

Assessment of the spread of florfenicol resistant enterococci harboring oxazolidinone resistance genes – a “One Health Approach”

Background:

Linezolid is a highly effective last-resort antibiotic used for the treatment of infections caused by multi-drug resistant Gram-positive pathogens such as vancomycin-resistant enterococci. Linezolid belongs to the oxazolidinone antibiotics, which inhibit protein synthesis by binding to the 23S ribosomal RNA of the 50S subunit. Linezolid resistance mechanisms include mutations in the 23S rRNA binding site, mutations in the genes encoding ribosomal proteins L3, L4, or L22, and the acquisition of the transferable genes *cfr*, *optrA*, or *poxxA*.

WP1: Faecal carriage of enterococci harbouring oxazolidinone resistance genes in the community in Switzerland

Hypothesis: Healthy humans are already carriers of cfr⁺, optrA⁺ and poxA⁺ enterococci. If so, might there be a foodborne link?

paper is submitted

WP2: Dissemination of florfenicol resistant enterococci harboring oxazolidinone resistance genes in the environment

Hypothesis: The aquatic ecosystem contributes to the dissemination of linezolid-resistant enterococci.

River water

Ten florfenicol resistant *Enterococcus* spp. isolates recovered in 2020 from streams of different regions in Switzerland were genetically characterized in this study. All isolates underwent short-read sequencing (Illumina MiniSeq), and seven isolates with suspected plasmid-encoded linezolid-resistance determinants additionally underwent long-read sequencing (MinION, ONT). The 10 florfenicol-resistant enterococci isolates all carried phenicol-oxazolidinone-resistance genes, i.e., *optrA* (3x *E. faecalis*, 2x *E. faecium*, 2x *E. hirae*, 1x *E. raffinosus*), *poxxA* (1x *E. faecium*), or both (1x *E. faecium*). Most isolates were also non-susceptible to linezolid. In most genomes, *optrA* and *poxxA* were embedded in transposition units integrated into plasmids or into the chromosomal *radC*. For the first time a chromosomally integrated *optrA* in an *Enterococcus raffinosus* isolate is described.

Conclusion: Our findings suggest that the aquatic ecosystem contributes to the dissemination of linezolid-resistant enterococci which is a worrisome aspect from a public health perspective.

- Biggel et al. (2021). Genetic context of *optrA* and *poxxA* in florfenicol-resistant enterococci isolated from flowing surface water in Switzerland. *Antimicrobial Agents and Chemotherapy* 65:e01083-21. <https://doi.org/10.1128/AAC.01083-21>
- Nüesch-Inderbilen et al. (2021), M. Linezolid-resistant *Enterococcus faecalis* ST16 harbouring *optrA* on a Tn6674-like element isolated from surface water. *Journal of Global Antimicrobial Resistance* 25, 89–92. <https://doi.org/10.1016/j.jgar.2021.02.029>

WP3: Farm animals as a potential reservoir of florfenicol resistant enterococci harboring oxazolidinone resistance genes in Switzerland

Hypothesis: The use of florfenicol and other antibiotics in farm animals may co-select enterococci that carry acquired transferable resistance genes which confer resistance to linezolid.

Fattening pigs at slaughter

A total of 31 florfenicol-resistant enterococcal isolates were obtained from 27 (5%) of 565 cecal samples of fattening pigs from seven (11%) of 62 farms. Screening by PCR for the presence of the oxazolidinone resistance genes revealed the presence of *cfr/poxxA* in 1/31, *optrA* in 15/31 and *poxxA* in 15/31 enterococcal isolates, respectively. WGS analysis of ten selected isolates (one *Enterococcus* species per herd) showed the presence of *E. faecalis* (n=1), *E. faecium* (n=1), and *E. hirae* (n=1), harboring *optrA18*, *optrA7* and a new *optrA* allele, respectively. *E. durans* (n=1), *E. faecium* (n=4), and *E. hirae* (n=1), carried the wild-type *poxxA*, and *E. faecalis* (n=1) co-harbored *cfr(D)* and *poxxA2*. With the exception of *optrA7*, all oxazolidinone resistance genes were found on plasmids. MLST analysis identified *E. faecalis* ST19 and ST376, *E. faecium* ST80 belonging to hospital-adapted clade A1, and *E. faecium* ST21, ST55, ST269 and ST416, belonging to clade A2 which represents human commensals and animal strains.

Conclusion: The occurrence of oxazolidinone resistance determinants in porcine enterococci including genotypes belonging to major human pathogens is concerning. Cross-selection through the use of florfenicol in animal farming has emerged as a problem that needs to be addressed in order to mitigate the dissemination to humans, especially through the food chain.

- Nüesch-Inderbilen et al. (2022). Fattening pigs are a reservoir of florfenicol resistant enterococci harboring oxazolidinone resistance genes. Journal of Food Protection, accepted 22.2.2022

Cattle at slaughter

In total, 618 fecal samples from beef cattle (258 from veal calves and 360 from adult beef) were collected at slaughterhouse level. The prevalence of florfenicol resistant Enterococci was 2% in adult beef cattle and 34% in veal calves. The differences in prevalence and the occurrence of herd clustering of FRE harboring samples assume that risk factors are involved. *E. faecalis* (77%), *E. faecium* (19%) and other *Enterococcus* species (4%), namely *E. durans*, *E. gallinarum* and *E. dispar*, were detected. Every florfenicol resistant *Enterococcus* isolate harbored at least one of the three oxazolidinone resistance genes *optrA*, *poxxA* and *cfr*.

Conclusion: The high occurrence of oxazolidinone resistance determinants in enterococci from veal calves is worrisome. Co-selection through the use of florfenicol in animal farming has emerged as a problem that needs to be addressed in order to mitigate the dissemination to humans, especially through the food chain.

- Haussmann, A. (2021). vet. med. master thesis

The further genetic characterization of the strains from this master thesis is part of an ongoing WGS based study...

Poultry at slaughter

Project is ongoing...

WP4: Potential spread of florfenicol resistant enterococci harboring oxazolidinone resistance genes along the foodchain

Hypothesis: Ready-to-eat products can be vectors of florfenicol resistant enterococci harboring oxazolidinone resistance genes. However, due to a lack of data a risk assessment for different food products is not yet possible.

Swiss alpine hard cheese made with unpasteurized cow's milk

The aim of this study was to provide further microbiological data for Swiss alpine hard cheese made using raw cow's milk. A total of 100 cheese samples were collected between July 2021 and January 2022 from dairies, cheese shops, supermarkets or directly on alps throughout Switzerland and were tested for the occurrence of foodborne pathogens (*Salmonella*, *L. monocytogenes*, STEC). Since raw milk can also introduce antimicrobial resistant bacteria in the cheese making process, all samples were further tested for the occurrence of florfenicol-resistant enterococci harbouring transferable resistance genes *cfr*, *optrA*, and/or *poxxA*. Florfenicol-resistant enterococci were not detected in any of the 100 cheese samples.

Conclusion: Swiss alpine hard cheese does not represent a relevant risk for the transfer of florfenicol resistant enterococci harboring oxazolidinone resistance genes along the foodchain.

- Beth, A. (2022). vet. med. master thesis

Further projects on different other food products are ongoing....