Dissemination of hospital-adapted lineages of vancomycin-resistant *Enterococcus faecium* in wastewater

Theo Gouliouris
University of Cambridge, Department of Medicine
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Importance of *Enterococcus faecium* as a human pathogen

- *E. faecium*: Gut commensal of humans and animals
- Indicator organism of faecal contamination
- Increasingly important cause of healthcare associated infections over last 30 years
- Emergence of hospital-associated multidrug resistant lineages (CC17)
  - intrinsic ampicillin and quinolone resistance
  - horizontally acquired vancomycin resistance as a transposon usually on a plasmid
- Vancomycin-resistant *E. faecium* (VREfm) classified as a serious antibiotic resistance threat by CDC and WHO

Lebreton et al, MBio 2013
Aims

• Cross sectional study investigating the prevalence of hospital-associated lineages of *E. faecium* and vancomycin resistance in wastewater treatment plants across the East of England

• Phylogenetic analysis comparing wastewater and bacteraemia isolates from the same region
Sampling

20 wastewater treatment plants sampled in 2014-5
-10 plants (blue) located downstream of hospitals (squares)
-10 plants (orange) unrelated to acute hospitals

Wastewater collected from Cambridge University Hospital sewer on 4 occasions (2014-5) (triangle)

Bacteraemia isolates from Cambridge University Hospital (2014-6, n=23) and from the East of England (2010-2012, n=164) (red dots)
Wastewater treatment

- Raw wastewater
- Preliminary treatment (large solids screening)
- Primary treatment
- Secondary treatment (activated sludge or biological filter beds)
- Tertiary treatment (only some WWTPs)
- Final Effluent
- Surface waters

Images:
- Raw wastewater sampling point
- Activated sludge
- Secondary settlement tank
- Treated wastewater sampling point
- Terminal UV light
Wastewater treatment

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Raw wastewater sampling point | Activated sludge | Secondary settlement tank | Treated wastewater sampling point

Terminal UV light
Wastewater processing
VREfm is disseminated in wastewater

Wastewater treatment reduces but does not eliminate *E. faecium*
VREfm is disseminated in wastewater

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VREfm is disseminated in wastewater

Wastewater treatment reduces but does not eliminate *E. faecium*
Wastewater contains a high diversity of *E. faecium* with hospital-adapted clones representing a minority population. 

n=620 isolates
Dominant subclades shared between wastewater plants and hospitals

n=481

Subclades according to location
Dominant subclades shared between wastewater plants and hospitals

Network using 5-SNP cut-off
Resistance genes shared between wastewater, hospital sewer and invasive isolates

Resistance genes (n=28)

Glycopeptides  Aminoglycosides  Phenics  MLS genes  Tetracyclines

Resistome profiles (n=197)

- Wastewater
- Hospital sewer
- Bacteraemia

<table>
<thead>
<tr>
<th>Location</th>
<th>Waste</th>
<th>Resistome profiles</th>
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<tr>
<td>Wastewater</td>
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<td>Hospital sewer</td>
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<td>Bacteraemia</td>
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Resistance genes shared between wastewater, hospital sewer and invasive isolates

Resistance genes (n=28)
- trimethoprim
- glycopeptides
- aminoglycosides
- phenicols
- MLS genes
- tetracyclines

Resistome profiles (n=197)

Wastewater: 77
Hospital sewer: 68
Bacteraemia: 24

Legend:
- Brown: Bacteraemia
- Purple: Hospital sewer
- Green: Wastewater
Putative virulence genes detected in wastewater
Conclusions

- *E. faecium* (and VREfm) of the hospital-adapted lineage in wastewater is:
  - closely related to clinical isolates causing bacteraemia
  - is widely disseminated in both hospital and non-hospital-related wastewater in the East of England, indicating that hospital effluent is not its sole source

- Findings suggest there may be widespread VREfm carriage in the community with implications for introduction and spread into hospitals

- Wastewater may represent a useful reservoir for VREfm surveillance

- Wastewater treatment is insufficient to prevent downstream environmental contamination with VREfm
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