Linezolid (and phenicol) resistance (*optrA*) in *Enterococcus*

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Linezolid and Florfenicol

https://en.wikipedia.org/wiki/Linezolid

https://en.wikipedia.org/wiki/Florfenicol
Resistance to Oxazolidones (Linezolid, tedizolid)

• Mutations
  – 23S rDNA (G2576T)
  – mutations in ribosomal proteins L3, L4 or L22
    encoded by *rplC*, *rplD* and *rplV*, respectively

• Transferrable genes
  – *Cfr* and *cfrB* – encode methyl transferases – resistance to phenicols, oxazolidones, lincosamides, streptogramin A and pleuromutilins
  – *optrA* – ABC transporter – active efflux of oxazolidones and phenicols
**optrA**

- Encodes an ABC transporter mediating active efflux
- Reduces susceptibility to: oxazolidones (linezolid and tedizolid) and phenicols (chloramphenicol and florfenicol)
- Found first in China by Wang et al in 2015, quite widespread in Chinese strains from human and animal origin
- Found in Enterococci: *E. faecium* and *E. faecalis* but also in Staphylococci
- Found since then in Europe, Asia and South America
- Found in isolates from humans and animals as well as from food products and soils
**Colombia study**

- **Background:**
  - Large study performed in 2011 as part of Colombian monitoring (COIPARS)
  - 40 strains linezolid resistant
  - Check testing methods (automatic MIC determination vs other MIC methods)
  - Check at DTU - confirmed resistance in three strains
  - Nested PCR and further searches - no mutation found
  - Sequencing of three strains using NGS - no mutations or *cfr* found in 2012
  - Transfer and cloning was not attempted as they were close to breakpoint / lack time 😞

- **Unanswered question** - What is the mechanism of resistance???

- **Aha moment:** ECCMID 2016
- **Re-analyses in 2016**
Methods and results

- COIPARS screening: 556 Enterococci from poultry meat 2010-2011
- Origin Colombia, from retail stores in diverse Colombian departments

- 40 strains with linezolid results close to breakpoint - 3 strains resistant to linezolid after re-checks and submitted to WGS- 2012

- Analyses (2016):
  - Sequence data was available, re run on updated ResFinder revealed \textit{optrA} gene in all three strains
  - Set up PCR for \textit{optrA}, the same three were positive out of the 40, so the remaining 37 were negative
  - Analysis of the WGS data for plasmids, resistance genes, virulence genes and typing
  - genetic surroundings of \textit{optrA}
    - Comparison to plasmid pE349 found in China
## Results

<table>
<thead>
<tr>
<th>ID</th>
<th>Species</th>
<th>Resistances found (MIC)</th>
<th>Resistance genes found (Resfinder)</th>
<th>Efflux pump and H-M resistance genes found (RAST)</th>
<th>Virulence genes found (VirulenceFinder)</th>
<th>MLST</th>
<th>SNP analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>12E</td>
<td><em>E. faecalis</em></td>
<td>CHL, ERY, LZD, STR, TET, FFN, TZD*</td>
<td><strong>optR</strong>A, erm (B), lsa (A), tet (L), tet (K), fexA</td>
<td>copZ, cutC, corC, czeD, czrR, EF-G, TR, CIA</td>
<td>ebpA, SrtA, ebpB, cCF10, ebpC, efaAfs, tpX, gelE, cad, ace, elrA, cOB1, hylA, fsrB, hylB, camE, agg</td>
<td>59</td>
<td>0*</td>
</tr>
<tr>
<td>34E</td>
<td><em>E. faecalis</em></td>
<td>ERY, LZD, TET, TZD**</td>
<td><strong>optR</strong>A, erm (B), lsa (A), tet (L), fexA</td>
<td>copZ, cutC, corC, czeD, czrR, EF-G, TR, CIA</td>
<td>elrA, ebpA, SrtA, tpX, ebpB, cCF10, ebpC, efaAfs, gelE, cad, ace, cOB1, hylA, fsrB, hylB, camE</td>
<td>59</td>
<td>61</td>
</tr>
<tr>
<td>745E</td>
<td><em>E. faecalis</em></td>
<td>CIP, ERY, GEN, KAN, LZD, STR, TET, TZD*</td>
<td>aph (3’)-III, aac(6’)-Iapl, <strong>optR</strong>A, lnu (B), lsa (A), tet (L), fexA</td>
<td>copZ, cutC, corC, czeD, czrR, EF-G, TR, CIA</td>
<td>elrA, ebpA, tpX, ebpB, cCF10, ebpC, efaAfs, gelE, cad, ace, cOB1, hylA, fsrB, hylB, camE, SrtA</td>
<td>489</td>
<td>12242</td>
</tr>
</tbody>
</table>
• Plasmid comparisons
  - Large similarity between the three plasmids and the plasmid from Wang et al.

• *optrA* gene sequence
  - Two *optrA* genes (12E and 745E) identical to previously described sequence
  - The sequence of the *optrA* in E34 was found slightly different from the others
Conclusions

• First finding in South America- gene more widespread than previously thought

• Poultry meat at retail implicated – origin??

• New resistance to important antimicrobial class so there was need to alert WHO and local authorities

• Monitoring needed to continuously see the picture development
Key learning points and Future perspectives

- We learned that:
  - WGS data storage will help to solve old cases
  - Data analysis will evolve and some times when there are new findings data need to be reanalysed

- Future Perspectives:
  - optrA found in meat- other reservoirs?
  - Potential for spread is likely- similar plasmid as in China (travelled a lot or is widespread, why?)
  - Virulence genes present- do these strains have potential to be pathogenic?
  - Closely related , but also more distant strains harbouring similar plasmid- spread mediated by clones or plasmids or both?
Denmark project (awaiting publication)

- Background- findings of linezolid resistance in one instance without finding of resistance cause. optrA found in Colombian isolates prompted alert.

- Database retrospective screening (DANMAP data)
  - 12 650 data entries checked for MIC of linezolid tested between 2002 and 2016
  - Sequencing suspected strains

- Resfinder update and Genome screening (566 genomes)
  - Diverse Gram positive species and project- genomes kept at DTU- no positives found
Methods and results

• Methods
  – Strains 12650 strains (5,605 *E. faecium* and 7,045 *E. faecalis* isolated from 2002-2016)
  – Sequencing of resistant strains found (MIC >4 mg/L)
  – Analysis using genomic tools

• Results
  – 5 strains above ECOFF linezolid (MIC=8mg/L)
  – two oldest strains (2006) unavailable

<table>
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<tr>
<th>ID</th>
<th>Species</th>
<th>Origin</th>
<th>Resistances found (MIC)</th>
<th>Resistance genes found (Resfinder)</th>
<th>Plasmids found (Plasmid finder)</th>
<th>MLST</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012-70-467-7</td>
<td><em>E. faecium</em></td>
<td>Imported turkey meat</td>
<td>CHL, CIP, ERY, LZD, FFN, STR, TZD*</td>
<td><em>aph(3')-III</em>, <em>aadE</em>, <em>spc</em>, <em>msr(C)</em>, <em>erm (B)</em>, <em>lnu (B)</em>, <em>optrA</em></td>
<td>Rep2, rep14</td>
<td>ST-22</td>
</tr>
<tr>
<td>2013-70-487-9</td>
<td><em>E. faecium</em></td>
<td>Imported broiler meat</td>
<td>AMP, LZD, TET</td>
<td><em>spc</em>, <em>msrC</em>, <em>tet(L)</em>, <em>tet(M)</em></td>
<td>Rep22, repUS15</td>
<td>ST-873</td>
</tr>
<tr>
<td>15090820</td>
<td><em>E. faecalis</em></td>
<td>Danish veal</td>
<td>ERY, FFN, LZD, TET, TZD*</td>
<td><em>str</em>, <em>lsa(A)</em>, <em>erm(B)</em>, <em>optrA</em>, <em>fexA</em>, <em>tet(M)</em>, <em>tet (L)</em></td>
<td>Rep7, rep9</td>
<td>ST-22</td>
</tr>
</tbody>
</table>
Genetic location of $optRA$

- **The *E. faecalis*** isolate from veal had a sequence similar to plasmid pE349 containing $optRA$ and $fexA$ (phenicol resistance)

- **The *E. faecium*** isolate matched the genetic surrounding of *E. faecalis* E016 described by He *et al.* 2016- chromosomal?
Key learning points and future perspectives

• We learned that:
  – Re-analysing phenotypical data might be needed sometimes when new resistances come up or cut-offs change
  – WGS data storage might help
  – Even in low-use countries we still find emerging resistances if we look for them

• Future perspectives:
  – Third strain – which mechanism causes the phenotype??
  – Linezolid resistance is on the rare/unusual resistance list and we should follow up on resistance mechanisms
  – Origin of the resistant strains or plasmids?
  – Different species carrying *optrA*, transfer is likely however the genetic environment of one strain indicate it might be located on the chromosome while the other is on a plasmid
General Discussion

• This resistance might be relatively widespread
  – Geographical dispersion seems global
  – Different reservoirs (different bacterial species, human, animal, food and environmental)

• Findings show optrA carried in different plasmids and even on chromosome in different bacterial species and types

• Found associated on plasmids with cfr genes

• Potential for transfer to human clones or even other pathogens

• Potential consequences in treatment of serious MDR infections (Staphylococci, MRSA and Enterococci)

• One Health approach needed

• Prudent use of drugs that might select (phenicols) or co-select
Recommendations for the laboratories

- Include linezolid and phenicols in Gram positive panels
- Include linezolid resistance in the unusual resistance lists to follow up on each case
- Consider to do a retrospective database check
- Please note the MIC can be close to breakpoint
- In case it is detected, act on it!
- Alert national and international authorities as it is a critical resistance, communicate with human sector on data sharing
- EURL can help in the characterization/confirmation
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