D-PhD02-2.2: Poster presentation at an international conference of the first results obtained from NGS analysis of linezolid-resistant strains.

WP2- Next Generation Sequencing

Responsible Partner: E27 P04

Contributing partners: E27 P09 and E27 P01

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Dissemination *Author's suggestion to inform the following possible interested parties.*	OHEJP WP 1 ☐ OHEJP WP 2 ☐ OHEJP WP 3 ☐ OHEJP WP 4 ☐ OHEJP WP 5 ☐ OHEJP WP 6 ☐ OHEJP WP 7 ☐ Project Management Team ☐ Communication Team ☐ Scientific Steering Board ☐ National Stakeholders/Program Owners Committee ☐ EFSA ☐ ECDC ☐ EEA ☐ EMA ☐ FAO ☐ WHO ☐ OIE ☐ Other international stakeholder(s): …… Social Media: ..... **Other recipient(s): Confidential, will be disseminated through a research paper publication (under writing)**.....

D-PHD02-2.2: POSTER PRESENTATION AT AN INTERNATIONAL CONFERENCE OF THE FIRST RESULTS OBTAINED FROM NGS ANALYSIS OF LINEZOLID-RESISTANT STRAINS (PRESENTED AT THE OH-EJP ASM 2019 IN DUBLIN)

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Project "LIN-RES": Molecular Basis, Origin, Transferability and Risk Factors Associated with Linezolid-Resistance in Gram-Positive Bacteria of Human and Animal Origin.

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Linezolid belongs to the oxazolidinone family of antimicrobials and is one of the last resort drug used to fight human infections caused by multi-resistant Gram-positive bacteria such as Staphylococci and Enterococci. It is commercially available since 2000 and has not been licensed for use in animals. Outside of clinical care settings, resistance to Linezolid is rare and attributable to a transferable 23SrRNA methylase encoded by the *cfr* gene (for Chloramphenicol Florfenicol Resistance). Two other genes confer resistance to linezolid, *optrA* and *poxtA* and code respectively for an ABC protein family and for an ARE ABC-F family protein. In this work, we investigated Livestock-associated Gram positive (staphylococcus and enterococcus) and strains isolated from human samples characterized by a Linezolid Minimal Inhibitory Concentration (LZD-MIC) >4 µg ml-1.

METHODS

Animal MRSA (Methicilin Resistant *Staphylococcus aureus*) strains (n=31) were isolated from pools of nasal swabs taken from healthy Belgian pigs sampled at farm in 2019. Animal enterococcus strains (n=198) were isolated from pigs, bovines or poultry faeces/caecal content on Slanetz and Bartley plates in 2019 in Belgium. Strains were then isolated on Columbia Sheep Blood plates supplemented with linezolid at 4µg ml-1. More strains were collected via collaborations or came from previous monitoring campaigns. Susceptibility to linezolid is determine by the micro dilution method (Sensititre®). Resistant isolates were sequenced by Illumina MiSeq sequencing to determine which gene is responsible of the linezolid resistance and to analyze the genetic environment of these genes.

RESULTS

Isolates	Isolation year	MIC LZD	species	Animal origin
MSA-06488-1	2016	8	*S. aureus*	Pig
MSA-08895-1	2016	8	*S. aureus*	Pig
36656	2018	4	*E. faecium*	Human
36079	2017	8	*E. faecalis*	Human
S421	2015	16	*S. aureus*	Human
U1901103	2019	16	*E. faecalis*	Bovine
U1901112	2019	16	*E. faecalis*	Poultry
U1903107	2019	8	*E. faecium*	Pig

Table 1: Minimal Inhibitory Concentrations (µg ml⁻¹) determined for linezolid by Sensititer. MICs scoring above ECOFF are in red.

Within the animal samples collected since January 2019 (n= 229) in Belgium, 3 (1.31%) contains strains resistant to linezolid according to the epidemiological cut-off recommended by EUCAST (ECOFF = 4µg ml^{-1}). The origin of the strains are multiple as shown in table 1 and all of them originated from healthy animals. The 3 Staphylococcus aureus strains belong to the clonal complex 398 and spa type t011 which is very common in livestock. One of the MRSA was isolated from a human farmer clinical case from Belgium. According to table 1, one of the strains is sensitive to linezolid but grown on medium supplemented with linezolid 4µg ml^{-1} suggesting a MIC close to the ECOFF value. Five of the 8 strains have been sequenced and all of the five strains carried a resistance gene against oxazolidinone, *cfr* or *optrA* (table 2). Interestingly, *cfr* was found in our study in Staphylococcus strains and *optrA* in Enterococcus strains. This observation fits with previous publications where *cfr* was mostly present in Staphylococcus and *optrA* mostly in enterococcus. In both cases, *fexA* was associated with the *cfr/optrA* genes suggesting a co-selection.

Resistance gene	Contigs in isolate 36656		Contigs in isolates 36079		Contigs in isolate S421		Contig in isolate MSA-06488-1		Contig in isolate MSA-08895-1		Res phenotype	Accession number
	%identity	Contig number	%identity	Contig number	%identity	Contig number	%identity	Contig number	%identity	Contig number		
optrA	100	NODE_16_length_53811_cov_22.6569	100	NODE_28_length_18559_cov_22.4199	-	-	-	-	-	-	Oxazolidinone, Phenicol	KT862781
cfr	-	-	-	-	100	NODE_50_length_11230_cov_23.3777	100	NODE_26_length_36288_cov_13.2848	100	NODE_26_length_35972_cov_45.2769	Phenicols, Lincosamides, Oxazolidinones, Pleuromutilins, Streptogramin A	AM408573
fexA	99.65	NODE_16_length_53811_cov_22.6569	99.65	NODE_28_length_18559_cov_22.4199	100	NODE_50_length_11230_cov_23.3777	99.72	NODE_26_length_36288_cov_13.2848	99.72	NODE_26_length_35972_cov_45.2769	Phenicol	AJ549214

Table 2: Antimicrobial resistance genes found in the genome of 5 of the 8 LZD-RES strains from the table 1: expected phenotype and genome mapping positions.

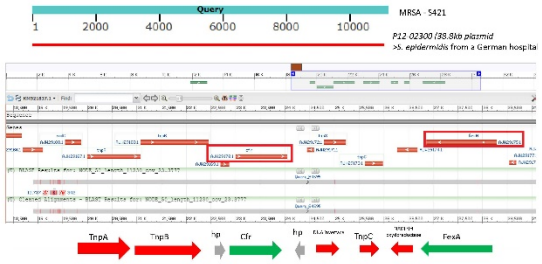

Figure: ORF alignment and structural organisation of the highly conserved *cfr*-containing DNA region identified in one of the 3 LZD-RES MRSAs. The schematic representation is the same for the 3 MRSA strains.

The 3 MRSA strains have the same genetic organization. Indeed, *cfr* was found associated with *fexA* and surrounded by transposon elements. The contigs containing *cfr* and *fexA* hit with a plasmid of 38.8Kb isolated from a *S. epidermidis* from a German hospital. The 3 contigs containing *cfr* have more than 98% of identities with the common part of this plasmid. This association of *cfr* and *fexA* is found in multiple Staphylococcus species from different geographical regions of the globe. The transferability of this element containing *cfr* and *fexA* still need to be proved experimentally.

The genetic environment of the Enterococci still need to be analyzed to know which element are present around the *optrA* and *fexA* genes but the first observations showed a Tnp associated element (data not shown). More analysis are necessary to analyze the genetic environment of the Enterococci strains.

CONCLUSION AND PERSPECTIVES

The data presented here demonstrate the co-occurrence of *cfr* or *optrA* and *fexA*. The *cfr/fexA* element is carried on a conserved transposon-like element carried by different plasmids hosted in various Staphylococci species isolated from both animal and human isolates. The *optrA/fexA* element is present in the 2 Enterococci sequenced strains. Further analysis will be done on the other strains to investigate the observed resistances. The presence of linezolid resistance in animal strains is worrying as this antibiotic is not licensed for animal use and is a last resort drug for human infections. This observation and the fact that *fexA* is found associated with *cfr* and *optrA* suggest a co-selection of these genes, potentially due to the use of phenicols. The transferability of these elements will be analyzed in the future.

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