

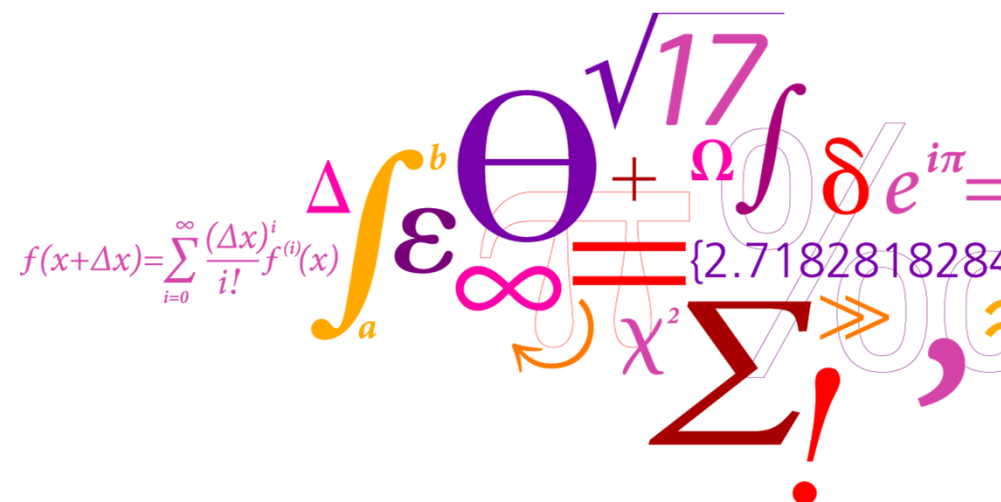


# Linezolid (and phenicol) resistance (*optrA*) in Enterococcus

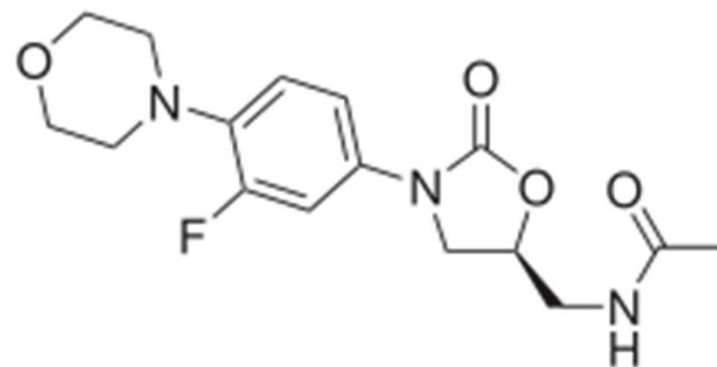
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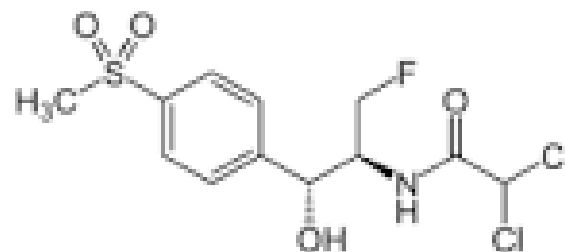
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# Linezolid and Florfenicol



<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 *cfz* 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

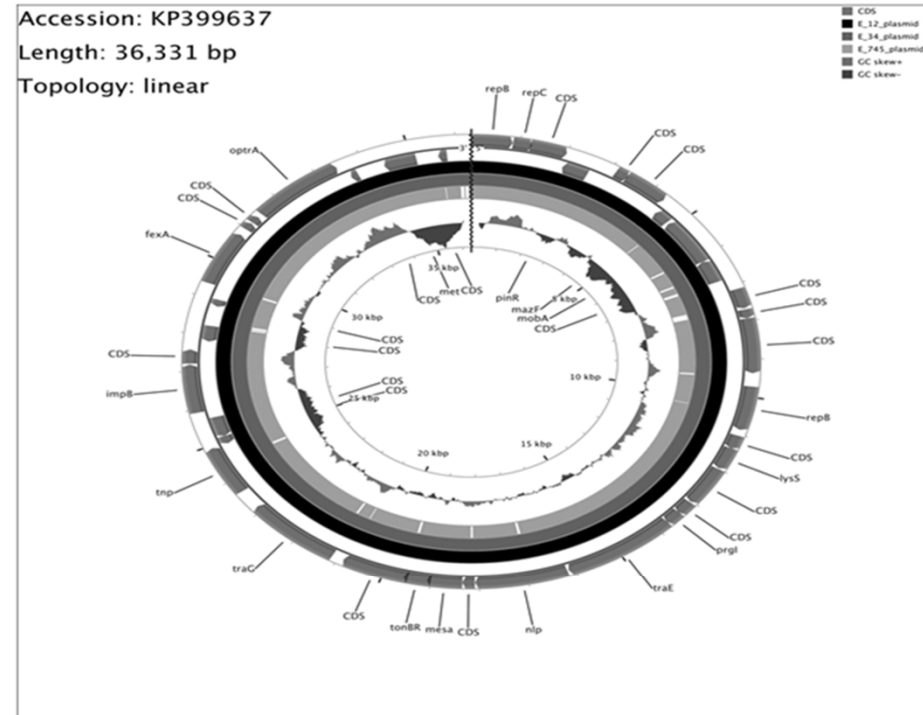
- COIPARS screening: 556 Enterococci from poultry meat 2010-2011
- Origin Colombia, from retail stores in diverse Colombian departments
  
- 40 strains with linezolid results close to breakpoint - 3 strains resistant to linezolid after re-checks and submitted to WGS- 2012
  
- Analyses (2016):
  - Sequence data was available, re run on updated ResFinder revealed *optrA* gene in all three strains
  - Set up PCR for *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 *optrA*
    - Comparison to plasmid pE349 found in China

# Results

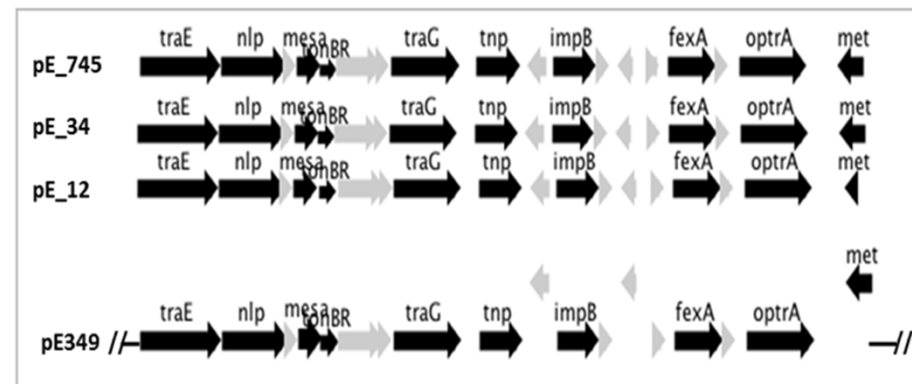
ID	Species	Resistances found (MIC)	Resistance genes found (Resfinder)	Efflux pump and H-M resistance genes found (RAST)	Virulence genes found (VirulenceFinder)	MLST	SNP analysis
12E	<i>E. faecalis</i>	CHL, ERY, LZD, STR, TET, FFN, TZD*	<i>optrA</i> , <i>erm (B)</i> , <i>lsa (A)</i> , <i>tet (L)</i> , <i>tet (K)</i> , <i>fexA</i>	<i>copZ</i> , <i>cutC</i> , <i>corC</i> , <i>czcD</i> , <i>czrR</i> , <i>EF-G</i> , <i>TR</i> , <i>CIA</i>	<i>ebpA</i> , <i>SrtA</i> , <i>ebpB</i> , <i>cCF10</i> , <i>ebpC</i> , <i>efaAfs</i> , <i>tpx</i> , <i>gelE</i> , <i>cad</i> , <i>ace</i> , <i>elrA</i> , <i>cOB1</i> , <i>hyla</i> , <i>fsrB</i> , <i>hylB</i> , <i>camE</i> , <i>agg</i>	ST59	0*
34E	<i>E. faecalis</i>	ERY, LZD, TET, TZD**	<i>optrA</i> , <i>erm (B)</i> , <i>lsa (A)</i> , <i>tet (L)</i> , <i>fexA</i>	<i>copZ</i> , <i>cutC</i> , <i>corC</i> , <i>czcD</i> , <i>czrR</i> , <i>EF-G</i> , <i>TR</i> , <i>CIA</i>	<i>elrA</i> , <i>ebpA</i> , <i>SrtA</i> , <i>tpx</i> , <i>ebpB</i> , <i>cCF10</i> , <i>ebpC</i> , <i>efaAfs</i> , <i>gelE</i> , <i>cad</i> , <i>ace</i> , <i>cOB1</i> , <i>hyla</i> , <i>fsrB</i> , <i>hylB</i> , <i>camE</i>	ST59	61
745E	<i>E. faecalis</i>	CIP, ERY, GEN, KAN, LZD, STR, TET, TZD*	<i>aph (3')-III</i> , <i>aac(6')apl</i> , <i>optrA</i> , <i>lnu (B)</i> , <i>lsa (A)</i> , <i>tet (L)</i> , <i>fexA</i>	<i>copZ</i> , <i>cutC</i> , <i>corC</i> , <i>czcD</i> , <i>czrR</i> , <i>EF-G</i> , <i>TR</i> , <i>CIA</i>	<i>elrA</i> , <i>ebpA</i> , <i>tpx</i> , <i>ebpB</i> , <i>cCF10</i> , <i>ebpC</i> , <i>efaAfs</i> , <i>gelE</i> , <i>cad</i> , <i>ace</i> , <i>cOB1</i> , <i>hyla</i> , <i>fsrB</i> , <i>hylB</i> , <i>camE</i> , <i>SrtA</i>	ST489	12242

- 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

a)



b)





## 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

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

## 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 *cfp* 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



## Acknowledgements

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