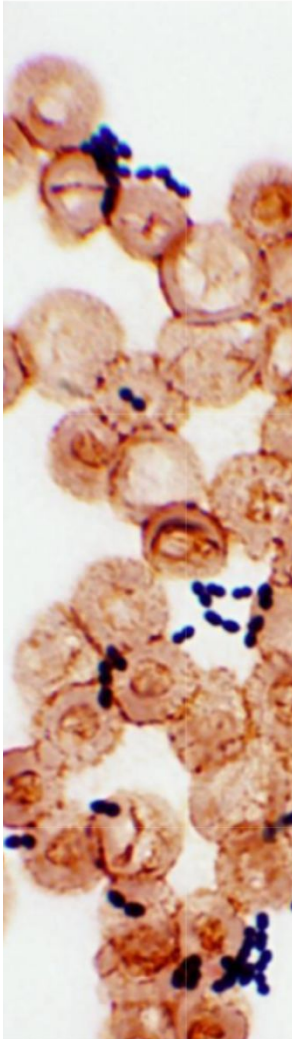


DIFFICULT-TO-TREAT ENTEROCOCCAL INFECTIONS & THEIR PHAGE ADVERSARIES

MADISON STELLFOX, MD, PHD
INFECTIOUS DISEASES FELLOW
POSTDOCTORAL SCHOLAR

UNIVERSITY OF PITTSBURGH
MENTOR: DARIA VAN TYNE, PHD

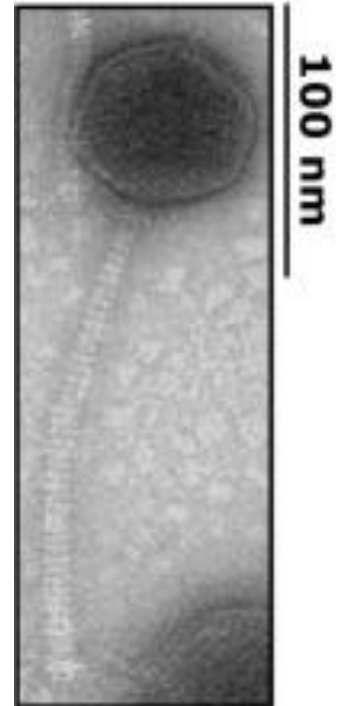
CLINICAL CHALLENGES OF ENTEROCOCCAL INFECTIONS



- Enterococci are ubiquitous, gram-positive GI-tract commensals that have become concerning clinical pathogens (*E. faecium* and *E. faecalis*)
 - Hardy and intrinsically resistant to many classes of antibiotics
 - Growing percentage of healthcare-associated infections are VRE
- VRE is a significant concern in our 3-hospital, 2220-bed healthcare system:
 - Averaging 6 – 8 VRE blood stream infections per month
 - ~10% are recurrent infections
 - Up to 30% of VRE infections may experience treatment failure

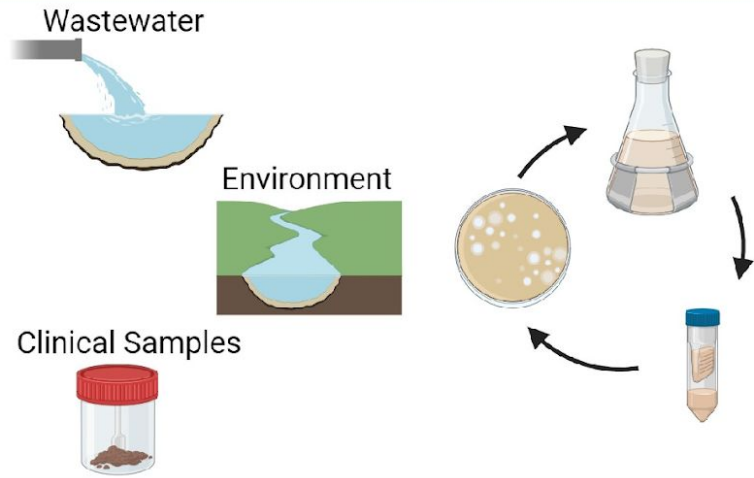
PHAGE THERAPY FOR DIFFICULT-TO-TREAT ENTEROCOCCAL INFECTIONS

- Bacteriophages (phages) are viruses that infect bacteria and can lyse their hosts
- Clinical potential of phage therapy was recognized in the early 1900s but was largely supplanted by broad-spectrum antibiotics
- Resurgence of interest as clinical practice is increasingly challenged by:
 - Antimicrobial resistance
 - Increasing use of immunosuppressive therapies
 - Challenges associated with long-term indwelling medical devices
- Clinical experience with phage therapy for enterococcal infections is limited:
 - Small case series & individual compassionate use protocols for salvage regimens
 - *In vitro* studies in clinically relevant enterococcal strains



Preclinical Pipeline

Novel ϕ Isolation For Target Pathogens



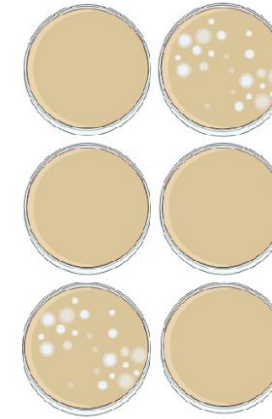
Maintain Species-Specific ϕ Libraries

Clinical Enterococcal Phage Library:

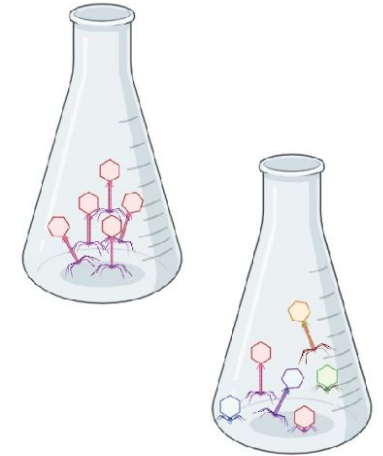
E. faecium – 19

E. faecalis – 17

Screen Patient Isolates

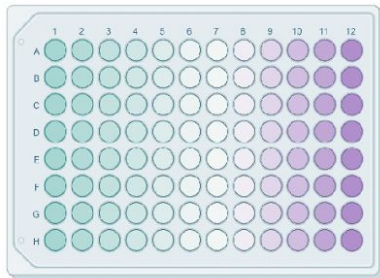


Patient-Specific ϕ Preparations

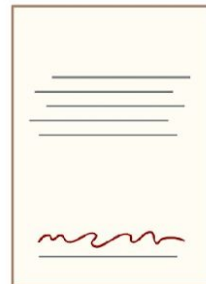


Clinical Preparation and Use

ϕ Preparation Testing



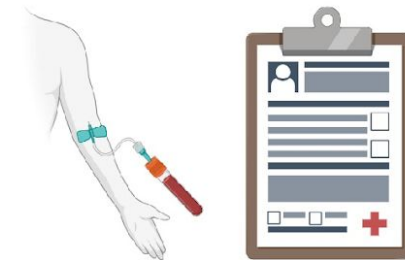
Approval & Consent



Initiate Treatment

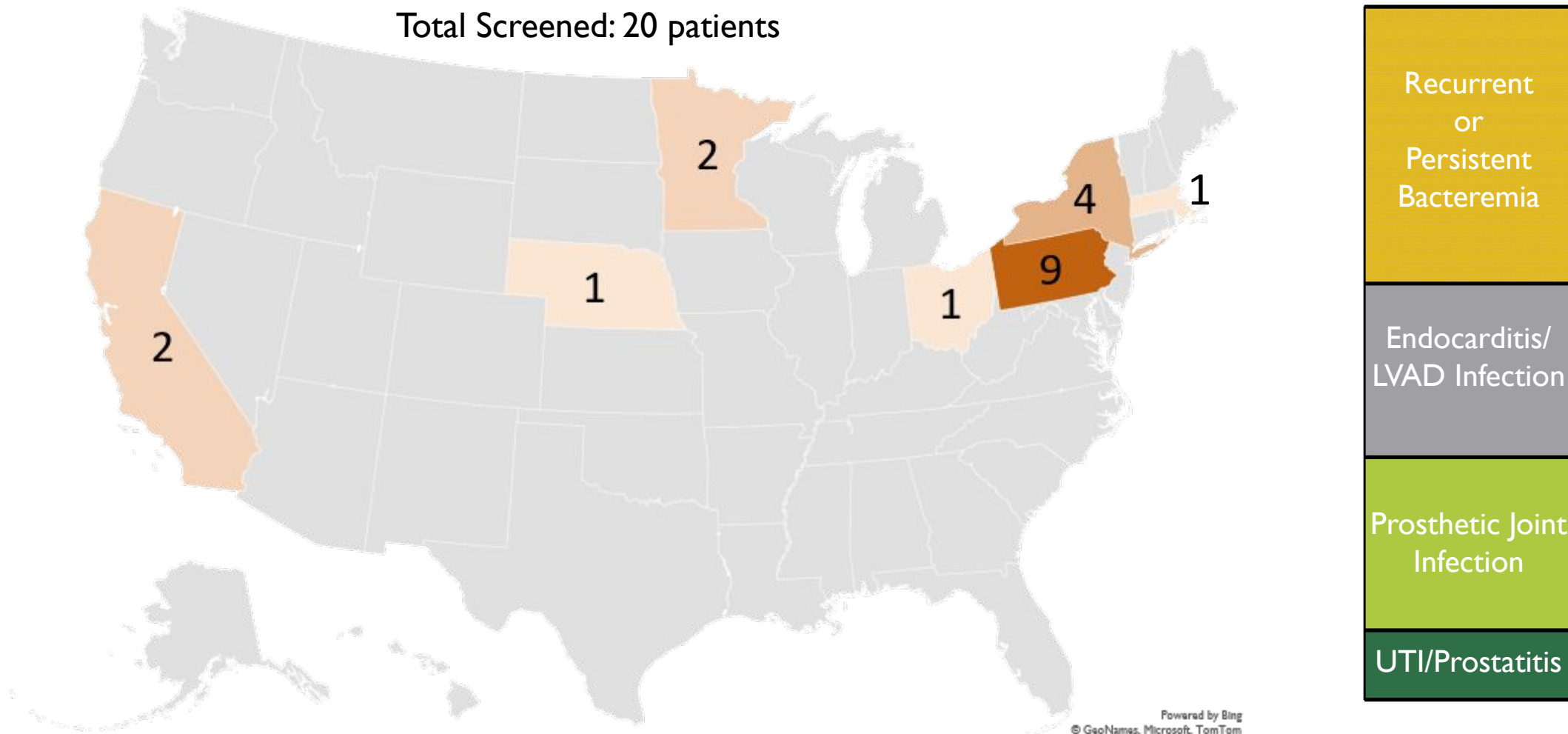


Monitoring



CLINICAL Φ SCREENING FOR ENTEROCOCCAL INFECTIONS

Total Screened: 20 patients



Recurrent
or
Persistent
Bacteremia

Endocarditis/
LVAD Infection

Prosthetic Joint
Infection

UTI/Prostatitis

Enterococcal Φ Therapy Requests (20)

E. faecalis (3)

E. faecium (17)

Φ Found (3)

No Φ Found (7)

Susceptible to Φ Library (10)

Pursuing Therapy (3)

Declined/Unable (5)

Resolution w/o Φ (2)

Treated (3)

TREATMENT OUTCOMES

Patient	Organism	Clinical Scenario	Phages	Dosage (PFU/mL)	Route	Duration	Outcome
1	VSE & VRE <i>faecium</i>	Recurrent bacteremia	Φ9184 ΦHi3	1-2 x 10 ⁹	IV, PO	6 months	
2	VRE <i>faecium</i>	Endovascular infection & Persistent bacteremia	Φ9184 ΦHi3	1 x 10 ⁹	IV, PO	8 weeks (planned)	
3	VRE <i>faecium</i>	Prosthetic joint infection	Φ9184 ΦHi3	2 x 10 ⁹	IV, OR lavage	4 weeks (IV)	

All preparations were well tolerated
No phage-related adverse events

IMPROVING ENTEROCOCCAL PHAGE THERAPY



Broaden our Φ library through collaboration & environmental sampling

↑ screening success rate

↑ options to prepare clinical-quality Φ preparations

Develop clinically relevant enterococcal strain collections

Design *in vitro* studies to answer outstanding questions to improve our use of phages in clinical practice

WHEN IS THE BEST TIME TO PROVIDE PHAGE THERAPY?

RECURRENT VRE BLOOD STREAM INFECTION COHORT (VRE-BSI)

Central microbiology lab serving 3 hospitals (~2200 beds)



Collect VRE isolates from (+) blood cultures



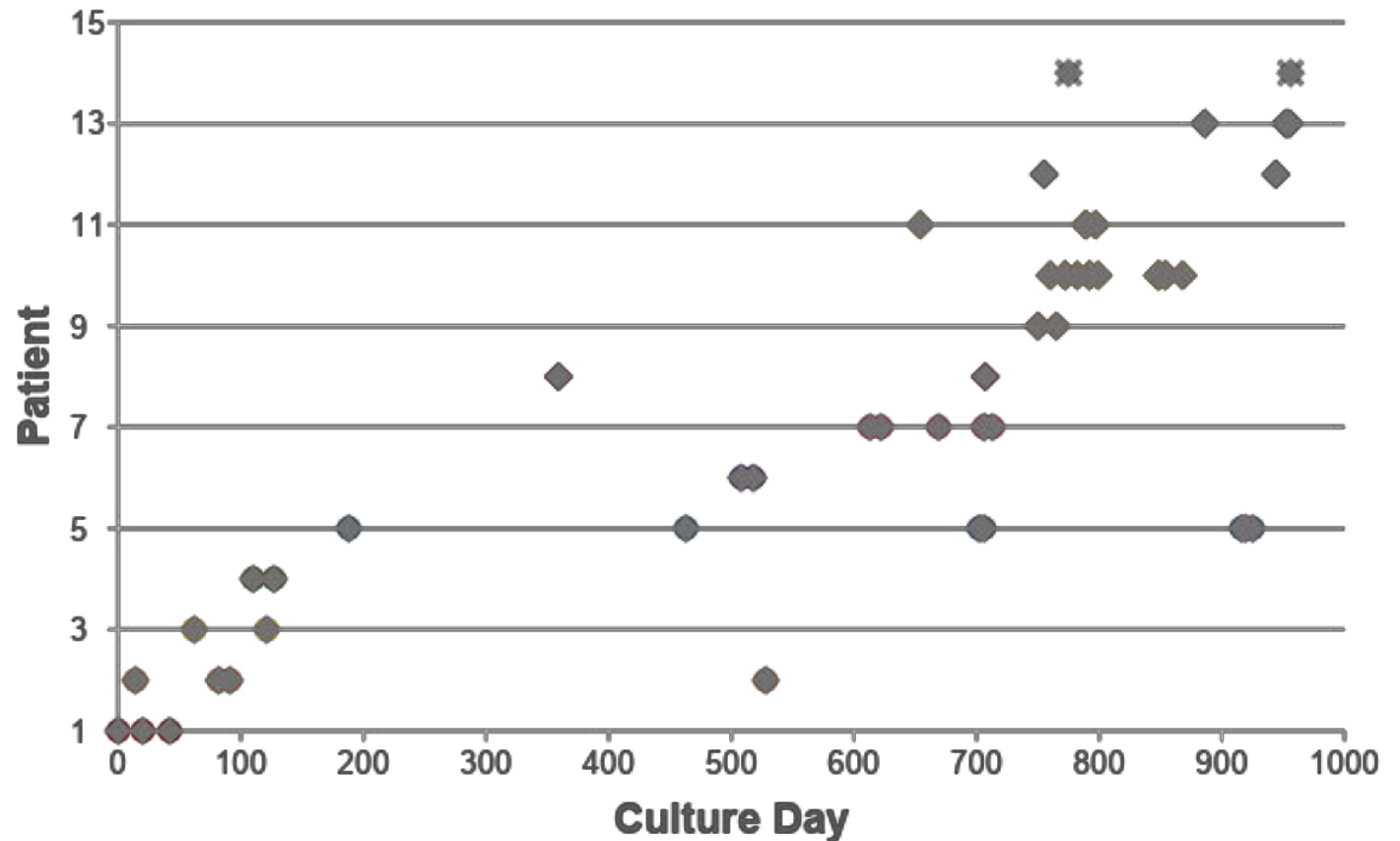
> 150 patients with VRE-BSIs



Collect serial isolates from patients with > 1 VRE-BSI event

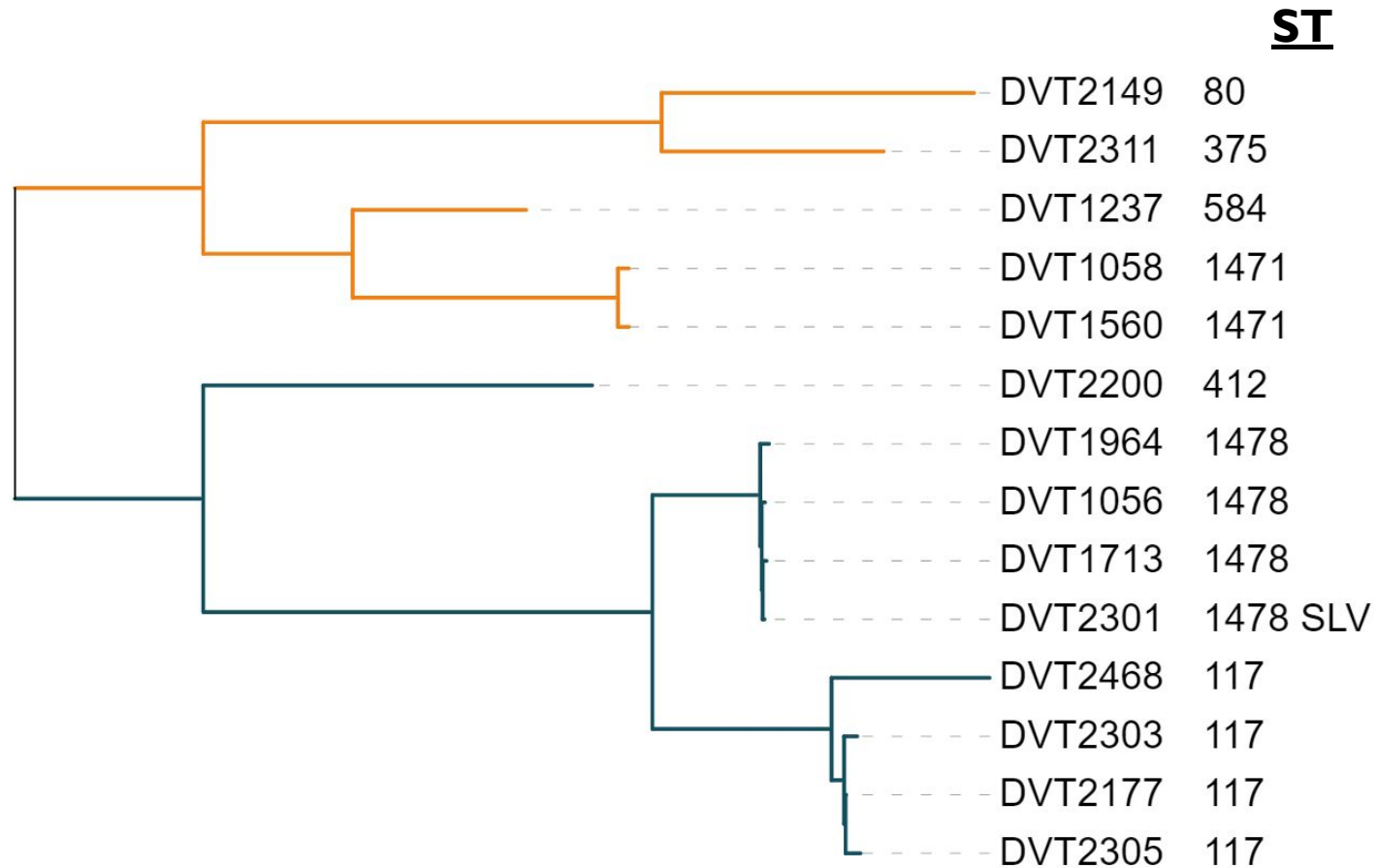
RECURRENT VRE-BSI COHORT

- 14 patients with >1 VRE-BSI event
- 55 serial *E. faecium* isolates
- 2 – 13 isolates per patient
- Up to 437 days between recurrences
- Majority of the patients (13/14) had recurrences with highly related isolates by SKA analysis



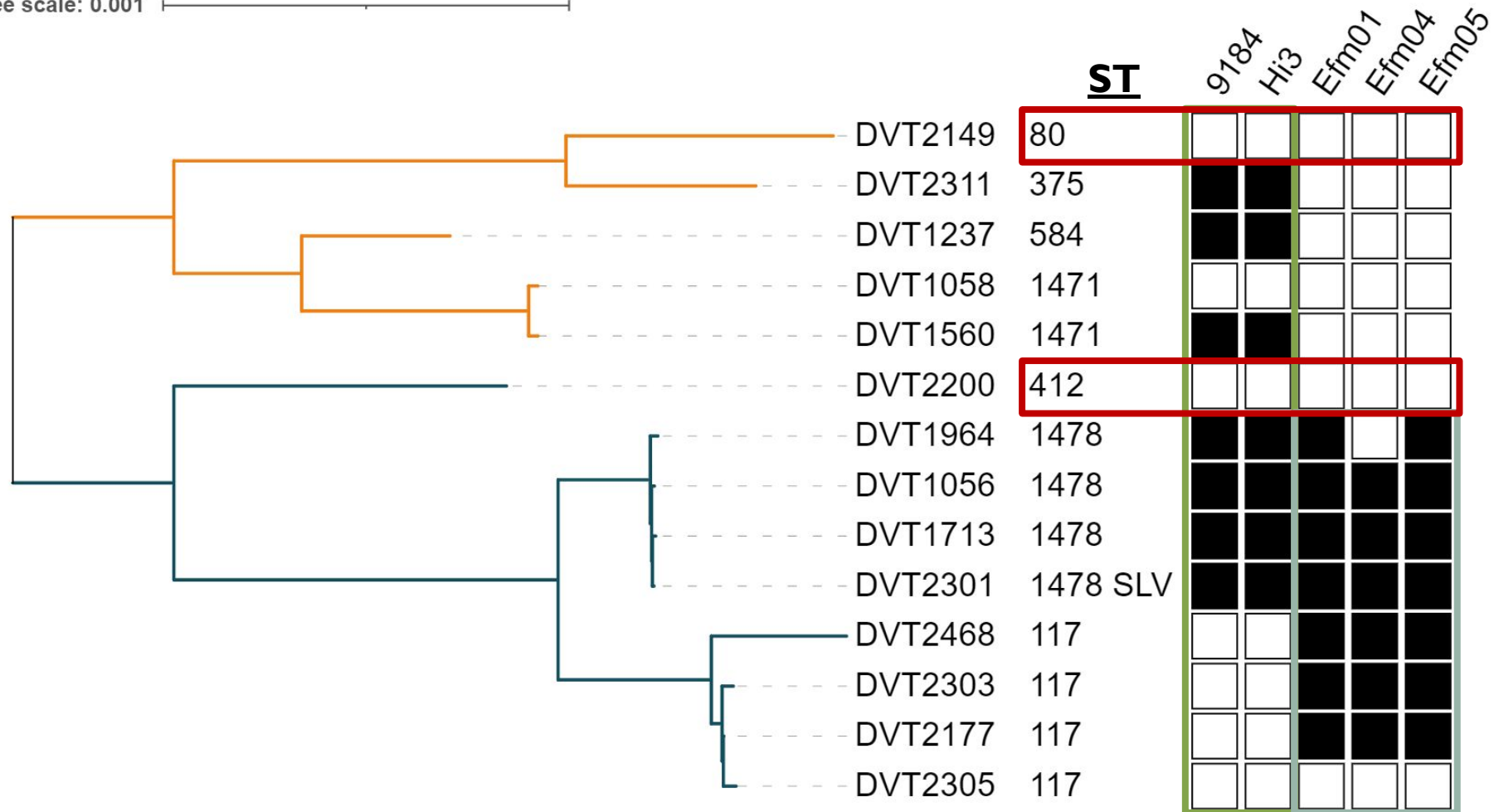
RECURRENT VRE-BSI COHORT: INITIAL ISOLATE PHYLOGENY

Tree scale: 0.001

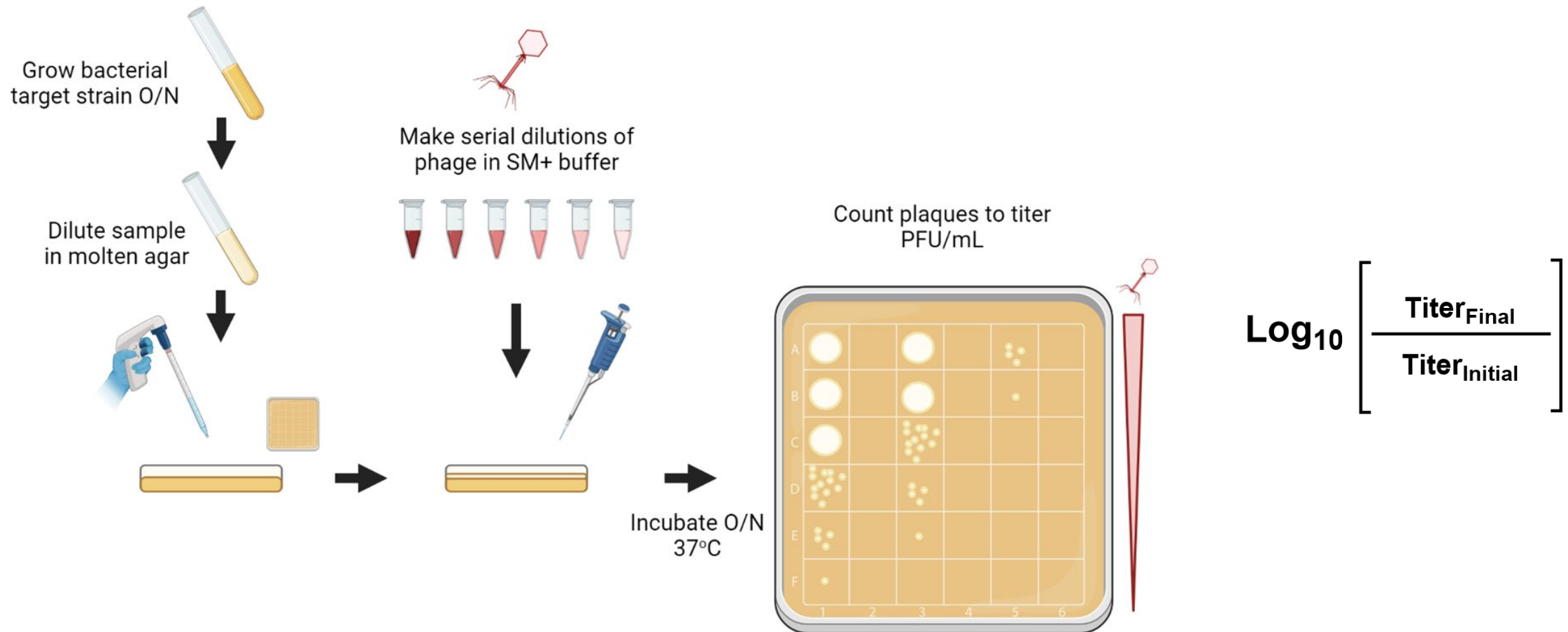


RECURRENT VRE-BSI COHORT: INITIAL ISOLATE PHYLOGENY & PHAGE SUSCEPTIBILITY

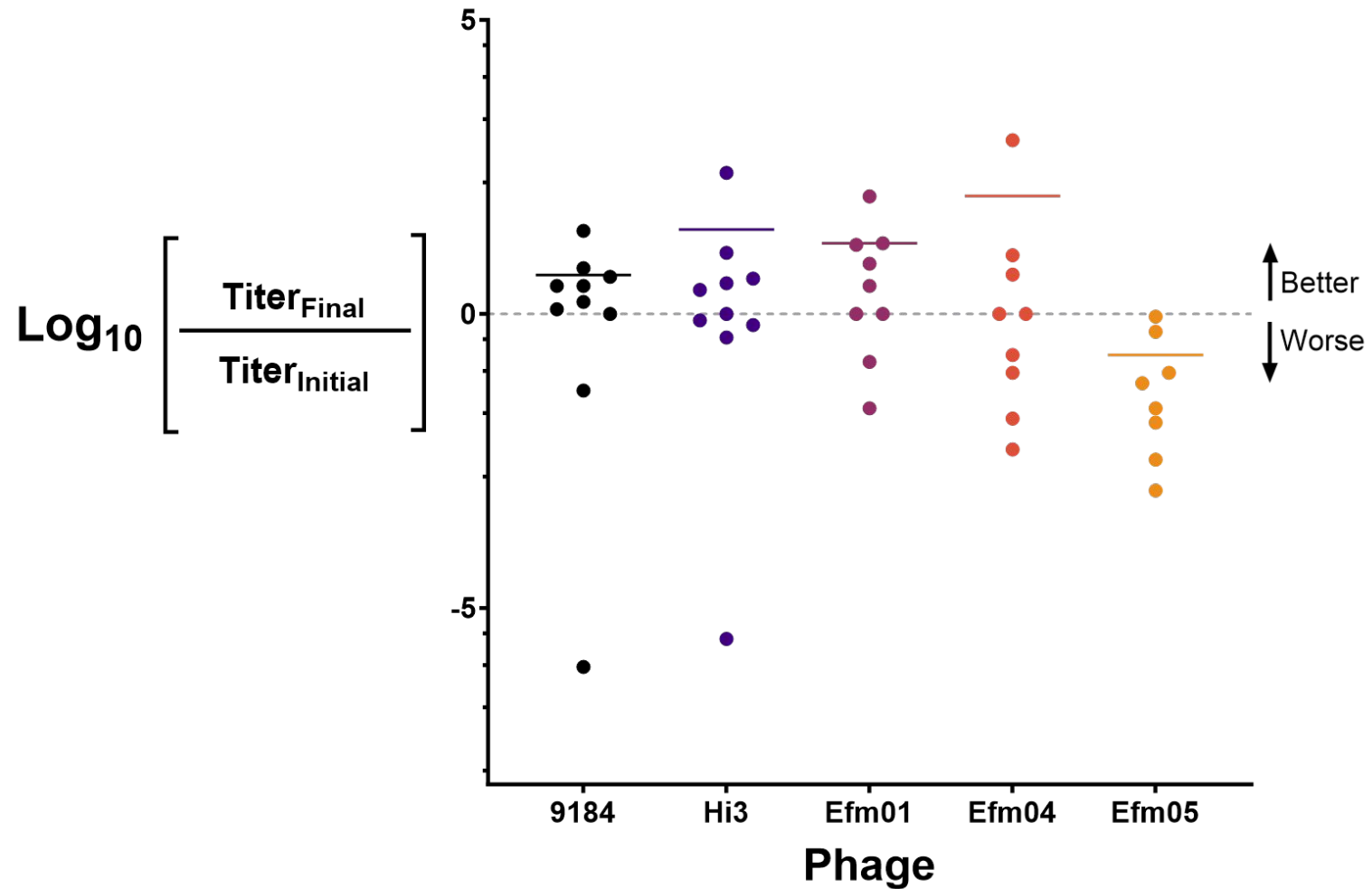
Tree scale: 0.001



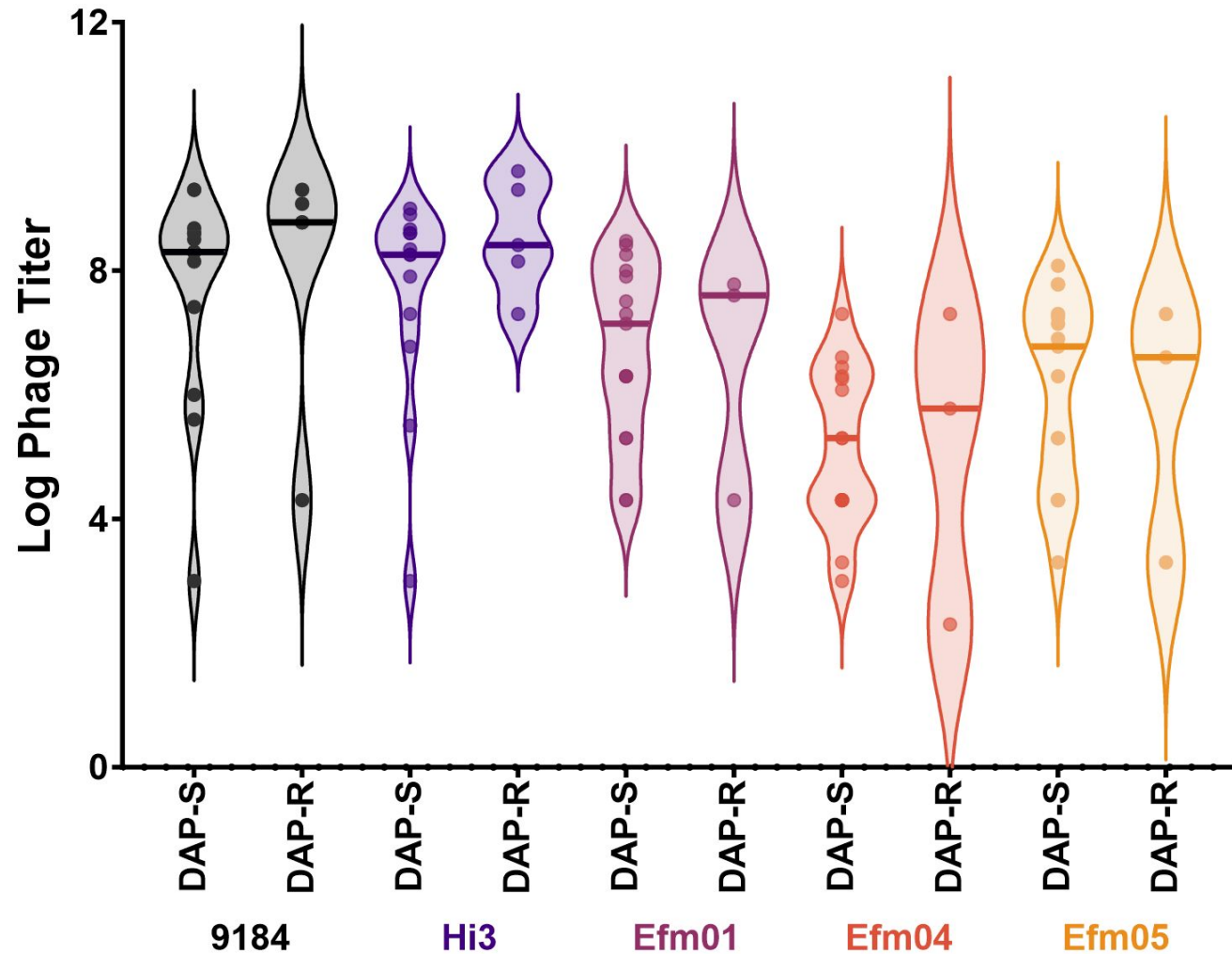
QUANTIFYING PHAGE SUSCEPTIBILITY OVER TIME



↑ PHAGE SUSCEPTIBILITY OF RECURRENT VRE-BSI ISOLATES



↑ PHAGE SUSCEPTIBILITY OF DAP-R VRE-BSI ISOLATES



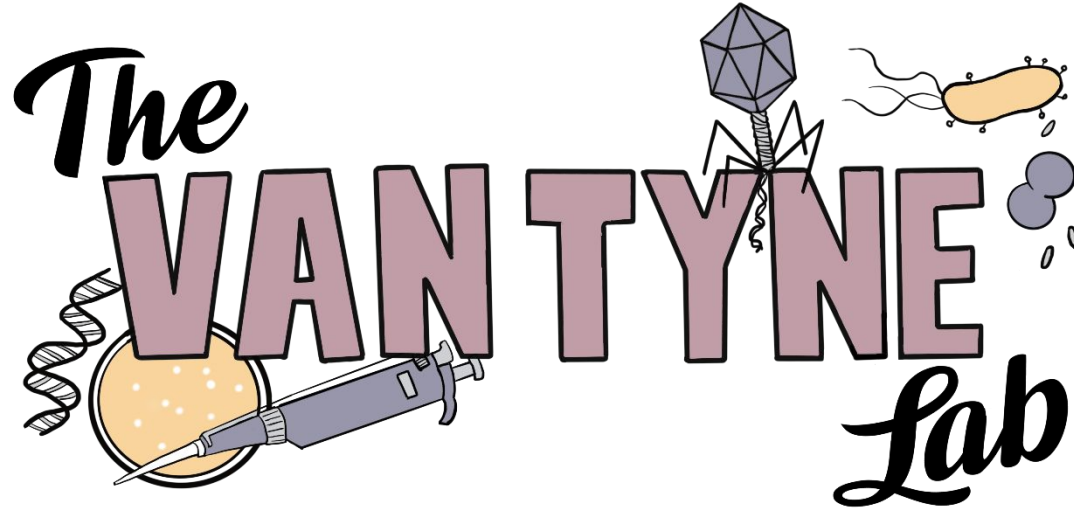
CONCLUSIONS

- Clinical phage therapy continues to be safe and well tolerated
- Recurrent VRE-BSI infections are not isolated to one genetic lineage
- Within individual patients, recurrent isolates tend to be closely related
- Current use of phage therapy as a salvage or rescue regimen remains a viable strategy
 - Phage susceptibility tends to remain stable or improve over time
 - More drug-resistant isolates trend towards increased phage susceptibility

FUTURE DIRECTIONS

- Expand analyses to increase both bacterial isolate cohort size & phage diversity
- Leverage clinical data to better understand phage-bacterial host dynamics in the context of antibiotic exposures during standard-of-care therapies
- Use these highly related serial isolates to better understand
 - Phenotypic and genotypic adaptations of VRE during recurrent infections
 - Phage-bacterial host dynamics throughout the course of recurrent disease

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 - Nate Wallace
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 - Greg Canfield, MD, PhD
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 - Marissa Griffith
- **Ryan Shields Lab – University of Pittsburgh**
 - Kevin Squires
 - Ava Dorazio
- **Graham Stafford Lab – University of Sheffield, UK**