6%) (19), thus at least some discrepancies are probably due to incorrect phenotyping results. In contrast, concordance between genotypic predictions made using a single method but based on WGS generated from 5 different laboratories was recently shown to be >99.8% (40).

Limitations

This comparison was based on a pre-specified set of resistance or virulence associated genes: some genetic traits previously associated with resistance were omitted (eg. *IleS* mutations linked to low-level mupirocin resistance). Despite this, we found good agreement between genotypic predictions and phenotype. Typewriter used Velvet de novo assemblies: other newer assemblers (e.g. SPADES (41)) might have improved predictions further. We included data which had been used in development of two of the methods compared, which could potentially have led to over-fitting, although performance of all three methods was in fact similar on this dataset (Supplementary Table 3). All analysis was undertaken on short-read Illumina data. The increasing use of long-read sequences will require further software testing, although Mykrobe has been successfully used for initial resistance calling in *Mycobacterium tuberculosis* from Nanopore sequencing in a small number of samples (42). However, it has not been comprehensively tested, nor have Typewriter or Genefinder, with long-read sequences generated using Nanopore or PacBio technology. The greatest differences detected in this study were between phenotype and genotype, which could be partly due to the method of phenotypic testing and recognised issues with reproducibility. We did not have resources to re-phenotype all or a subset of the isolates; well-characterised sets of repeatedly phenotyped isolates would be useful for further studies. We found no suggestion that missing calls in one program were associated with scores just below a threshold, but did not undertake a more detailed assessment of specific sequence coverage and quality around discrepant genetic predictions.

Conclusion

In summary, in this study the choice between three specific bioinformatic methods to identify resistance-determinants or other genes in *S. aureus* did not prove critical. All demonstrated a high concordance with each other, and phenotypic methods, and can be recommended for genotype prediction. However, each has some limitations and therefore consensus methods provide at least some assurance. Due to computational speed, Mykrobe (de Bruijn graph-based) and Genefinder (or equivalent mapping-based program such as SRST2 (16)) are a sensible combination to use as an initial consensus method, followed by Typewriter (BLAST-based) if these two methods disagree. As a set of 34 diverse bacteria have been made available for whole genome sequencing validation (43), the study strains and genotypic predictions are available as a resource for other studies investigating different bioinformatic analysis methods which will become increasingly important as this technique is more widely used to inform clinical management, though bacterial identification, antimicrobial susceptibility prediction and virulence profiling. External quality control of clinical laboratory performance in predicting antibiotic resistance is provided by UK proficiency testing schemes such as UK NEQAS (United Kingdom National External Quality Assessment Service for Microbiology) (44); a similar set of standards will need to be created to accredit whole genome sequencing methods.

Materials and Methods

Three sets of *S. aureus* isolates with known high-quality phenotypes were analysed: a derivation, n=501, and validation, n=491, set (denoted “Oxford derivation/validation”) from blood cultures and nasal swabs isolates at the Oxford Radcliffe Hospitals NHS Trust and Brighton and Sussex University Hospitals NHS Trust, spanning a period of 13 years, sequenced for an initial assessment of genotypic prediction of susceptibility phenotype in *S. aureus* (9, 10)and 397 isolates that had been referred to the Public Health England reference laboratory for investigation (denoted "Colindale 397", available at NCBI: PRJNA445516). The Oxford derivation set had previously been used in the development of Typewriter and Mykrobe, but not Genefinder; the former methods were then applied to the Oxford validation set.

Phenotypes for "Oxford derivation/validation" isolates used disc diffusion and/or automated broth diffusion (BD Phoenix) with discrepancies between phenotype and genotype resolved as described previously (11). All PHE isolates (n=397) were subjected to MIC testing by the PHE Staphylococcal Reference Laboratory using the agar dilution method (45). In addition, the *mec*A/C status and virulence gene profile of the PHE isolates was determined by PCR or microarray testing as described previously (14). The European Committee on Antimicrobial Susceptibility Testing (EUCAST): thresholds were used to determine sensitivity or resistances for each phenotype (http://www.eucast.org/clinical_breakpoints).

All "Oxford derivation/validation" isolates were sequenced using the Illumina HiSeq 2000 platform as previously described (46). PHE samples were sequenced in an Illumina HiSeq 2500 platform as described previously (47) (both 150bp reads). Samples determined as mixed based on WGS were excluded from further analysis. Quality control of sequences at PHE used the trimmomatic software (Illumina adapter removed, leading and trailing quality threshold set to 30 and minimum length of read set to 50 bases) (48). Isolates from Oxford analysed by Typewriter were mapped and de novo assembled with exclusion parameters of <70% coverage of reference genome for mapping and <50% of the genome in contigs >1 Kb (10). Mykrobe processes raw sequence data with no prior cleaning of the data. Isolates came from 111 sequence types, including 29 new STs/alleles, covering the range of *S. aureus* genomic diversity as previously described in Oxfordshire.

Three programs, Genefinder (MD; PHE, not published), Mykrobe (PB; Version v0.3.13-2-gd5880fa, open-source at https://github.com/iqbal-lab/Mykrobe-predictor), and Typewriter (TG; version 2.0, MMM group, Oxford University, https://github.com/tgolubch/typewriter) (Table 1), were compared to determine presence/absence of resistance-determinants (genes or variants) and toxin genes (Tables 2, 3). Mykrobe is part of the automated processing with the Complete Pathogen Software Solution (COMPASS) developed at University of Oxford. This returns quality and depth of sequence metrics, maps against a reference (MRSA 252, GenBank Accession no: BX57186561) using Stampy (49) and performs *de novo* assembly using Velvet v1.0.18 (50). These de novo assemblies formed the basis for the Typewriter program, whereas Genefinder used the raw sequencing reads.

Although all three methods search for matches to a pre-defined list of alleles, they have different approaches to their identification (further details below). Genefinder and Mykrobe required fastq files whereas Typewriter used BLAST on de novo assemblies. All used pre-set thresholds to detect genes. Thresholds are adapted for certain genes (e.g. *blaZ* which can be chromosomally integrated or carried on plasmids) to improve prediction and for quality control. Both Typewriter and Mykrobe identified presence or absence of each target singly, whereas Genefinder identified which of closely related homologs is most plausibly present. Genefinder and Mykrobe were very fast, between one and three minutes, and can be used on a standard desktop computer (specification of 2.3 GHz processor and 16GB memory). Typewriter, as it requires de novo assembly, took up to three hours and used cloud computing or high-capacity servers.

Genefinder was written by MD. It used a mapping approach (similar to SRST2, https://github.com/katholt/srst2) to detect the presence or absence of predefined genes or variations in predefined genes using Bowtie. Thresholds were defined at 90% overall, but amended where required in order to distinguish between both variants where genes were represented with multiple reference sequences and the level of diversity expected for each gene sought. Genefinder also checked for premature stop codons and compared the average depth of read coverage to identify any potential sequence contamination.

Mykrobe was written by PB and ZI (9). A threshold frequency was generated for each gene (K minimum percentage) based on the empirical level of diversity observed in the training set described by Bradley (K=0.3 for *blaZ*, K=0.6 for *fusB*, *fusC*, K=0.8 otherwise). The maximum likelihood from 3 models (gene absent, gene present in minor proportion, gene present) was chosen. The models took into account expected proportion of kmers based on depth of coverage and empirical level of diversity (described in (9)). Mutations were genotyped by choosing the maximum likelihood model from 3 Poisson models comparing the depth of coverage across 63 base pair reference and alternate alleles while demanding 100% coverage across the allele, also described in (9).

Typewriter was developed by TG (described in (10)). It considered BLAST results over a query reference (blastn for sequence identity, tblastn for mutations). It used a "relative coverage" to determine presence/absence of a gene, a metric that gives equal weight to coverage and sequence identity. Typewriter reported this value for each query gene of interest and cutoffs were adjusted to optimize specificity/sensitivity for different genes. In this study, a relative cutoff of 90% for resistance and toxin genes was used except *blaZ* for which a cutoff of 80% was used. For variant reporting, mutations were reported above a given threshold of relative coverage (e.g. 90%) however, this could be changed or set to 0% to report all identified differences from the query sequence. Stop codons were predicted, as were novel mutations.

84 genes were included in the analysis; 46 acquired resistance genes, five sets of chromosomal variants within resistance-associated genes, five cassette chromosome recombinases (*ccr*) and 28 virulence genes (Tables 2, 3). Acquired resistance genes were classified as present (p,P) or absent (a, A), setting 3 missing Genefinder predictions ("ND" or "X") to absent. Chromosomal resistance variants were those listed in Supplementary Table 4; 23 other mutations were reported in the relevant genes but were not compared, as they are not considered resistance-determinants (Supplementary Table 4). For all methods, genotype predictions of susceptibility phenotype were based on the presence of any relevant resistance-determinant as shown in Tables 2 and 3 (as described in (10) with minor modifications and updates from (9)). Intermediate phenotype results were excluded from analysis (80 cases; 0.5%).

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Figure legends

Figure 1: Determinant-by-determinant disagreements between methods

Each panel shows percentage difference in proportion of detected presence of each determinant between the first method and the second.

Figure2: Antimicrobial susceptibility genotypic predictions compared to phenotype

Figure 3: Sensitivity and specificity of genotypic predictions of antimicrobial susceptibility

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Table 1 Overview of Genefinder, Mykrobe and Typewriter methods and requirements

	Genefinder	MyKrobe (9)	Typewriter (10)
Method	Maps raw reads to list of target alleles using Bowtie	Looks for list of target alleles in de Bruijn assembly graph	Blasts list of target alleles against de novo assemblies*
Input	Fastq file	Fastq file	Genome assembly output (Velvet)
Required homology to declare gene presence/absence	>90% to target allele	Based on Kmer recovery: K is minimum percentage expected to be recovered for a gene; K = 0.3 for blaZ, K=0.6 for Fus B, C, K= 0.8 otherwise **	>90% relative coverage (homologyXlength) (80% for *blaZ*)
Required homology to declare SNP	>90% to target: can be modified	100% of 63 kmers required to call a variant present	>90% to target: can be modified
Prediction of stop codons in genes present	Yes	No: there is no assembly	Yes
Reads can be mapped to	Multiple targets	Single target	Single target

	Genefinder	MyKrobe (9)	Typewriter (10)
Speed / processor	1 to 3 minutes on laptop with 2.3 GHz processor and 16GB memory†	2 minutes on laptop with 2.3 GHz processor and 16GB memory	3 hours for assemblies on cloud computational system, then few minutes for BLAST
Sequence quality control	Threshold adjusted if gene has multiple reference sequence or variable level of diversity, can detect potential contamination by comparing average depth of coverage	Can identify mixtures of difference species and same species	Thresholds for n50 and parallel reference-based mapping: nothing reported if below these thresholds

* using blastn for sequence identity and tblast for mutations.

† Genefinder speed is relative to the number of genes present in the database

Table 2: Predicted antibiotic susceptibility phenotype from WGS by Genefinder, Mykrobe, Typewriter (n=1379)

Antibiotic	**Susceptibility prediction for Genefinder, MyKrobe, Typewriter**						**Discordant across methods (n, %)**
	RRR	**SSS**	**RRS**	**RSR**	**RSS**	**SRS**	
Ciprofloxacin	304	1072	0	2	0	1	3 (0.2%)
Clindamycin	338	1024	7	0	0	10	17 (1.2%)
Erythromycin	354	1011	6	0	0	8	14 (1.2%)
Fusidic acid	151	1221	4	0	0	3	7 (0.5%)
Gentamicin	76	1300	1	0	0	2	3 (0.2%)
Methicillin	393	984	2	0	0	0	2 (0.1%)
Mupirocin	15	1362	0	0	2	0	2 (0.1%)
Penicillin	1,161	211	3	0	0	4	7 (0.5%)
Rifampicin	23	1,354	0	1	0	1	2 (0.1%)
Tetracycline	121	1,249	4	0	0	5	9 (0.7%)
Trimethoprim	175	1,199	3	1	0	1	5 (0.4%)
Vancomycin	0	1,379	0	0	0	0	0 (0.0%)
Total (% of 16548)	3111 (18.8%)	13,366 (80.8%)	30 (0.2%)	4 (0.02%)	2 (0.01%)	35 (0.2%)	71 (0.4%)

Table 3: Predicted genotype and phenotype

(a) Antimicrobial susceptibility

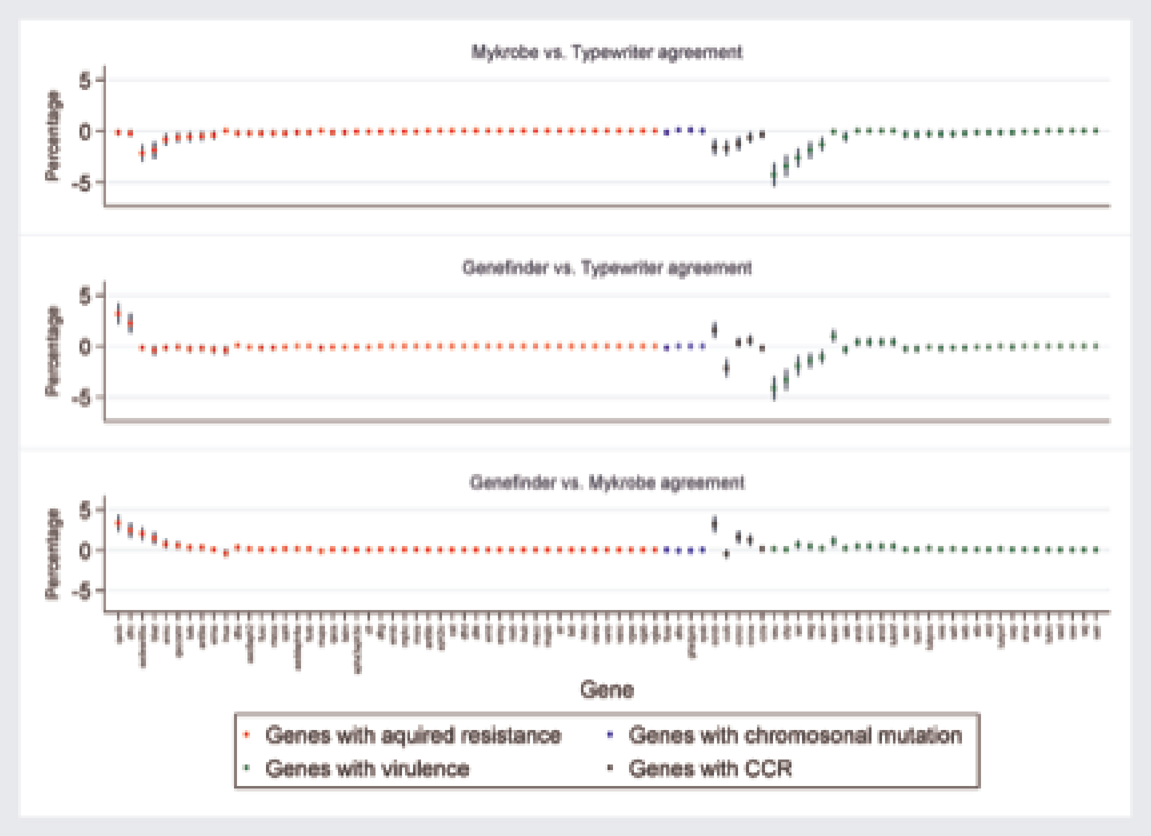
	Antimicrobial susceptibility prediction from Genefinder, Mykrobe, Typewriter						
Laboratory phenotype	RRR	SSS	RRS	RSR	RSS	SRS	Total
R	2720	89	9	3	0	4	2825
S	97	11504	13	1	2	22	11639
Total	2817	11593	22	4	2	26	14464

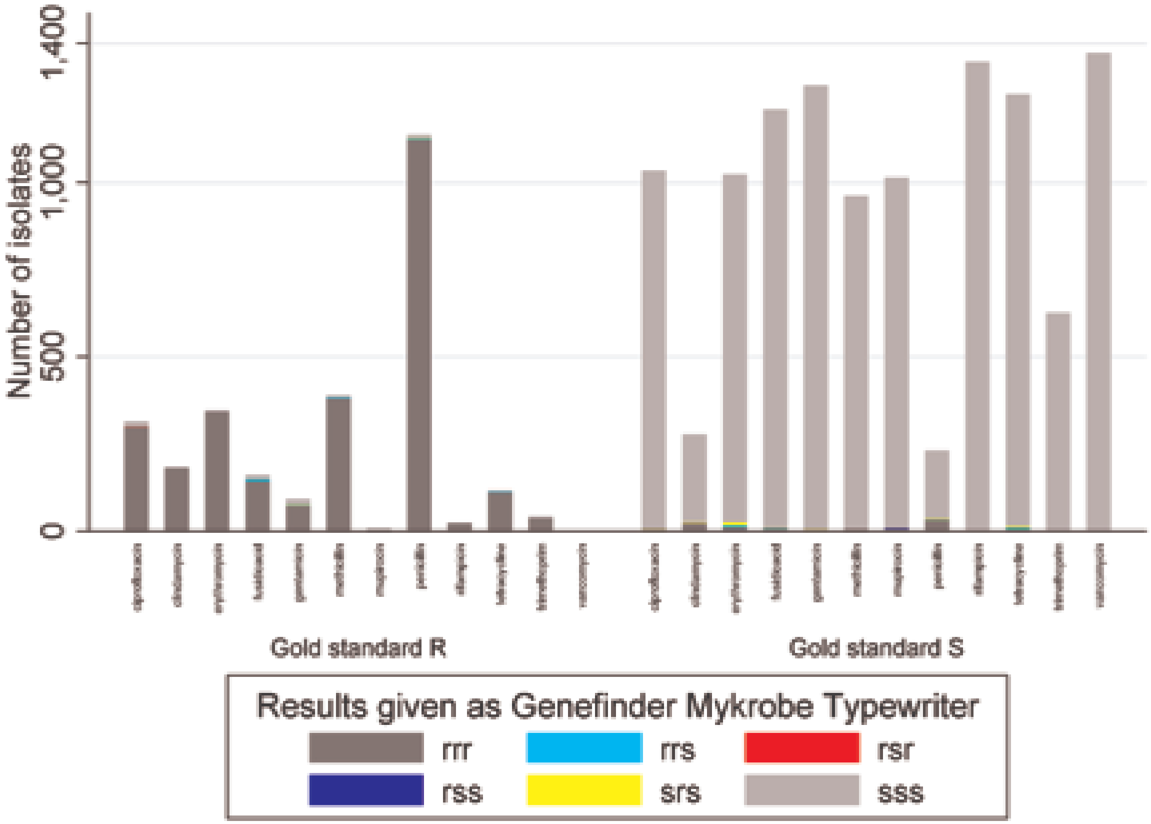
(b) Virulence genes, *ccr* genes and *mecA*/*mecC*

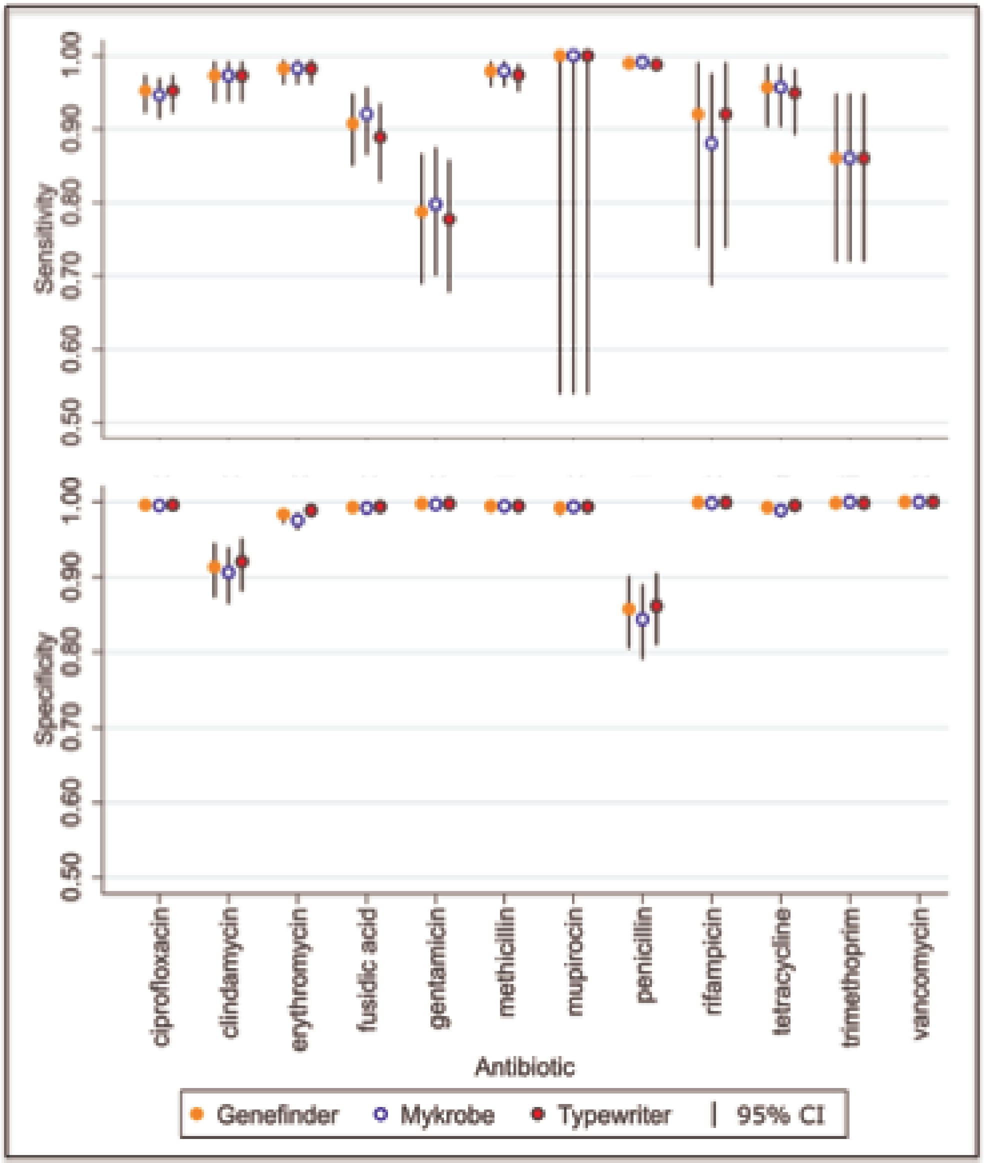
	Prediction from Genefinder, Mykrobe, Typewriter				
PCR	AAA	PPP	APA	PPA	Total
A	3362	82	10	17	3475
P	14	618	2	10	643
Total	3376	700	12	27	4115

Note: not all isolates were phenotyped for all antimicrobials, and therefore total with phenotypes (14464) is less than the total with genotypic predictions (16548) in Table 2. Only PHE isolates had PCR results for some virulence genes. Dark grey shading shows complete concordance, and light grey majority concordance between predictions.

R=resistant, S=susceptible, A=absent, P=present


Mykrobe vs. Typewriter agreement
Genefinder vs. Typewriter agreement
Genefinder vs. Mykrobe agreement
Percentage
5
0
-5
Gene
Genes with aquired resistance
Genes with chromosonal mutation
Genes with virulence
Genes with CCR


Number of isolates
0
500
1,000
1,400
ciprofloxacin
clindamycin
erythromycin
fusidic acid
gentamicin
methicillin
mupirocin
penicillin
rifampicin
tetracycline
trimethoprim
vancomycin
ciprofloxacin
clindamycin
erythromycin
fusidic acid
gentamicin
methicillin
mupirocin
penicillin
rifampicin
tetracycline
trimethoprim
vancomycin
Gold standard R
Gold standard S
Results given as Genefinder Mykrobe Typewriter
rrr
rrs
rsr
rss
srs
sss


Sensitivity
1.00
0.90
0.80
0.70
0.60
0.50
Specificity
1.00
0.90
0.80
0.70
0.60
0.50
ciprofloxacin
clindamycin
erythromycin
fusidic acid
gentamicin
methicillin
mupirocin
penicillin
rifampicin
tetracycline
trimethoprim
vancomycin
Antibiotic
● Genefinder
○ Mykrobe
● Typewriter
| 95% CI