LIN-RES project: Genomic analysis of linezolid resistance in strains isolated from healthy animals and human patients.

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Linezolid is a critically important antibiotic (AB) to fight human infections caused by multi-resistant Gram-positive bacteria such as MRSA and VRE. Linezolid resistance can be caused by point mutations in the 23SrRNA gene and by plasmid-borne genes : *cfr*, *optrA* and *poxtA*. While linezolid is not licenced for use in food-producing animals, linezolid resistant (LZD-R) isolates were retrieved from food-producing animals in different European countries including Belgium. In this context, the project aims to assess the occurrence of linezolid resistance in enterococci and staphylococci in 2019 in Belgium with a selective culture method. Another endpoint of the project is to investigate the genetic environment associated with the LZD-R phenotype and the relatedness of the isolates.

METHODS

Healthy animal faecal samples ((broilers n=295), turkeys (n=86), laying hens (n=205), breeding poultry (n=163), veal calves (n=293) and pigs (n=283)) and sows and fattening pigs nasal swabs samples (n=78 and 70 respectively) were collected during 2019 through the official Belgian monitoring of antimicrobial resistance for enterococci and staphylococci and subcultivated on blood agar plates supplemented with linezolid (4mg/L) for 44h to 48h at 37°C. Two colonies of each positive samples were isolated and identified by MALDI-TOF. For each sample, one isolate per bacterial species was conserved and the linezolid resistance was quantified by broth microdilution (Sensititre®). Three MRSA from previous monitoring and isolates recovered from human samples (4 enterococci and 1 MRSA) were added to the collection. Whole genome sequencing was performed on all the isolates to analyse the genetic organisation surrounding LZD-R genes and for further relatedness analysis.

RESULTS

Linezolid resistance occurrence was ranging in faecal samples from 0% in turkeys to 16.4% in veal calves and reaching up to 25.7% in nasal samples from fattening pigs, all bacterial species combined.
WGS and Resfinder results showed that LZD-R staphylococci (n=7) carried only the *cfr* gene (n=7) while the three genes were found in LZD-R enterococci (n=143): *cfr* (n=3), *poxtA* (n=36) and *optrA* (n=124). One *poxtA* was observed in a *Pediococcus pentosaceus*. All LZD-R isolates lacking LZD-R genes (n=4) contained 23S rRNA mutation. *poxtA* was not observed in the investigated human isolates. WGS results showed that *cfr* and *optrA* were often associated with *fexA* gene (resistance to phenicols) and that *poxtA* was associated with *fexB* (according to reads mapping analysis) (figure). Investigation of genetic organisation revealed 4 different organisations for *cfr*, 20 for *optrA* and one for *poxtA*. No differences were observed between animal and human isolates regarding the genetic organisations. In all cases, several resistance genes were carried by the isolates and in most cases, contigs carrying LZD-R genes carried also other resistance genes (mostly resistance genes to phenicols and/or macrolides and/or aminoglycosides).

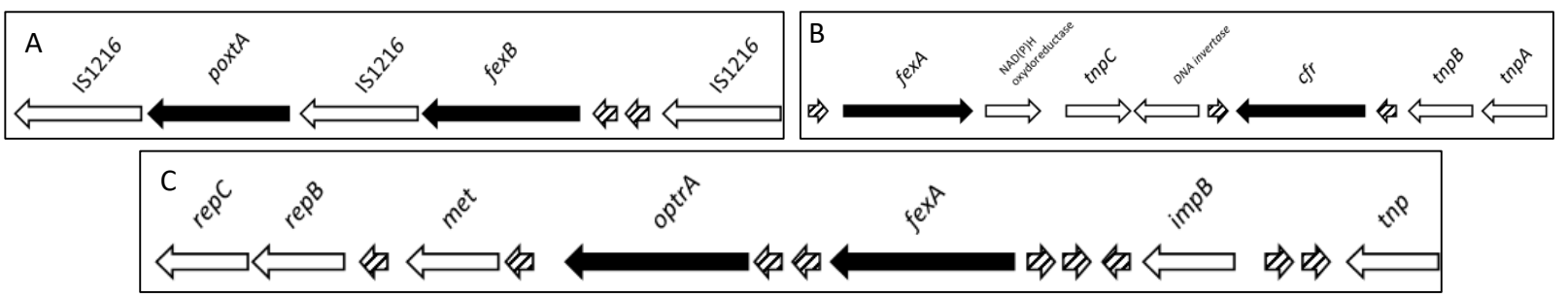

Figure: most observed organisation of (A) *poxtA* (n=35/37), (B) *cfr* (n=7/10) and (C) *optrA* (n=24/124)

MLST analysis was performed on the *Staphylococcus aureus* (n=6), *Enterococcus faecalis* (n=80) and *Enterococcus faecium* (n=48) isolates. Only one sequence type (ST) was observed for *S. aureus*, ST398. Regarding enterococci, 28 and 32 different STs were observed for *E. faecalis* and *E. faecium* respectively including new STs for both species.

CONCLUSION AND PERSPECTIVES

Linezolid resistance is not so rare in animal isolates, revealing a reservoir of plasmid-borne LZD-R genes in the agricultural sector. Moreover, the majority of the isolates carried also phenicol resistance genes. Several different organisations and sequence types were observed in the isolates collection. These observations revealed a large diversity of linezolid resistant isolates and a reservoir of bacteria susceptible to share linezolid resistance genes that could be cross-selected by other antibiotics including phenicols. Further investigation of relatedness between the isolates will be done through cgMLST and phylogenomic analysis. Conjugation assays will be done to investigate the transferability of the observed linezolid resistance genes.

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