Whole genome sequencing analysis of *Enterococcus faecium* clinical isolates reveals high strain diversity and accurate prediction of antimicrobial resistance

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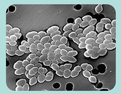
1- Background on *E. faecium*



» Gram-positive bacterium with 2.5 - 3.1 Mb genome size.
» A major cause of hospital-acquired infections.
» Difficult to treat, due to high rates of multidrug resistance.

» Whole genome sequencing (WGS) is a powerful tool to uncover transmission patterns and antimicrobial resistance (AMR) mechanisms of *Enterococcus faecium*, but most *E. faecium* genomic studies focus on isolates from outbreak investigations rather than routine sampling.

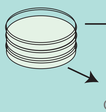
» The use of WGS to predict *E. faecium* AMR has not been tested systematically.

» Here we use WGS to characterize over 400 unique *E. faecium* clinical isolates to assess their strain diversity and AMR mechanisms.

2- Methods

Derivation set

193 MGH clinical isolates
Collected 1/2016-12/2017
55% from screening swabs (colonization), remaining from clinical infections



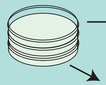
WGS → Sequence typing and analysis of antibiotic resistance genes → Determination of gene/SNP-based prediction rules
Including literature review

Routine AST
Clinical microbiology lab

SRST2 with supplemental analysis

Validation set

226 MGH clinical isolates
Collected 1/2018-9/2019
28% from screening swabs (colonization), remaining from clinical infections
Enrichment for more resistant organisms

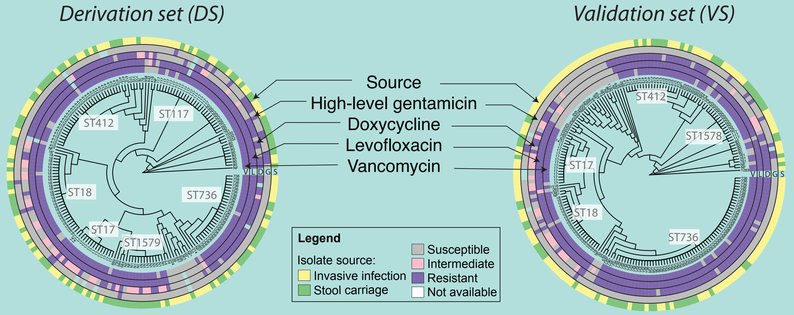


WGS → Sequence typing and analysis of antibiotic resistance genes → Application of gene/SNP-based prediction rules to determine model accuracy

Routine AST
Clinical microbiology lab

SRST2 with supplemental analysis

3- Results: High diversity and novel STs



» >85% of DS and 75% of VS isolates belonged to hospital-associated clonal complex (CC) 17, most frequently ST736, ST18, ST412, ST17, and ST117.

» The sixth most common MLST type was novel (now ST1578), most closely related to ST117.

» The DS also included 12 unique isolates of an additional novel type (now designated ST1579), most closely related to ST17 and predominantly found in rectal screening samples.

4- Results: Accurate rules-based predictions

Antimicrobial Drug	Genotype used for prediction	Overall suscep. rate	Categorical agreement	Very major error rate (FN) (95% CI)	Major error rate (FP) (95% CI)	PPV	NPV
Ampicillin	Mutation of *pbp5* 485M	13%	98.9%	1.1% (0.13, 4.0)	0% (0, 13)	100.0%	92.8%
Vancomycin	Presence of *vanA* or *vanB*	21%	99.0%	0.0% (0, 2.2)	2.3% (0.06, 12)	99.4%	100.0%
Gentamicin high-level	Presence of *aac(6')-Ie-aph(2")-Ia*	95%	100.0%	0.0% (0, 28)	0.0% (0, 1.9)	100.0%	100.0%
Ciprofloxacin*	Mutation of *gyrA* 84S or *parC* 82S	15%	100.0%	0.0% (0, 2.1)	0.0% (0, 11)	100.0%	100.0%
Levofloxacin*	Mutation of *gyrA* 84S or *parC* 82S	15%	100.0%	0.0% (0, 2.0)	0.0% (0, 11)	100.0%	100.0%
Tetracycline	Presence of *tetL*, *tetM*, or *tetS*	24%	96.1%	0.0% (0, 2.3)	14% (5.8, 27)	95.7%	100.0%
Doxycycline	Presence of *tetM*	28%	90.8%	1.4% (0.16, 4.8)	27% (16, 40)	90.1%	95.6%
Linezolid	Mutation of *23S rRNA* G2576T	99%	n/a	n/a	n/a	n/a	n/a

» After resolving genotyping or phenotyping errors, the genotypic-phenotypic categorical agreement was generally excellent. All drugs surpassed the FDA performance metric of a percent categorical agreement above 89.9%.

» The very major error (VME) rate, also known as the false negative rate, was 1.4% or lower for all drugs.

5- Conclusions

» In a diverse and challenging set of clinical *E. faecium* isolates, known AMR genes and SNPs can be simply applied to predict phenotypic susceptibility with an average categorical agreement of 97.8% across seven commonly used antibiotics.

» Our findings can be used to improve molecular VRE screening methods and existing WGS-based bioinformatics tools.

6- Acknowledgements

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7- References

Baym et al. "Inexpensive multiplexed library preparation for megabase-sized genomes." *PLoS One*, 2015.

PubMLST, https://pubmlst.org/efaecium

Inouye et al. "SRST2: Rapid genomic surveillance for public health and hospital microbiology labs." *Genome Medicine*, 2014.