Epidemiological Analysis of Vancomycin Resistant Enterococci Carriage among Patients in an Acute Hospital.

Caoimhe Lynch
BSc (Hons) Biomedical Science
Vancomycin Resistant Enterococci (VRE)

- GI commensals, traditionally defined as low grade pathogens
- Acquisition linked to length of hospital stay, presence of feeding tubes/catheters
- Persistent hospital environmental contaminants (CUH study, O’Leary, 2006)
- Emerging nosocomial pathogen
- Limited treatment options
- Tn1546 - associated with mobile genetic elements, transferred via conjugation.
- Clonal Complex 17 (CC17)
45.1% of invasive Enterococci isolates were vancomycin resistant, in Ireland \[1\]
Background to this research project

- Concern regarding high incidence of Vancomycin Resistant Enterococci (VRE) bloodstream infections in CUH

- Prevalence study of patient enteric carriage in patient cohort – Cork University Hospital (CUH) undertaken in MSc research project (Eileen Whelton), cross sectional study[2]

- May-August 2014: 350 sequential faecal samples screened from patient samples submitted for routine enteric pathogen detection request for VRE, using Chromogenic culture (chromID VRE™, BioMérieux)

- Hospital + community patients sampled
Background to this research project continued...

- Speciation (MALDI-TOF): 100% *E. faecium* (n=67)
- Resistance Determinants: *vanA* 86%, *vanA + vanB* 14%; Resistant also to ampicillin (100%), high level gentamicin (82%)

<table>
<thead>
<tr>
<th>COHORT</th>
<th>PREVALENCE %</th>
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<tbody>
<tr>
<td>CUH (n = 194)</td>
<td>31.4</td>
</tr>
<tr>
<td>Other Hospitals (n = 27)</td>
<td>22.2</td>
</tr>
<tr>
<td>GP (n = 129)</td>
<td>0</td>
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</table>

- Female carriers (58%)
- Median age: 73 years
- Highest incidence multi-bedded diabetic ward
Aims of current project

• To conduct an epidemiological study of this population of clinical isolates

• To investigate for indications of clonal spread among the isolates
# Materials and Methods

<table>
<thead>
<tr>
<th>Manual re-extraction of DNA from 67 overnight cultures of VRE</th>
<th>Omega® E.N.Z.A. Bacterial DNA Extraction Kit</th>
</tr>
</thead>
</table>
| Primer selection and optimisation of protocol for Random Amplified Polymorphic DNA (RAPD) PCR analysis. | **OPL-07**, *AB106, AB111, P1245*,  
*ISIS-R, ISII16F, OPE-06, OPK-11 and OPK-12*  
Annealing temperature: 35°C |
| Fingerprinting by RAPD analysis of the entire collection of strains.  
(Compares favourably with gold standard) | • 1.5% agarose gel, 90 V, 1X TAE  
• Sybr®Safe DNA gel stain, Midori Green nucleic acid stain  
• Techne Prime Thermal Cycler |
| Construction of a phylogenetic tree | PyElph 1.4 Software |
| Investigation for presence of Clonal Complex 17 | PCR-based detection of insertion sequence 16 |
## Results (I)

**Validation of primers for use in fingerprinting**

<table>
<thead>
<tr>
<th>PRIMER</th>
<th>EFFICACY i.e. NO. OF BANDS GENERATED</th>
<th>BAND PATTERN</th>
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Results (II)

RAPD PCR fingerprinting using OPL-07: appearance on gel electrophoresis \(^4\)
Two most prevalent types: II and XIV

PyElph 1.4. analysis: band matching
Results (III)

**RAPD Fingerprint Analysis**

- 18 types determined on the basis of single band difference
- Reproducible & differentiated types
- Type II (n = 15) most prevalent, type XIV (n = 12) second most prevalent

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<th>V</th>
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**Total**

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</thead>
</table>

Cork University Hospital

Instituto Tecnológico de Chorcai
Results (IV)

Phylogenetic Analysis using Neighbour Joining on PyElph 1.4 software [5]
Results (V)

Determination of Clonal Complex 17 among the collection of isolates using PCR detection of synonymous Insertion Sequence 16 (IS16)
Subsequent Investigations

- IS16 PCR-based DNA testing of the remaining isolates (conducted by Shauna Keane), to support the hypothesis of the universal presence of CC17.
- Sequencing of the 547 bp product as a positive control for the method
- BLASTn analysis

Table: Sequences producing significant alignments:

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<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
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IS16 Sequence

Enterococcus faecalis insertion sequence IS16 putative transposase (tnp) gene, complete cds
Sequence ID: gbJU35366.1|EFU35366 Length: 1466 Number of Matches: 1

Range 1: 343 to 833

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<td>491/491(100%)</td>
<td>0/491(0%)</td>
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Query 1
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Sbjct 833
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Query 61
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Sbjct 773
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Sbjct 713
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Query 181
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Sbjct 653
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Query 241
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Sbjct 593
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Sbjct 533
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Query 361
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Sbjct 473
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Query 421
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Sbjct 413
GGTTCCTCTCGCTCTGCATCTTTTCTCAAGATAGATAGGACTAACTATACTGGTCCTCTGTT

Query 481
CGTGGAAACATG

Sbjct 353
CGTGGAAACATG

CUH
Ospedal Ollscoill Chorcaí
Cork University Hospital

CORK INSTITUTE OF TECHNOLOGY
INSTITUTO TECNOLOGICO CHORKAI
Conclusion

• Epidemiological fingerprinting of the 67 VRE isolates demonstrated considerable heterogeneity (18 types)
• High incidence of BSI at CUH not linked strongly to clonal dissemination
• CC17 detected in all VRE hospital samples (CUH and others) suggests gut related horizontal gene transfer & environmental reservoirs
• Epidemiological typing of VRE of questionable use in this case, regardless of selected typing method
• Suggests a role for the detection of specific virulence or resistance genes, or mobile genetic elements, as a first line screening tool, prior to microbe identification or epidemiological typing
Further Considerations

- Antibiotic usage – conferring a selective pressure on microbes
- IS16 associated with a survivalist advantage: enhanced genome plasticity
- This study supports the hypothesis that the acquisition of IS16 (and therefore CC17) contributes to ecological success
- Risk of spread of resistance determinants to MRSA

Finally:
- This project generated novel findings that suggest that epidemiological typing results should be interpreted with caution
- Further applied research to combat VRE spread in hospitals may save lives
Vancomycin-resistant enterococci carriage in an acute Irish hospital

E. Whelton, C. Lynch, B. O’Reilly, G.D. Corcoran, B. Cryan, S.M. Keane, R.D. Sleator, B. Lucey

Department of Medical Microbiology, Cork University Hospital, Cork, Ireland
Department of Biological Sciences, Cork Institute of Technology, Cork, Ireland
Department of Pathology, Cork University Hospital, Cork, Ireland

SUMMARY

Background: Ireland has been shown to have the highest rate of vancomycin-resistant enterococci (VRE) in cases of bacteraemia in Europe, according to a report in 2014 from the European Antimicrobial Resistance Surveillance System Network.

Aim: To investigate the prevalence of VRE gut colonization in a cohort of patients in 2014 at Cork University Hospital (CUH) by performing a cross-sectional study using faecal samples submitted to the microbiology laboratory for routine investigation from both hospital inpatients and community-based patients.

Methods: Faeces were examined for VRE colonization using selective cultivation, antimicrobial susceptibility testing, and speciation using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. All VRE isolates were evaluated by molecular means for resistance determinants, type, and Insertion Sequence 16 as an indicator of Clonal Complex 17 (CC17).

Findings: From the 350 specimens investigated, 67 (19.1%) specimens were positive for VRE [95% confidence interval (CI): 15.0–23.2]. The prevalence of VRE colonization among CUH patients tested in this study (N = 194) was 31.4% (95% CI: 24.7–38.1). By contrast, the general practitioner patient samples (N = 29) showed a prevalence of 0%, whereas 22.2% of samples from other hospitals (N = 27) were positive for VRE. All isolates were Enterococcus faecium (VREFm) and were indicated to contain CC17, though with considerable heterogeneity among the isolates.

Conclusion: This high prevalence goes some way towards providing an explanation for the current high rates of VRE bacteraemia in Ireland, as well as highlighting the benefits of screening and enhanced infection control practices by all hospitals to control the high rates of VRE observed.

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References

  http://dx.doi.org/10.1016/j.jhin.2016.03.005
  http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3299638/
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• Brendan O’ Reilly (Laboratory Manager, CUH) and Eddie McCullagh (Medical Scientist, CUH) for provision of updated BSI VRE statistics