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Comparing two whole genome sequencing bioinformatic software for identifying Enterococcal antibiotic-resistant genes.

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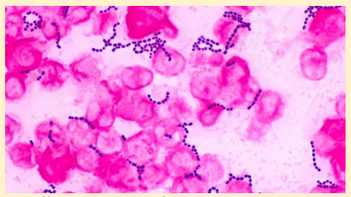
E. faecium (n=60)	*E. faecalis* (n=29)	
Genes		Drug class resistance
aph(2'')-Ia, ant(6)-Ia, aac(6')-I, aad(6), aac(6')-Ii, aacA/aphD, aph(3')-IIIa, ant(9)-Ia	ant(6)-Ia, aad(6), aacA/aphD, aadD1, aph(3')-IIIa	Aminoglycosides
sat4	sat4	Aminoglycosides, Nucleoside
dfrF	dfrC, dfrE, dfrF	Pyrimidine analogs, Trimethoprim/sulfonamides
eat(A)		Pleuromutilins
	emeA	Acridine, Quinolones, Tetracyclines
efmA		Beta-lactams, Macrolides/lincosamides/streptogramins, Nitroimidazole, Nucleoside, Peptides, Quinolones, Tetracyclines
pbp5		Beta-lactams
vanA, vanR, vanS, vanX-A, vanY-A, vanZ-A, vanH-A	bleO, vanA, vanR, vanS, vanX-A, vanY-A, vanZ-A, vanH-A	Glycopeptides
liaR		Glycopeptides, Lipopeptides
liaS		Glycopeptides, Lipopeptides, Peptides
dfrG	dfrG	Pyrimidine analogs, Trimethoprim/sulfonamides
msrC		Macrolides/lincosamides/streptogramins, Tetracyclines
erm(A), erm(B)	erm(B), erm(C)	Macrolides/lincosamides/streptogramins, Peptides, Polyketides
tet(M), tet(S)	tet(M)	Tetracyclines
tet(L)		Beta-lactams, Fosfomycins, Macrolides/lincosamides/streptogramins, Nitroimidazole, Quinolones, Tetracyclines
	lsa(A)	Macrolides/lincosamides/streptogramins, Pleuromutilins, Tetracycline

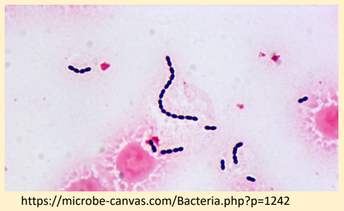
EPISEQ
ResFinder
Both

Table 1. Genetic mutations identified by EPISEQ and ResFinder arranged by resistance to its respective drug class.



https://microbe-canvas.com/Bacteria.php?p=1241

E. faecalis gram stain on blood culture.



https://microbe-canvas.com/Bacteria.php?p=1242

E. faecium gram stain on blood culture.

BACKGROUND

Enterococcus is a multidrug-resistant organism and a leading cause of healthcare-associated infections (HAI). Identification of antibiotic resistance can be determined either phenotypically or genotypically. The methods for phenotypic resistance are well established and steered by the Clinical & Laboratory Standards Institute (CLSI). However, the determination of genotypic resistance is an emerging area in antimicrobial stewardship with no set standards. Here we performed whole genome sequencing (WGS) on clinical isolates of Enterococci (both *E.faecalis* and *E.faecium*) and utilized two software: EPISEQ CS TM (BIOMÉRIEUX, Marcy l 'Etoile, France), a proprietary software and ResFinder, an open source to identify different genetic mutations which confers resistance to various classes of antibiotics.
The objective of this study was to compare the antibiotic resistance gene outputs from these software.

METHODS

- We performed WGS on 89 clinical isolates of Enterococci (both *E.faecalis* and *E.faecium*) from two disparate, geographically distinct tertiary care Detroit hospitals admitted to 16 intensive care units (ICU) and non-ICU wards between 2017-2019.
- The samples were obtained 48 hours after admission and WGS was performed using the Illumina NextSeq instrument (Illumina, Inc., CA). The FASTQ files were initially subjected to analysis using EPISEQ CS. EPISEQ CS allows users to download FASTA files along with providing detailed WgMLST analysis and their resistome output.
- The FASTA output from EPISEQ CS was further uploaded into ResFinder with default settings. Outputs from both software were arranged using Excel program to identify antibiotic genes that were identified from both software or one software alone by color coding the output.
- The variations and outputs were tallied to identify similarities and differences.

RESULTS

We analyzed 89 isolates using both software. There was a significant difference in the genetic mutation output from both software.

***Enterococcus faecalis* (N = 29)**

- EPISEQ was able to identify 13 different gene mutations which were not identified by ResFinder.
- ResFinder was able to identify only 1 different gene mutation which was not identified by EPISEQ.
- There were 9 common genetic mutations which were identified by both software for Enterococcus faecalis.

***Enterococcus faecium* (N = 60)**

- EPISEQ was able to identify 15 different gene mutations which were not identified by ResFinder.
- ResFinder was able to identify 2 different gene mutations which were not identified by EPISEQ.
- There were 12 common genetic mutations which were identified by both software for Enterococcus faecium.

CONCLUSION

The ability to identify different genetic mutations by both software likely depends on databases used to determine the resistant antibiotic genes. EPISEQ CS uses **4 different databases** including the open source ResFinder and other proprietary databases making it more sensitive and hence able to identify more genetic mutations compared to ResFinder alone. The costs for analyzing the WGS data through proprietary software is steep but provides additional benefit for detection of more mutations. The value and practical utility of detecting such mutations for routine clinical practice is not well established. More standards are needed by regulatory agencies such as CLSI before these methods can be adapted for practical and or clinical applications.

ANTIBIOTIC RESISTANCE

		Resfinder		EPISEQ	
Gene mutation	Drug class	E. faecalis (N=29)	E. faecium (N=60)	E. faecalis (N=29)	E. faecium (N=60)
sat4	Aminoglycoside	0%	0%	95%	95%
aad(6)		0%	0%	97%	97%
aac(6')-I		0%	0%	100%	100%
ant(6)-Ia		72%	97%	0%	0%
aph(2'')-Ia		3%	12%	0%	0%
aacA/aphD		69%	73%	73%	73%
aadD1		83%	0%	86%	0%
aph(3')-IIIa		65%	93%	92%	92%
ant(9)-Ia		0%	25%	25%	25%
vanA	Glycopeptides	0%	0%	100%	100%
vanR		0%	0%	85%	85%
vanS		0%	0%	85%	85%
vanX-A		0%	0%	100%	100%
vanY-A		0%	0%	97%	97%
vanZ-A		0%	0%	98%	98%
bleO		0%	0%	86%	0%
vanH-A		96%	100%	97%	97%
liaR	Glycopeptides, Lipopeptides	0%	0%	17%	17%
liaS		0%	0%	17%	17%
dfrC	Pyrimidine analogs, Trimethoprim/sulfonamides	0%	0%	7%	0%
dfrE		0%	0%	100%	0%
dfrF		0%	0%	55%	55%
dfrG		3%	57%	57%	57%
erm(A)	Macrolides/lincosamides/streptogramins, Polyketides	0%	22%	22%	22%
erm(B)		100%	97%	93%	93%
erm(C)		3%	0%	3%	0%
lsa(A)	Macrolides/lincosamides/streptogramins, Pleuromutilins, Tetracyclines	96%	0%	100%	0%
msrC		0%	100%	100%	100%
tet(L)	Beta-lactams, Macrolides, Quinolones, Tetracyclines	0%	20%	20%	20%
efmA	Beta-lactams	0%	0%	100%	100%
pbp5		0%	0%	100%	100%
tet(M)	Tetracycline Resistance	95%	48%	42%	42%
tet(S)	Tetracyclines	0%	13%	18%	18%
emeA	Acridine, Quinolones, Tetracyclines	0%	0%	97%	0%
eat(A)	Pleuromutilins	0%	0%	100%	100%

EPISEQ
ResFinder
Both

Table 2. Software-specific prevalence for specific antibiotic resistance genes for both E. faecalis(N = 29) and E. Faecium isolates (N = 60).

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