ACQUISITION AND TRANSMISSION NETWORKS OF ENTEROCOCCUS FAECIUM REVEALED BY WHOLE GENOME SEQUENCING: A LONGITUDINAL COHORT STUDY

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Declaration

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- No other conflicts of interest to declare

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**E. faecium** as a human pathogen

- Leading cause of infection in immunocompromised and critically ill patients
- *E. faecium*: Healthcare-associated lineages are multidrug resistant – ampicillin, quinolone – and can acquire resistance to vancomycin
- Previously known as CC17, now named clade A1
- Whole genome sequencing of *E. faecium* bloodstream infection isolates shows evidence of hospital transmission
- Detailed epidemiological studies lacking

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Raven *et al.* (CID 2017) CUH *E. faecium* bacteraemias 2006-12
Aims

Conduct a prospective observational study over 6 months on two adult Haematology wards at Cambridge University Hospitals (CUH) from May till November 2015

• Determine the rate of carriage of *E. faecium* in the Haematology inpatient population at CUH and the level of environmental contamination

Use whole genome sequencing to:

• Determine rates of acquisition and transmission of *E. faecium*
Methods - Participants

- Setting: Tertiary referral centre for Haemato-oncology/regional haematopoietic stem cell transplant unit
- Two wards
  - 16-bedded unit (11 single-rooms), HEPA-filtered, positive pressure
  - 11-bedded unit (4 single-rooms)
- Informed consent required
- Stool samples on admission, weekly and on discharge
- If no stool available on discharge or if patient did not participate in study, environmental sample was obtained on discharge
Methods - Stool

Stool sample

Unselected enterococcal culture (admission sample only)

- Direct culture (SB)

- Enriched culture (Enterococcosel broth)

  Subculture (SB) plate

  Pick 2 isolates

  Save 1 isolate if ASVSEfm

  Isolate sequenced

  5 isolates sequenced
  - if first positive sample and no VRE grown
  - from all samples up to first VRE growth in patients who acquired VRE
  - 1 isolate sequenced from all other samples

Ampicillin resistance selective culture

- Direct culture (Amp-Enterococcosel) plate

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Vancomycin resistance selective culture

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Methods - Environment

• Terminal pooled swabs: one from bedside and one from bathroom
• Fortnightly from:
  – communal bathrooms and toilets
  – non-touch surfaces (air vents and HEPA filters)
  – medical devices (computers on wheels, handheld devices, etc)
• Plating on ampicillin and VRE selective media
• Sequenced 1 VREfm colony, or 1 VSEfm colony if VRE plate was negative
Recruitment and results

335 patients admitted (486 admissions)

174 (52%) patients enrolled
269 (55%) admissions

149 (86%) participants screened at least once
376 stool samples
(Median 3, IQR 2-5, range 1-8)

101 (68%) participants screened more than once

161 (48%) not enrolled
For 131/217 (60%) admissions
environmental swab obtained

For 23/25 (92%) participants
only environmental samples obtained

94/149 (63%) VREfm positive patients
212/376 (56%) VREfm positive samples

922 environmental swabs performed

116/149 (78%) AREfm positive patients
271/376 (72%) AREfm positive samples

127/149 (85%) Efm positive patients

116/149 (78%) AREfm positive patients
271/376 (72%) AREfm positive samples

94/149 (63%) VREfm positive patients
212/376 (56%) VREfm positive samples
Phylogenetic tree of 1560 isolates (1001 stool, 559 environmental)

Analysis confined to 1477 (943 stool and 534 environmental isolates) belonging to clade A1 (CC17) (95%)
Subtypes during study period

- Stool – 1st occurrence per participant
- Stool – subsequent occurrence
- Patient’s environment – 1st occurrence
- Patient’s environment – subsequent occurrence
- Communal bathroom – 1st occurrence
- Communal bathroom – subsequent occurrence
- Medical device – 1st occurrence
- Medical device – subsequent occurrence
- Non-touch areas – 1st occurrence
- Non-touch areas – subsequent occurrence

115 subtypes – 91 found in patients (median 2, range 1-6 subtypes per patient)
n=115 subtypes identified

Two major subtypes identified in 25 and 30 patients

91/115 (78.4%) in stool samples

Subtypes colonising multiple patients were more likely to be found in the hospital environment (89%) than those colonising a single patient (51%) (Fisher exact test, p<2.3x10^{-4})
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Epidemiological relationships - Network

N=26 transmission clusters (size 2 to 8 patients)

Type of node
- Patient
- Patient's own environment
- Communal area
- Medical device
- Non-toach area
- Non-sampled patient's environment

Type of edge
- Acquired subtype with strong epidemiological link
- Index subtype with strong epidemiological link
- Acquired subtype with weak epidemiological link
- Index subtype with weak epidemiological link

Subtype colour-coding (patients involved in transmission)

- 47A (30)
- 15A (25)
- 26B (7)
- 5A  (6)
- 49A (5)
- 46A (5)
- 43B (6)
- 26G (6)
- 37C (4)
- 33B (4)
- 21A (4)
- 9A  (3)
- 60A (3)
- 58A (3)
- 4A  (3)
- 45A (3)
- 42A (3)
- 38A (3)
- 37B (3)
- 33A (3)
- 29A (3)
- 28B (3)
- 29A (3)
- 46A (5)
- 58A (3)
- 4A  (3)
- 19A (3)
- 36A (2)
- 37A (2)
- 27A (2)
- 25A (2)
- 23A (2)
- 12A (2)
- 12A (2)
Summary

- WGS provides unprecedented detail of *E. faecium* colonisation dynamics
- VREfm colonises the majority of patients admitted to the Haematology wards with evidence of mixed subtype carriage and multiple acquisition events
- Pathogen with highest rate of hospital transmission at CUH
- Spread is facilitated by contamination of communal areas and healthcare devices, and high patient turnover, movement and readmission rates
- Current infection control practices are not effective
- Improved cleaning protocols and hand hygiene are required
- Complete eradication will be difficult without draconian measures
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• Dr David Enoch

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• Anne Green
• Claire Cowling
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• Dr Direk Limmathurotsakul

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Copan Italia Spa for providing the swabs

The patients
How to define a subtype based on pairwise distance after removal of recombination (same sample comparisons)
Sample Genetic distance (# of SNPs)

- Same ST and 50SNP cluster
- Different ST, same 50SNP cluster
- Different ST and 50SNP cluster
- Same ST, different 50SNP cluster
Genetic distance (# of SNPs)

- Same ST and 50SNP cluster
- Different ST, same 50SNP cluster
- Different ST and 50SNP cluster
- Same ST, different 50SNP cluster

Sample
SNP threshold for inferring transmission (same and different sample comparisons for each patient over time)
Environmental sampling – VREfm positivity during study

[Bar chart showing positivity percentages for different areas and locations]
N=11/26 single transmission event of 2 patients
hospital environment not involved (1 exception)
N=15/26 transmission clusters involved 3 to 8 patients hospital environment involved (2 exc.)
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Phylogenetic tree of 1560 isolates (1001 stool, 559 environmental)

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Stool positivity increased over time

33 VREfm-negative patients (33%) acquired VREfm
41 patients (41%) acquired ARVSEfm and/or ARVREfm