

Detection of linezolid-resistant coagulase-negative staphylococci carrying *cfr* gene and PVL-positive *S. simulans* in environmental samples

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Background

Linezolid is an important therapeutic option for the treatment of infections caused by multidrug-resistant (MDR) pathogens, including methicillin-resistant coagulase-negative staphylococci (MRCoNS)¹. The *cfr* gene mediates transferable resistance to linezolid and it also confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A antimicrobials. Moreover, the Pantan-Valentine leucocidin (PVL) is the most important toxin produced by *Staphylococcus aureus*, but there are few reports describing PVL-producing coagulase-negative staphylococci (CoNS)².

Objective: To analyze the diversity of CoNS in environmental samples of swine farms, to characterize the antimicrobial resistance phenotype/genotype and to determine the presence of the PVL in recovered isolates.

Methodology

Sampling and staphylococci recovery: In 2015, air and slurry pound samples of two Spanish pig farms, of intensive (Farm A) and semi-extensive (Farm B) production were collected. Samples were inoculated in mannitol salt agar (MSA) plates for staphylococci recovery and the species identification was performed by MALDI-TOF³.

Antimicrobial resistance phenotype and genotype: The susceptibility for 13 antimicrobials was determined by disk-diffusion method and the Minimum Inhibitory Concentration (MIC) to linezolid was performed by E-test (EUCAST, 2017). The presence of 32 antimicrobial resistance genes was determined by PCR and sequencing. In addition, mutations in the subunit GyrA were determined by PCR and sequencing in ciprofloxacin-resistant isolates⁴.

Virulence factors: The presence of the virulence factor *lukS/F-PV* encoding the PVL was likewise investigated by PCR⁵.

References

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Aknowledgements

This work was supported by project SAF2016-76571-R from the Agencia Española de Investigación (AEI) and FEDER. L. Ruiz-Ripa has a pre-doctoral fellowship from the Universidad de La Rioja (Spain). O. M. Mama has a pre-doctoral fellowship from Mujeres por África-Universidad de La Rioja (Spain).

Results

67 CoNS isolates (thirty from Farm A and thirty-seven from Farm B) were recovered, belonging to 14 species with predominance of *S. arlettae* (n=18), *S. cohnii* (n=11), *S. sciuri* (n=9) and *S. saprophyticus* (n=5) (Figure 1). *S. arlettae* and *S. cohnii* were the species more frequently detected in the intensive and the semi-extensive farm, respectively.

Seven isolates (*S. saprophyticus*, 5; *S. haemolyticus*, 1; *S. sciuri*, 1) showed methicillin-resistance and harbored the *mecA* gene, being negative for *mecB* and *mecC* (Figure 2). Moreover, four isolates belonging to the *S. sciuri* group also carried the *mecA* gene but they did not show a methicillin-resistance phenotype. Only one isolate (*S. chromogenes*) was susceptible to all the antimicrobials tested and 70% of the strains were MDR. The percentage of resistant strains and the resistance genes detected are shown in Figure 2. Four ciprofloxacin-resistant isolates were detected, but only one *S. lentus* showed mutations in *gyrA* (S84L).

Figure 2. Percentage of resistant isolates depending on the swine farm and antimicrobial resistance genes detected.

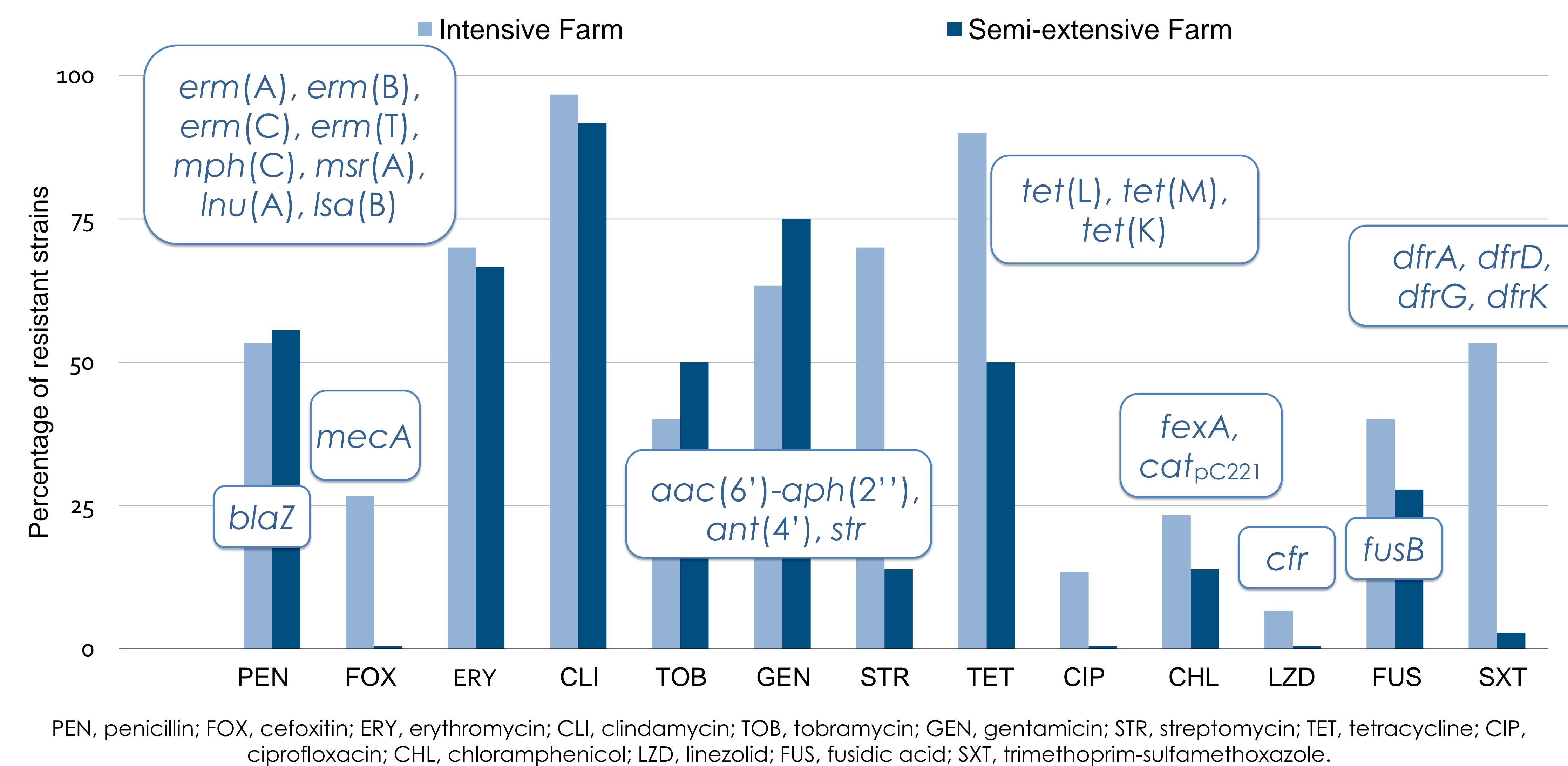
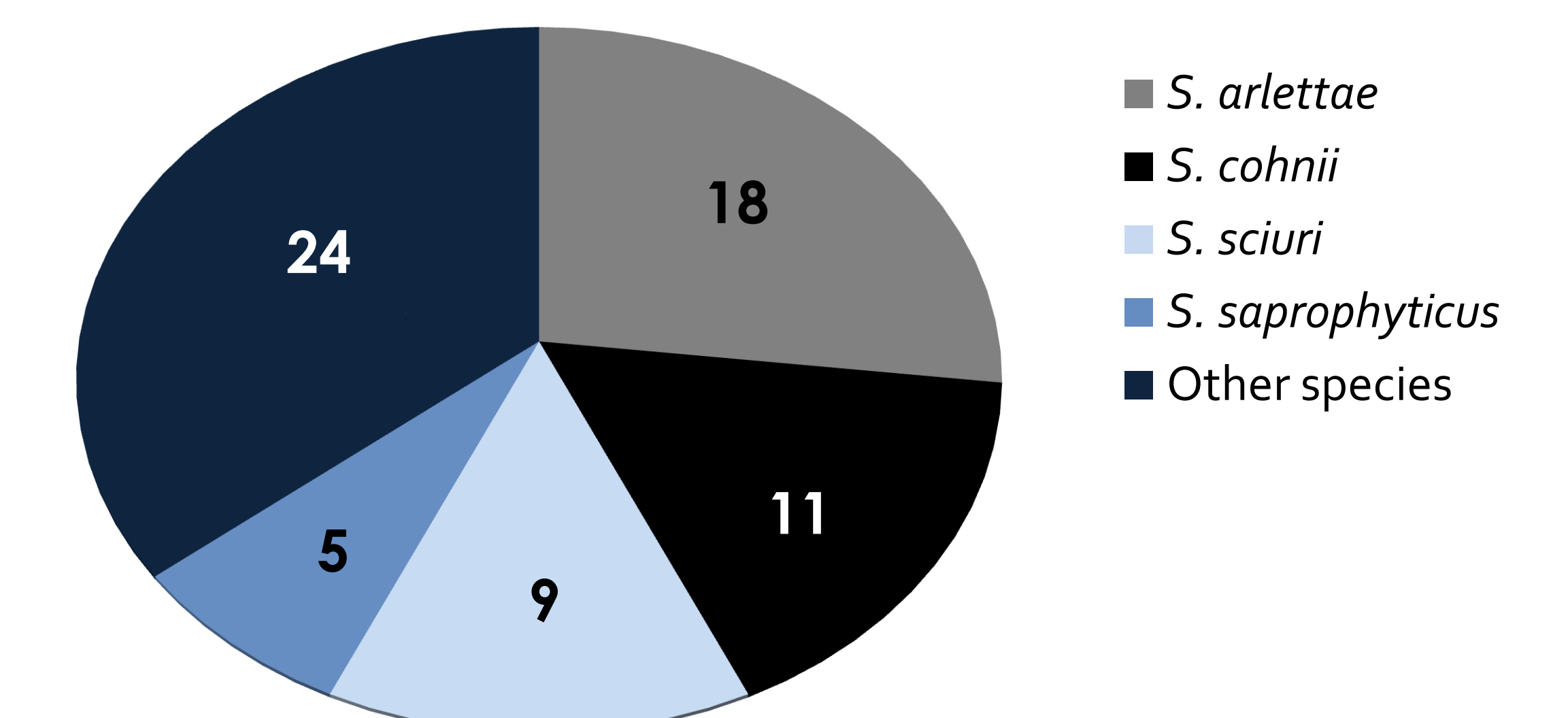


Figure 1. N° of isolates of each species recovered from the swine farm.



Other species include *S. haemolyticus*, *S. xylosus*, *S. epidermidis*, *S. equorum*, *S. nepalensis*, *S. warneri*, *S. lentus*, *S. chromogenes*, *S. simulans* and *S. succinus*.

The two linezolid-resistant isolates *S. equorum* and *S. arlettae* (MIC=8 µg/ml) recovered from the farm with intensive production, carried the *cfr* gene and were negative for both *optrA* and *poxA* genes (Table 1). The genes *lukS/F-PV* encoding the PVL were detected in one *S. simulans* isolate.

Table 1. Resistance genes detected in the linezolid-resistant isolates.

Isolate	Resistance genes detected
<i>S. equorum</i>	<i>cfr</i> , <i>mecA</i> , <i>lsa(B)</i> , <i>erm(B)</i> , <i>str</i> , <i>cat_{PC221}</i> , <i>tet(M)</i>
<i>S. arlettae</i>	<i>cfr</i> , <i>lsa(B)</i> , <i>mph(C)</i> , <i>msr(A)</i> , <i>lnu(A)</i> , <i>str</i> , <i>tet(K)</i> , <i>tet(M)</i> , <i>fexA</i> , <i>dfrD</i>

Conclusions

1. A high rate (70%) of multidrug-resistant CoNS was detected among environmental samples of swine farms.
2. The presence of *cfr*-positive linezolid-resistant CoNS in the air of pig farms, although this antimicrobial is not used in livestock, stressed the importance of coselection of resistances.
3. The detection of a PVL-positive *S. simulans* makes it necessary to monitor these virulent microorganisms in the environment.
4. CoNS could act as a source of important antimicrobial resistance and virulence genes in environments with high selective pressure, like in the case of swine farms.