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Use of online tools for antimicrobial resistance prediction by whole-genome sequencing in methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE)

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ABSTRACT

Objectives: The antimicrobial resistance (AMR) crisis represents a serious threat to public health and has resulted in concentrated efforts to accelerate development of rapid molecular diagnostics for AMR. In combination with publicly available web-based AMR databases, whole-genome sequencing (WGS) offers the capacity for rapid detection of AMR genes. Here we studied the concordance between WGS-based resistance prediction and phenotypic susceptibility test results for methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) clinical isolates using publicly available tools and databases.

Methods: Clinical isolates prospectively collected at the University of Pittsburgh Medical Center between December 2016 and December 2017 underwent WGS. The AMR gene content was assessed from assembled genomes by BLASTn search of online databases. Concordance between the WGS-predicted resistance profile and phenotypic susceptibility as well as the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each antibiotic/organism combination, using the phenotypic results as gold standard.

Results: Phenotypic susceptibility testing and WGS results were available for 1242 isolate/antibiotic combinations. Overall concordance was 99.3%, with a sensitivity, specificity, PPV and NPV of 98.7% (95% CI 97.2–99.5%), 99.6% (95% CI 98.8–99.9%), 99.3% (95% CI 98.0–99.8%) and 99.2% (95% CI 98.3–99.7%), respectively. Additional identification of point mutations in housekeeping genes increased the concordance to 99.4%, sensitivity to 99.3% (95% CI 98.2–99.8%) and NPV to 99.4% (95% CI 98.4–99.8%).

Conclusion: WGS can be used as a reliable predicator of phenotypic resistance both for MRSA and VRE using readily available online tools.

1. Introduction

The antimicrobial resistance (AMR) crisis represents a serious threat to public health and the economy, claiming an estimated 23 000 deaths in the USA each year [1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) are among the most common healthcare-associated antimicrobial-resistant pathogens, causing significant morbidity and mortality. The impact of increasing AMR has resulted in concerted efforts to develop rapid molecular diagnostics of resistant pathogens, as current culture-based phenotypic susceptibility assays require up to 48–72 h for the results to become available [2,3].

With current advances in sequencing technology, there has been increasing utilisation of whole-genome sequencing (WGS) for species identification, antimicrobial susceptibility prediction and outbreak detection [4]. WGS has the potential to deliver sequencing data from clinical samples in a short time frame, allowing for earlier tailored therapy [5,6]. Previous studies have revealed high

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concordance between WGS and conventional methods of detecting AMR among Gram-positive pathogens [7–10].

In parallel with the advancement of sequencing methods, development of bioinformatics tools and web-based databases with acquired resistance genes provides user-friendly methods for identification of resistance genes in whole genome data [11,12]. These web-based databases require little formal training and can become practical tools for clinical microbiology laboratories. In addition, there have been recent breakthroughs in developing platforms that can create automatic genotype-based AMR reports from raw sequencing data, providing further proof of concept [9].

The purpose of the current study was to examine the concordance of AMR predicted by WGS and web-based resistance databases with conventional phenotypic susceptibility testing methods among contemporary MRSA and VRE clinical isolates.

2. Materials and methods

2.1. Study setting and population

The study isolates were prospectively collected at the University of Pittsburgh Medical Center Presbyterian Hospital (Pittsburgh, PA) between December 2016 and December 2017. The isolates were sequenced in batch routinely as part of a larger study to assess the utility of prospective WGS surveillance linked with electronic medical record mining in the early detection of hospital outbreaks [13,14]. The isolates included represent unique patient isolates. The study was approved by the institutional review board at the University of Pittsburgh.

2.2. Whole-genome sequencing, assembly and antimicrobial resistance gene detection

Genomic DNA was extracted from pure overnight cultures of single bacterial colonies using a QIAGEN DNeasy Tissue Kit (QIAGEN, Germantown, MD, USA) according to the manufacturer's instructions. Library construction was conducted using an Illumina Nextera DNA Sample Prep Kit (Illumina, Inc., San Diego, CA, USA) with 150-bp paired-end read length, and sequencing was performed on a NextSeq whole-genome sequencing platform (Illumina, Inc.). Reads were trimmed using CutAdapt in Trim Galore v.0.4.1 [15] and were then de novo assembled using SPAdes v.3.10 [16] from filtered short-read sequences. Sequence types (STs) were identified using multilocus sequence typing (MLST) (https://github.com/tseemann/mlst), and new STs were submitted to the PubMLST website (https://pubmlst.org/).

All MRSA genomes and the majority (96/100; 96.0%) of VRE isolates underwent core-genome single nucleotide polymorphism (SNP) analyses. Four *Enterococcus faecalis* genomes were excluded from core-genome analyses owing to the small sample size. SNPs were identified using Snippy [17] with default parameters using the best available genome assembly from our hospital for each species as reference genomes. The VRE reference genome (VRE32553) was assembled from PacBio long-read sequencing, whilst the MRSA reference (MRSA10173) was assembled from short-read Illumina sequences. A phylogenetic tree based on the core SNP alignment was generated using RAxML v.8.2.9 [18] by running 100 bootstrap replicates under the generalised time-reversible model of evolution, a categorical model of rate heterogeneity (GTR-CAT) and Lewis correction for ascertainment bias and was visualised using Interactive Tree of Life (iTOL) v.4 [19].

2.3. In silico prediction of antimicrobial resistance using online tools

The AMR gene content was assessed by BLASTn of assembled contigs against downloaded ResFinder and the Comprehensive Antibiotic Resistance Database (CARD) with 80% sequence identity and 90% sequence coverage cut-off [11,12]. These online platforms analyse WGS data directly from uploaded sequence files (in FASTQ format) and/or or assembled genome sequences (contigs in FASTA format). Isolates were considered resistant by genotyping if they contained at least one AMR gene known to confer resistance to that class of antibiotic (Table 1).

2.4. Phenotypic antimicrobial susceptibility testing

Routine antimicrobial susceptibility testing was performed by MicroScan WalkAway™ (Siemens Healthcare Diagnostics, Los Angeles, CA, USA) and susceptibility was determined using reference Clinical and Laboratory Standards Institute (CLSI) breakpoints [20]. For the purposes of this study, intermediate results were considered resistant. Antibiotics included in the analysis were those routinely tested at our institution, deemed of highest clinical relevance and have established genetic resistance determinants that can be readily identified from the online databases mentioned above. These included erythromycin, clindamycin, gentamicin, linezolid, methicillin, rifampicin, trimethoprim/sulfamethoxazole (SXT), tetracycline and vancomycin. Not all isolates were tested against all drugs as susceptibility panels used on isolates depended on specimen types.

2.5. Resolution of discordance between phenotypic and genotypic resistance testing

For isolates that showed discordance between genotypic and phenotypic methods, repeat susceptibility testing was performed by the Kirby–Bauer disk diffusion method. Susceptibility was

Table 1
Frequency of mechanisms of genotypic resistance among clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).

Antimicrobial agent/resistance gene	No. (%) of isolates[a]	
	MRSA	VRE
Erythromycin/clindamycin		
ermA	42 (42.0)	0 (0)
ermC	6 (6.0)	0 (0)
ermB	0 (0)	48 (85.7)
ermG	0 (0)	1 (1.8)
efmA	0 (0)	39 (69.6)
emeA	0 (0)	3 (5.4)
msrA	55 (55.0)	1 (1.7)
msrC	0 (0)	54 (90.0)
mphC	55 (55.0)	0 (0)
Gentamicin		
aph(2″)-I	2 (1.9)	n/a
Linezolid		
23S rRNA mutation[b]	0 (0)	2 (2.1)
Oxacillin		
mecA	105 (100)	0 (0)
Tetracycline		
tetK	6 (5.6)	0 (0)
tetM	3 (2.8)	28 (58.3)
tetU	0	42 (81.3)
tetL	0	25 (52.1)
Trimethoprim/sulfamethoxazole	7 (6.5)	n/a
dfrG	6 (5.7)	0 (0)
dfrC	1 (1.0)	0 (0)
Rifampicin		
rpoB gene mutations[c]	4 (3.9)	
Vancomycin		
vanA	0 (0)	96 (96.0)

[a] Percentages were determined by dividing the number of isolates harbouring the gene by the total number of isolates (per species) with phenotypic testing for the drug of interest.
[b] Linezolid 23S rRNA gene mutations (T1547C and T1245C).
[c] Rifampicin *rpoB* gene mutations (A477D, R484H and H481 N).

determined using reference CLSI breakpoints [20]. For isolates with discordant results for antibiotics known to have resistance conferred by amino acid substitutions in particular housekeeping genes (Table 1), comparison with the reference strains *S. aureus* MSSA476 (GenBank accession no. BX571857.1) or *E. faecalis* ATCC 29212 (GenBank accession no. CP008816.1) using CLC Genomics Workbench v.11 (QIAGEN) and Molecular Evolutionary Genetics Analysis (MEGA) software [21] was undertaken. Raw sequencing reads for all isolates were deposited in GenBank under accession nos. **SAMN09400893**–**SAMN09401217**.

2.6. Statistical analysis

Concordance between WGS-predicted resistance and phenotypic susceptibility was determined. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for WGS-predicted resistance were calculated for each antibiotic/organism combination, with the phenotypic results as gold standard, using the Wilson–Brown method [22]. In cases of discordance, disk diffusion results were considered the gold standard. Statistical calculations were performed using GraphPad Prism v.7 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

Phenotypic susceptibility testing and WGS results were available for 108 and 100 unique MRSA and VRE isolates, respectively (Figs. 1 and 2). Of 1242 isolate/antibiotic combinations, overall concordance was 99.3%, with a sensitivity, specificity, PPV and NPV of 98.7% [95% confidence interval (CI) 97.2–99.5%], 99.6% (95% CI 98.8–99.9%), 99.3% (95% CI 98.0–99.8%) and 99.2% (95% CI 98.3–99.7%), respectively. Additional identification of point mutations in housekeeping genes increased the concordance to 99.4% and the sensitivity and NPV to 99.3% (95% CI 98.2–99.8%) and 99.4% (95% CI 98.4–99.8%), respectively.

3.1. Concordance in methicillin-resistant Staphylococcus aureus

Phenotypic susceptibility testing and WGS results were available for 108 unique MRSA isolates (Fig. 1). Of 942 isolate/antibiotic combinations, initial concordance between the two methods was 96.7%. There were 31 discrepancies between WGS-predicted resistance and automated phenotypic susceptibility testing. Of the 31 discrepant results, 23 were reconciled by disk diffusion testing, increasing concordance to 99.5%. As a result, WGS-based prediction had an overall sensitivity, specificity, PPV and NPV of 98.5% (95% CI 96.2–99.6%), 99.9% (95% CI 99.2–100%), 99.7% (95% CI 97.4–100%) and 99.4% (95% CI 98.5–99.8%), respectively (Table 2).

3.1.1. Methicillin

The *mecA* gene encodes penicillin-binding protein 2a (PBP2a), which has a low affinity for β-lactam antibiotics and confers resistance to methicillin [23]. Initial susceptibility testing revealed

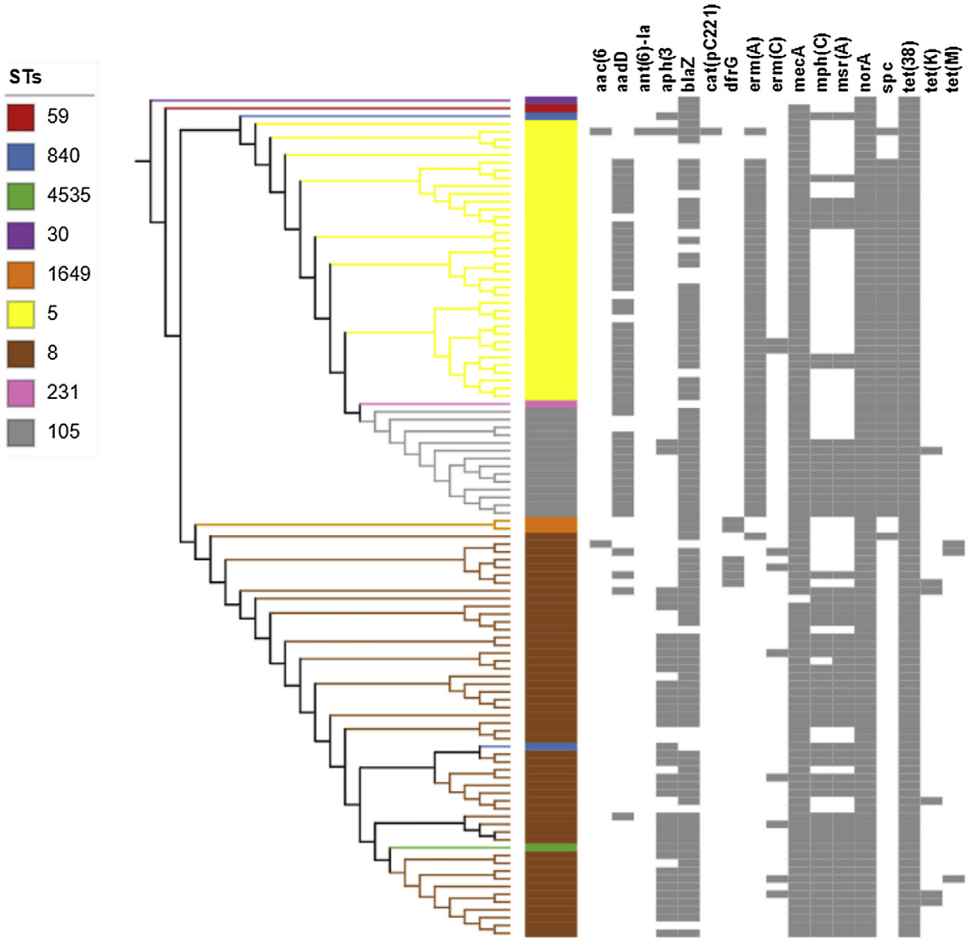

Fig. 1. Genetic relationship and antimicrobial resistance (AMR) gene content among 108 methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates. Maximum-likelihood phylogenetic tree constructed from aligned core-genome SNPs (left); coloured strip indicates multilocus sequence type (ST), while grey boxes (right panel) indicate the presence of the corresponding AMR gene. SNP, single nucleotide polymorphisms. *dfrC* and *rpoB* mutations are not represented in the figure.

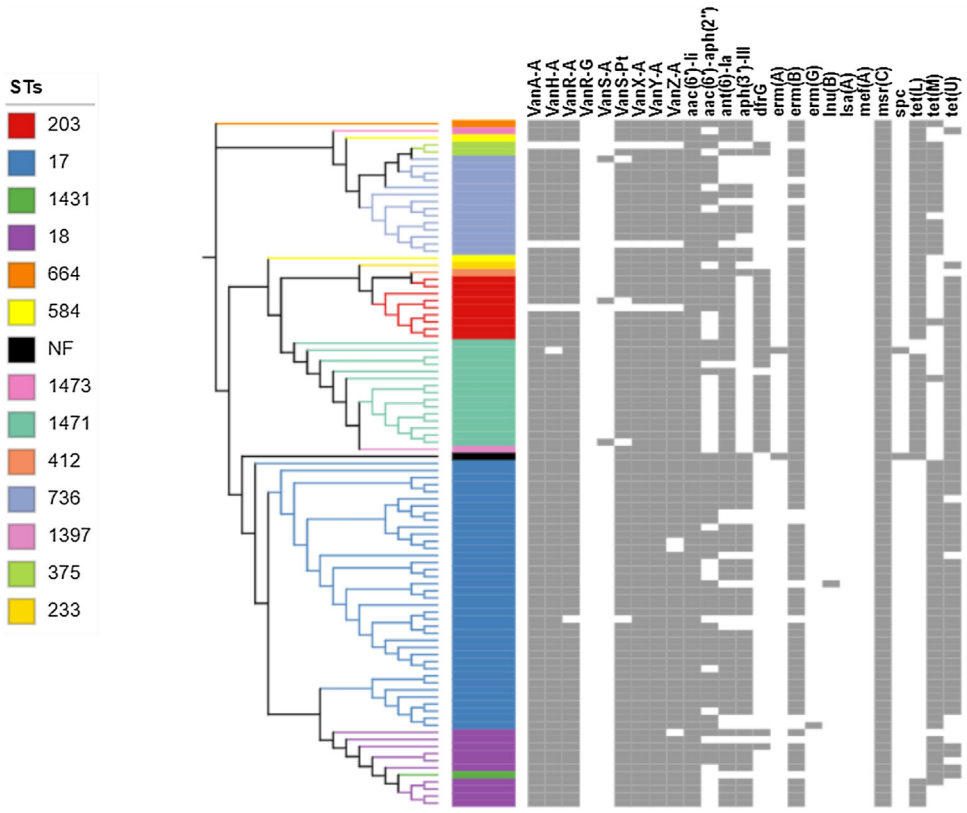

Fig. 2. Genetic relationship and antimicrobial resistance (AMR) gene content among 96 vancomycin-resistant *Enterococcus faecium* strains. Maximum-likelihood phylogenetic tree constructed from aligned core-genome SNPs (left); coloured strip indicates multilocus sequence type (ST), while grey boxes (right panel) indicate the presence of the corresponding AMR gene. SNP, single nucleotide polymorphisms. 23S rRNA gene mutations are not represented in the figure.

Table 2
Genotypic and phenotypic concordance among clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolates.

Antimicrobial agent	No. of isolates tested	Phenotypic resistance [*n* (%)]		Genotypic resistance inferred by WGS [*n* (%)]	Concordance with disk diffusion (%)	% (95% CI)			
		Automated	Disk diffusion			Sensitivity	Specificity	PPV	NPV
Methicillin	107	107 (100)	105 (98.1)	105 (98.1)	100	100 (96.6–100)	100 (15.8–100)	100 (96.6–100)	100 (15.8–100)
Erythromycin	100	88 (88.0)	87 (87.0)	84 (84.0)	96.0	96.6 (90.3–99.3)	92.3 (64.0–99.8)	98.8 (92.7–99.8)	80.0 (56.6–92.5)
Clindamycin	100	34 (34.0)	52 (52.0)	51 (51.0)	99.0	98.1 (89.9–100)	100 (92.5–100)	100 (92.5–100)	97.9 (87.1–99.7)
Tetracycline	105	10 (9.5)	9 (8.6)	9 (8.6)	100	100 (66.4–100)	100 (96.2–100)	100 (66.4–100)	100 (96.2–100)
Gentamicin	108	2 (1.9)	2 (1.9)	2 (1.9)	100	100 (15.8–100)	100 (96.6–100)	100 (15.8–100)	100 (96.6–100)
SXT	105	6 (5.7)	7 (6.7)	7 (6.7)	100	100 (59.0–100)	100 (96.3–100)	100 (59.0–100)	100 (96.3–100)
Rifampicin	103	5 (4.9)	4 (3.9)	4 (3.9)	100	100 (37.7–100)	100 (96.3–100)	100 (0.5–100)	100 (96.3–100)
Vancomycin	107	0	0	0	100	n/a	100 (96.6–100)	n/a	100 (96.6–100)
Linezolid	107	0	0	0	100	n/a	100 (96.6–100)	n/a	100 (96.6–100)
Total[a]	942	252 (26.8)	266 (28.2)	262 (27.8)	99.5	98.5 (96.2–99.6)	99.9 (99.2–100)	99.7 (97.4–100)	99.4 (98.5–99.8)

WGS, whole-genome sequencing; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; SXT, trimethoprim/sulfamethoxazole; n/a, not applicable.
[a] Total results for all MRSA isolate/antibiotic combinations.

that 107 (100%) isolates were resistant to methicillin. The *mecA* gene was identified in 105 (98.1%) MRSA isolates (Table 1). Repeat testing by disk diffusion revealed that two isolates without *mecA* were actually methicillin-susceptible *S. aureus*. Therefore, WGS showed 100% concordance with phenotypic methicillin susceptibility testing results, with two instances of initial discordance resolved by repeat susceptibility testing (Table 2).

3.1.2. Erythromycin

Resistance to the macrolide–lincosamide–streptogramin B group antibiotics can result either by (i) N6-dimethylation of an adenine residue in 23S rRNA by *erm* genes causing reduced affinity for the antibiotic, (ii) enzymatic modification (phosphorylation) of the antibiotic mediated by acquisition of *mph* genes or (iii) active transport of the antibiotic out of the cell via efflux proteins encoded by *msr* genes [24]. Initial susceptibility testing revealed that 88 (88.0%) MRSA isolates were resistant to erythromycin. By WGS, 169 macrolide resistance genes (Table 1) were identified, with the presence of at least one gene detected in 84 isolates (84.0%). Five isolates showed discordance. Four isolates were erythromycin-resistant with no macrolide resistance gene was identified. Repeat susceptibility testing by disk diffusion resulted in one isolate being reclassified as susceptible. Of the discordant isolates, one initially tested as susceptible despite the presence of the macrolide resistance genes *msrA* (98.0% identity) and *mph* [25] (99.3% identity) remained susceptible upon repeat disk diffusion testing. Overall, WGS was 96.0% concordant with phenotypic erythromycin susceptibility testing results, with one instance of discordance resolved by disk diffusion (Table 2).

3.1.3. Clindamycin

Resistance to lincosamides is mediated either by (i) inactivation by lincosamide nucleotidyltransferase enzymes encoded by *lnu* genes, (ii) methylation of 23S rRNA via *erm* genes or (iii) active transport of the antibiotic out of the cell by efflux pumps encoded by the *lsa* genes or ABC transporter proteins encoded by *vga* genes [24]. Initial susceptibility testing revealed 34 (34.0%) MRSA isolates to be resistant to clindamycin. By WGS, 53 lincosamide resistance genes were identified (Table 2) with 51 (51.0%) isolates having at least one lincosamide resistance gene. There were 18 discordant isolates. Seventeen isolates were susceptible with the presence of at least one resistance gene. All 17 isolates tested positive for inducible clindamycin resistance by disk diffusion. One isolate was resistant without the presence of a lincosamide resistance gene and repeat susceptibility testing confirmed it as resistant. WGS was therefore 99.0% concordant with phenotypic clindamycin susceptibility testing results (Table 2).

3.1.4. Tetracycline

Resistance to tetracyclines is conferred by ribosomal protection proteins that alter the binding of tetracycline to bacterial ribosomes or through active transport out of the cell by efflux pumps, both of which are encoded by *tet* genes [26,27]. Initial susceptibility testing revealed that 10 (9.5%) MRSA isolates were resistant to tetracycline. By WGS, tetracycline resistance genes were identified in nine (8.6%) isolates. One isolate initially tested as tetracycline-intermediate without the presence of a tetracycline resistance gene. Repeat susceptibility testing determined the isolate to be tetracycline-susceptible. WGS was therefore 100% concordant with phenotypic tetracycline susceptibility testing results (Table 2).

3.1.5. Gentamicin

Aminoglycoside resistance is mediated by *aac, ant* and *aph* genes encoding aminoglycoside *N*-acetyltransferases, *O*-adenylyltransferases and *O*-phosphotransferases, respectively [28]. Initial susceptibility testing revealed that two (1.9%) MRSA isolates were resistant and both possessed the *aph(2″)-I* gene. Thus, WGS was 100% concordant with phenotypic gentamicin susceptibility testing results (Table 2).

3.1.6. Trimethoprim/sulfamethoxazole

Resistance to trimethoprim is conferred either through the production of dihydrofolate reductase variants encoded by *dfr* genes or by an amino acid substitution in the *dfrB* housekeeping gene [29]. Initial susceptibility testing revealed that six (5.7%) MRSA isolates were resistant to SXT. WGS revealed seven (6.7%) isolates with SXT resistance genes. There was one discordant isolate with a *dfrG* gene that was susceptible on initial testing but tested resistant on repeat testing. Thus, WGS was 100% concordant with phenotypic SXT susceptibility testing results (Table 2).

3.1.7. Rifampicin

Resistance to rifampicin is mediated either through point mutations in the chromosomal *rpoB* gene that encodes the bacterial RNA polymerase [30] or, less commonly, by *arr* enzymes that catalyse ADP-ribosylation of rifamycins [31]. Initial susceptibility testing revealed that 5 (4.9%) MRSA isolates were resistant to rifampicin. WGS revealed four (3.9%) isolates with a resistance-conferring mutation in the *rpoB* gene (H481 N, R484H and A477D) [32,33]. Repeat susceptibility testing revealed that four (3.9%) MRSA isolates were rifampicin-resistant. WGS was 100% concordant with phenotypic rifampicin results (Table 2).

3.1.8. Vancomycin and linezolid

No MRSA isolates were resistant to vancomycin or linezolid and none harboured known vancomycin or linezolid resistance genes.

3.2. Concordance in vancomycin-resistant enterococci

Phenotypic susceptibility and WGS data were available for 100 unique VRE isolates (Fig. 2). Of a total of 300 isolate/antibiotic combinations, initial genotypic/phenotypic concordance was 96.3%. There were 11 discrepancies between WGS-predicted resistance and automated phenotypic susceptibility testing. Of the 11 discrepant results, 7 could be reconciled by disk diffusion testing, increasing concordance to 98.7%, with a sensitivity, specificity, PPV and NPV of 99.0% (95% CI 96.4–99.9%), 98.0% (95% CI 93.0–99.8%), 99.0% (95% CI 96.1–99.7%) and 98.0% (95% CI 92.6–99.5%), respectively. Additional identification of point mutations in housekeeping genes increased the concordance to 99.3% and the sensitivity and NPV to 100% (95% CI 99.1–100%) and 100% (95% CI 96.8–100%), respectively.

3.2.1. Vancomycin

Resistance to vancomycin is encoded by different clusters of genes referred to as the *van* gene cluster, which results in the replacement of D-Ala-D-Ala-ending peptidoglycan precursors with D-alanyl-D-lactate termini, to which vancomycin binds with substantially lower affinity [34]. Initial susceptibility testing revealed that all 100 (100%) VRE isolates, by definition, were resistant to vancomycin. WGS revealed the presence of the *vanA* gene in 96 (96.0%) isolates. Repeat susceptibility of four isolates without a *van* gene revealed them to be vancomycin-susceptible. Thus, WGS was 100% concordant with vancomycin susceptibility testing results (Table 3).

3.2.2. Linezolid

Resistance to linezolid is most frequently caused by a point mutation in 23S rRNA, with the G2576 T mutation being frequent [35]. In addition, resistance may occur by acquisition of the multidrug resistance gene *cfr* encoding an rRNA methyltransferase that adds a methyl group at the C-8 position of 23S rRNA nucleotide

Table 3
Genotypic and phenotypic concordance among clinical vancomycin-resistant enterococci (VRE) isolates.

Antimicrobial agent	No. of isolates	Phenotypic resistance [*n* (%)]		Genotypic resistance inferred by WGS [*n* (%)]	Concordance with disk diffusion (%)	% (95% CI)			
		Automated	Disk diffusion			Sensitivity	Specificity	PPV	NPV
Vancomycin	100	100 (100)	96 (96.0)	96 (96.0)	100	100 (96.2–100)	100 (39.8–100)	100 (96.2–100)	100 (39.8–100)
Linezolid[a]	96	3 (3.1)	2 (2.1)	0	97.9	0 (0–84.2)	100 (96.2–100)	n/a	97.9 (97.9–97.9)
Erythromycin	56	56 (100)	56 (100)	56 (100)	100	100 (93.6–100)	n/a	100 (93.6–100)	n/a
Tetracycline	48	44 (91.7)	45 (93.8)	47 (97.9)	95.8	100 (92.1–100)	33.3 (0.82–90.6)	95.7 (91.0–98.0)	100 (5.0–100)
Total[b,c]	300	203 (67.7)	199 (66.3)	199 (66.3)	98.7	99.0 (96.4–99.9)	98.0 (93.0–99.8)	99.0 (96.1–99.7)	98.0 (92.6–99.5)

WGS, whole-genome sequencing; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; n/a, not applicable.
[a] With the additional step of examining for point mutations in housekeeping genes, the concordance and NPV were increased to 100% (95 CI 96.2–100%).
[b] Total results for all VRE isolate/antibiotic combinations.
[c] Additional identification of point mutations in housekeeping genes increased the concordance to 99.3%, sensitivity to 100% (95% CI 99.1–100%) and NPV to 100% (95% CI 96.8–100%).

A2503, thus preventing the drug from binding to the target site [36]. Alterations in the ribosomal proteins L3, L4 and L22, encoded by *rplC, rplD* and *rplV*, respectively, have also been associated with increased resistance to linezolid [37]. Initial susceptibility testing revealed three (3.1%) isolates that were resistant to linezolid. WGS did not reveal the presence of any *cfr* genes. Repeat testing revealed one VRE isolate to be linezolid-susceptible. The two remaining discordant VRE isolates were found to have point mutations in 23S rRNA (T1547C and T1245C), which may have resulted in functional alteration in 23S rRNA. WGS (not including additional examination for housekeeping gene mutations) was 97.9% concordant with linezolid susceptibility testing results. With identification of point mutations in housekeeping genes, specificity and NPV were 100% (Table 3).

3.2.3. Erythromycin

Initial susceptibility testing revealed that all 56 (100%) VRE isolates were resistant to erythromycin. By WGS, 145 macrolide resistance genes were identified (Table 2), with the presence of at least one resistance gene in all 56 (100%) VRE isolates. WGS was 100% concordant with erythromycin phenotypic susceptibility testing with sensitivity and PPV of 100% (Table 3).

3.2.4. Tetracycline

Initial susceptibility testing revealed that 44 (91.7%) VRE isolates were tetracycline-resistant. By WGS, tetracycline resistance genes were identified in 47 isolates (97.9%). Three initial discordant results were found. After repeat susceptibility testing, two instances of discordance remained, with two isolates susceptible to tetracycline despite the presence of *tetU* (99.4% identity) in one isolate and *tetM* (97.3% identity) and *tetL* (96.6% identity) in another isolate. WGS was 95.8% concordant with phenotypic tetracycline susceptibility (Table 3).

4. Discussion

Infections caused by resistant organisms are associated with increased morbidity, mortality and economic burden [38,39]. The results of the current study show high concordance between WGS-predicted resistance and phenotypic susceptibility testing using publicly available online tools. Rapid determination of AMR profiles can lead to a decrease in time to appropriate therapy, as recently demonstrated by Tamma et al., and hence minimise the unintended consequences of antimicrobials and improve patient outcomes [2,40]. Although manual upload of genomes to online databases may be sufficient, significant improvement in throughput and workflow could be obtained by implementing command line versions of these tools using programmed analysis pipelines. With falling costs, reduced turnaround times and increased sequence quality, WGS has the potential to become a more routinely used tool in clinical microbiology laboratories [10]. And while rapid diagnostics of AMR detection have decreasing turnaround times (as little as 8 h), with sequencing one gains a host of valuable information that can provide high discriminatory power for prediction of AMR and molecular epidemiology, making it a valuable tool not only for clinical management but also for infection control and surveillance [25].

Whilst similar results have previously been demonstrated among MRSA isolates [8,9,41], to our knowledge validation of WGS for AMR prediction among human clinical VRE isolates is lacking. Most previous investigations have used in-house curated databases of resistance determinants [8,9,42]. Given the large body of literature on the genetic basis of resistance for MRSA and VRE, AMR determinants are well documented and represented in online databases [11,12]. In the current study, publicly available web-based AMR databases were used, achieving high concordance with gold-standard phenotypic methods. One notable exception was the low concordance rate for erythromycin resistance in MRSA. This may be due to the instability of *ermC*-containing plasmids, which may be lost during isolate passage in the laboratory [42,43]. In most instances of discrepancy between automated phenotypic susceptibility results and sequencing data, repeat susceptibility with disk diffusion validated the WGS data. Discrepancy between automated susceptibility testing and gold-standard methods such as broth microdilution and disk diffusion among *S. aureus* and *Enterococcus* has been reported, with higher minimum inhibitory concentrations (MICs) reported by automated methods [44,45]. These results can lead to increased use of broad-spectrum antibiotics, as confirmatory testing with broth microdilution and disk diffusion is an extra step that may be foregone.

A major barrier to the widespread adoption of genomic methods is the lack of bioinformatics expertise. In a recently developed software package 'Mykrobe Predictor', a known panel of AMR genes and point mutation sites are used in analysis of raw sequencing data to generate a user-friendly report, circumventing the need for bioinformatics expertise [9]. Similarly, even without the use of such a platform, with implementation of an automated pipeline one can receive a simple file format and, with basic

computer navigational skills, upload genome sequences directly to the different web-based databases and receive easy to interpret results [11,12].

There are several limitations of this study. First, although there were high overall levels of sensitivity, specificity, PPV and NPV, our process may fail to identify AMR conferred by point mutations in chromosomal housekeeping genes. With the additional step of manual sequence inspection, point mutations associated with resistance could be identified in a few instances and increased the sensitivity and specificity of WGS for detection of AMR. Nevertheless, this process can be time consuming and laborious and may requires additional bioinformatics software and skill. The more recently developed ARG-ANNOT is the first database to include detection of point mutations in chromosomal genes associated with AMR [46]. Furthermore, there was no vancomycin or linezolid resistance and only a low level of aminoglycoside resistance amongst the MRSA isolates in the current study, so we could not confidently evaluate resistance prediction for these agents. In addition, while we attempted to include most relevant antibiotics, daptomycin was not included in the analysis owing to the current gap in knowledge of genetic mechanisms of resistance for daptomycin and therefore these mechanisms are not fully represented in online databases [47]. Lastly, whilst we focused on MRSA and VRE owing to their clinical significance, further validation should be undertaken in susceptible organisms.

5. Conclusion

Using WGS and online AMR databases, we are able to achieve high concordance with phenotypic susceptibility testing among clinical isolates of MRSA and VRE. With increasing investment in furthering genomic analysis and investigation of genetic bases of resistance, additional prediction tools may soon become available. None the less, there remains a current need for a central comprehensive open-source, curated database containing all validated AMR genes as well as point mutations in housekeeping genes known to be associated with AMR.

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Competing interest

None declared.

Ethical approval

The study was approved by the institutional review board at the University of Pittsburgh (Pittsburgh, PA, USA).

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