Whole-Genome Analysis of VREfm Isolates with Daptomycin Resistance Using Oxford Nanopore and Illumina Sequencing

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Background

Vancomycin-resistant *Enterococcus faecium* (VREfm) is a major cause of nosocomial infections of the bloodstream and urinary tract. Bacteremia caused by VREfm is associated with increased mortality and and longer hospital stays. Daptomycin (DAP), a lipopeptide antibiotic, has become an important option for the management of VREfm infections [1].

Whole-genome analyses of VREfm revealed that VREfm harboring mutations in either *liaFSR*, *yycFG, gdpD*, or *cls* genes associated with DAP resistance [1].

Here, we use whole genome sequencing (WGS) with Oxford Nanopore Technologies (ONT) and Illumina to analyze clinical isolates of VREfm from 2 cases of prolonged VREfm with DAP resistance. In the case of ONT, we could perform real-time detection of Antimicrobial resistance genes using the ARMA workflow. Downstream whole-genome analysis of VREfm isolates was performed with the complete assembled genome sequences, obtained by combining ONT and Illumina sequencing. This technique allows examinanation of possible nosocomial VREfm transmission routes at University of Arkansas for Medical Sciences (UAMS), and to better understand molecular mechanisms of DAP resistance.

Objectives

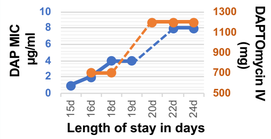
To perform whole-genome analyses of VREfm, using the ONT and Illumina platforms and to investigate the changes of genomic DNA during DAP treatment:

(1) Identify mutations in genes associated with DAP resistance
(2) *De novo* assembly of VREfm isolates from patients, using a combination of ONT and Illumina.
(3) Comparative *Enterococcus faecium* genomics

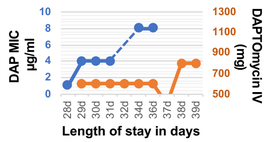
2 Cases of Prolonged VREfm Bacteremia

Two cases of prolonged VREfm bacteremia. The patients remained bacteremic despite high dose DAP therapy.

DAP MIC of each VREfm isolates collected two patients



- Case 1: A 63 year old male with acute myeloid leukemia developed neutropenic fever (NF) after chemotherapy.
- Admissions at E7 Unit – on Jun, 2018



- Case 2: A 64 year old male with myelodysplastic syndrome developed NF after a haploidentical stem cell transplant.
- Admissions at E7 Unit – on Aug, 2018

Experimental Design

Whole-Genome Analysis of VREfm Isolates

The real-time ARMA workflow

EPI2ME — ANTIMICROBIAL RESISTANCE

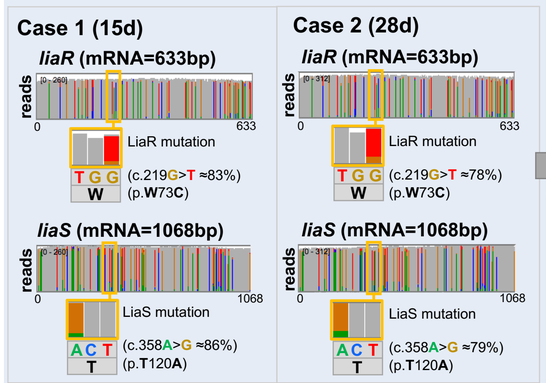
Case 1 (15d)

Alignments	Average Accuracy	Taxon	CARD Model
126	90.8%	Enterococcus faecium	protein variant model
117	85.2%	Enterococcus faecium DO	protein variant model
11	86.0%	Enterococcus faecium DO	protein variant model

Case 2 (28d)

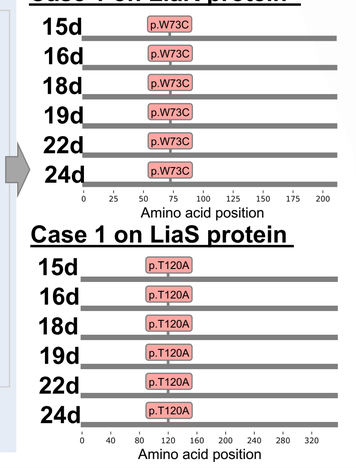
Alignments	Average Accuracy	Taxon	CARD Model
198	91.1%	Enterococcus faecium	protein variant model
162	85.4%	Enterococcus faecium DO	protein variant model
30	85.3%	Enterococcus faecium DO	protein variant model

VREfm from both cases harbor mutations on *liaR* and *liaS* detected by ONT

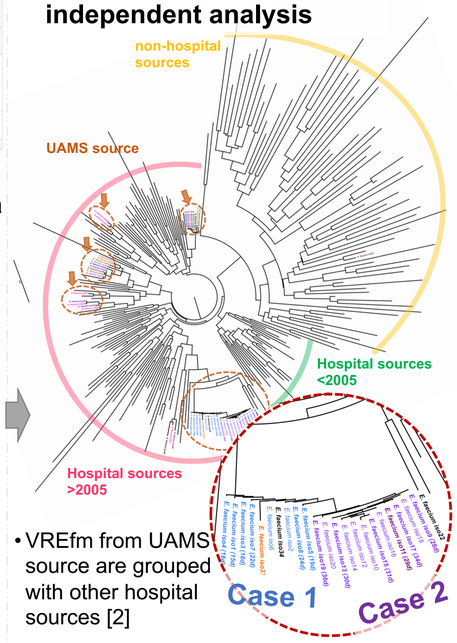


Co-mutations of LiaR and LiaS proteins were identified in VREfm from both cases before DAP treatment.

Confirm mutations on DAP resistance genes by Illumina



Comparative genomes using fastANI: reference-independent analysis



- VREfm from UAMS source are grouped with other hospital sources [2]
- The two patients got same VREfm strains with ≈99.98% average nucleotide identity.

Conclusion:

- Both patients in the same care unit with different admission time-point got infection from the same VREfm strain indicating nosocomial VREfm transmission.
- The VREfm harboring co-mutations of LiaR (p.W73C) and LiaS (p.T120A) protein before DAP treatment.
- The VREfm have no common mutations or accumulate mutations over time of DAP treatment.
- The increment of MICs for daptomycin with ≥4 µg/ml may be caused by gene expression or epigenomic.

Future works:

- Perform transcriptome or epigenome on the VREfm isolates.
- Use nanopore for screening daptomycin resistance genes in clinical samples to help direct appropriate antibiotic therapy.

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Reference

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