LiaX as a surrogate marker of daptomycin susceptibility in multidrug-resistant *Enterococcus faecium* recovered from cancer patients

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BACKGROUND

- *Enterococcus faecium* (*Efm*) are:
  - Leading causes of bloodstream infections (BSI) in patients (pts) with cancer, associated with increased morbidity and mortality[1,2]
  - Frequently multidrug-resistant, resulting in few treatment options
- Daptomycin (DAP) is a cyclic lipopeptide antibiotic that:
  - Is commonly used to treat vancomycin-resistant enterococci (VRE)
  - Rates of up to 20-40% of enterococcal isolates are reported resistant to in pts with cancer[3,4]
  - Has poorly reproducible minimum inhibitory concentrations (MICs)[5]
- The protein LiaX has been shown in *E. faecalis* to:
  - Serve as an extracellular sentinel molecule which binds and detects DAP and antimicrobial peptides.
  - Effect the LiaFSR cell membrane stress response pathway triggered by DAP
  - Increase DAP MICs when added to broth cultures[6]

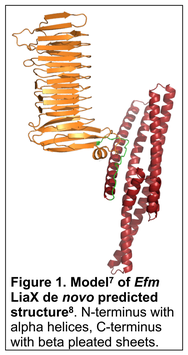

Figure 1. Model[7] of *Efm* LiaX de *novo* predicted structure[8]. N-terminus with alpha helices, C-terminus with beta pleated sheets.

Figure 2. The *liaFSR* and the *liaXYZ* genes are regulated by LiaR in *Efm*.* *Unpublished data

AIM

To demonstrate that detection of extracellular LiaX correlates with daptomycin resistance in clinical bacteremia isolates of *E. faecium*.

RESULTS

Characteristic	N=54
Age in years, median (range)	57.5 (22-82)
Male Gender, N (%)	33 (61.1)
Race	
White	39 (72.2)
Black/African American	10 (18.5)
Other	5 (9.3)
Hispanic/Latin Ethnicity, N (%)	7 (13)
Underlying Malignancy, N (%)	
Hematological	45 (83.3)
Solid Organ	9 (16.7)
Hematological cell transplant recipients, N (%)	16 (29.6)
VR*Efm*, N (%)	26 (48.1%)
Index Bacteremia	
E. faecium monomicrobial	39 (72.2)
Polymicrobial	15 (27.8)
Infection Source	
Gastrointestinal	34 (62.9)
Central-line associated	10 (18.5)
Genitourinary	5 (9.3)
Unknown	5 (9.3)

Table 1: Baseline demographic and clinical characteristics of patients at time of collection of blood culture growing *Efm*.

RESULTS

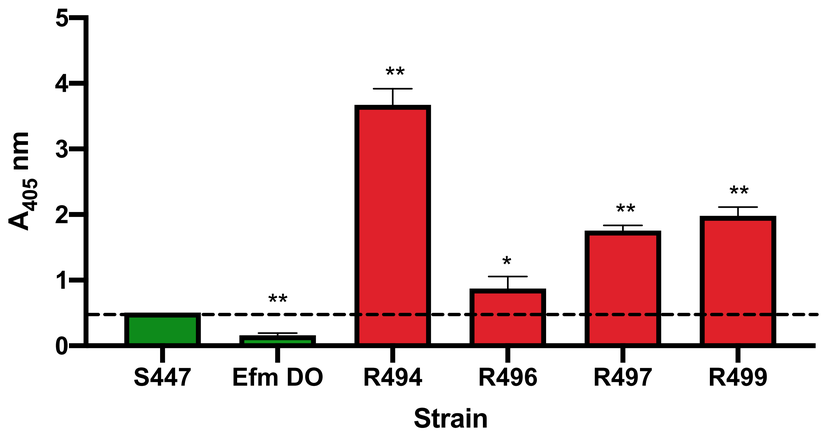

Figure 3. Whole-cell indirect LiaX ELISA absorbance (A_{405}nm) of *Efm* reference strains shows ability to differentiate DAP susceptible MICs from DAP resistant MICs. DAP susceptible (MIC=2 µg/ml) *Efm* strains are shown in green and DAP resistant (MIC≥8 µg/ml) strains in red. DAP-S reference strains have no LiaFSR mutations. The dotted line indicates an example cutoff for DAP-S/R in this assay. *p<0.05, **p<0.0001 by unpaired t-test. Coefficient of variance for each reference is <15%.

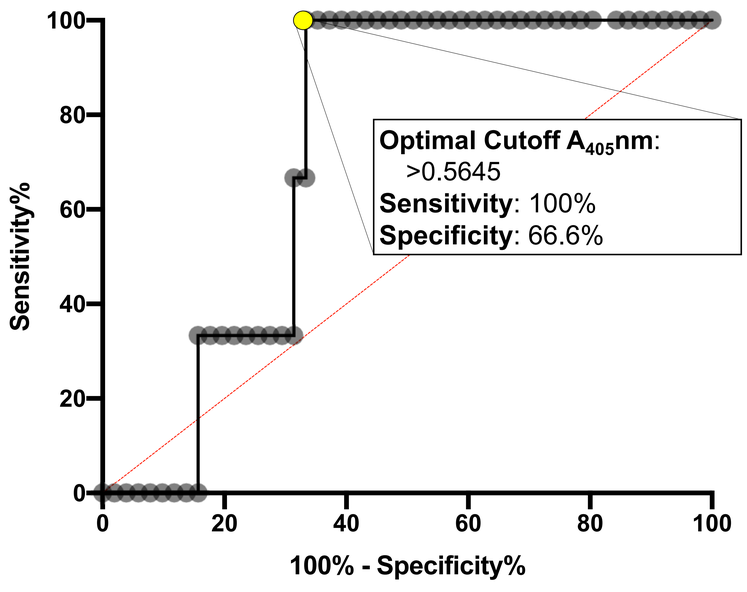

Figure 4. ROC Curve of ELISA LiaX A_{405}nm values compared to daptomycin categorical susceptibility as determined by broth microdilution. Of 54 clinical *Efm* isolates, 3 were DAP resistant by broth microdilution MIC. The optimal cutoff A405nm was determined to be >0.5645, which resulted in 100% sensitivity (95% CI: 43.85% - 100.00%) and 66.6% specificity (95% CI: 52.97% - 78.03%). The AUC of the curve is 0.7320.

ACKNOWLEDGEMENTS

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METHODS

Feasibility and Optimization

- We used 6 whole-genome sequenced, well-characterized[9] multidrug-resistant *Efm* BSI isolates to optimize a whole-cell enzyme-linked immunosorbent assay (ELISA) method.

Clinical *Efm* Isolates

- Clinical isolates from 54 pts with cancer and *Efm* bacteremia were obtained from the VENOUS *(**V**ancomycin-resistant **EN**terococci **OU**tcomes **S**tudy)* cohort between 2016 – 2018.
- Patient characteristics documented from time of first positive blood culture

ELISA method and MICs

- Each *Efm* isolate was grown to mid-exponential phase, normalized to cell density OD_{600} of 1.0, pelleted, and plated in high-binding microtiter 96-well plates. LiaX was detected by rat anti-sera (raised in-house), and goat anti-rat alkaline phosphatase-conjugated secondary antibody. Conjugates were incubated with chromogenic PNPP substrate and absorbance was read at 405 nm (A_{405} nm). Assays were conducted in triplicate for each isolate.
- DAP MICs were determined for each isolate by broth microdilution (BMD)

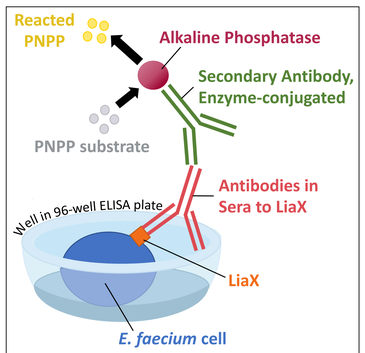

Figure 5. Whole-cell indirect ELISA. A representative *E. faecium* cell with LiaX on its surface is bound by LiaX antibodies in rat sera raised against LiaX. These antibodies are in turn bound by secondary antibodies conjugated to an enzyme that causes a yellow color change of substrate PNPP. The yellow color can be measured by light spectrophotometry.

Diagnostic Test Performance

- DAP MIC determined by BMD was used as the gold standard for categorical DAP susceptibility. DAP resistance was defined as MIC ≥ 8 µg/ml by CLSI standards.
- A Receiver Operating Characteristic (ROC) curve was generated to determine susceptible/resistant cutoff for optimal sensitivity and specificity.

DISCUSSION

1. **The detection of extracellular LiaX has important discrepancies with routine daptomycin MIC.**
   - Most discrepant isolates are susceptible by MIC but have high LiaX ELISA A_{405}nm values – the importance of this discrepancy will be assessed by clinical outcomes of patients with these isolates.
2. **Further evaluation of extracellular LiaX with an affinity-purified primary antibody is warranted.**
3. **E. *faecium* bacteremia in patients with cancer have a primarily gastrointestinal source, indicating the potential for screening tests based on LiaX.**

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