**Identification of the novel Macrolide-Lincosamide-Streptogramin B resistance gene *erm*(54) in a porcine LA-MRSA ST398**

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**Background and objectives:** Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) are mainly associated with sequence type (ST) 398 in many parts of the world and often multiresistant to antimicrobial agents [1]. Here, 178 porcine LA-MRSA from Germany were investigated for new antimicrobial resistance genes [2].

**Methods:** Whole-genome sequencing on Illumina MiSeq and PacBio Sequel II platforms was followed by hybrid assembly and sequence analysis. Plasmid pHKS3860 was transferred into *S*. *aureus* RN4220 via electrotransformation. Antimicrobial susceptibility testing via broth microdilution and agar disc diffusion was performed according to CLSI standards [3] to confirm the functionality of *erm*(54). An *erm*(54)-specific PCR assay was developed and applied to 30 macrolide-resistant staphylococcal isolates, which harbored next-related *erm* genes.

**Results:** A novel Macrolide-Lincosamide-Streptogramin B (MLSB) resistance gene, *erm*(54), was detected on the non-conjugative plasmid pHKS3860 of 36,929 bp in a porcine LA-MRSA ST398. The gene encoded a 23S rRNA methylase of 245 amino acids (aa) that was next-related to Erm(B) (72%). Moreover, *erm*(54) was expressed constitutively. A complex regulatory region composed of a small reading frame for a 30 aa protein and seven pairs of inverted repeats, which can form varying mRNA secondary structures, was detected upstream of *erm*(54). The transferred *erm*(54) caused a distinct increase in the minimal inhibitory concentrations of MLSB antibiotics in *S*. *aureus* RN4220. The new PCR assay detected *erm*(54) in the original strain and the transformants carrying pHKS3860, but none of the next-related *erm* genes available to us. Copper, mercury and cadmium resistance genes as well as an *ica* cluster for biofilm formation were also found on plasmid pHKS3860.

**Conclusions:** The new transferable and functionally active MLSB resistance gene *erm*(54) was identified in a porcine LA-MRSA ST398 isolate from Germany. The co-location of *erm*(54) on a plasmid with heavy metal resistance genes may increase the risk for co-selection and persistence under selection pressure imposed by heavy metals in animal feed or the environment.

**References**

[1] Butaye P, Argudín MA and Smith TC (2016). *Curr Clin Microbiol Rep* 3:19-31.

[2] Krüger-Haker H, Ji X, Hanke D, Fiedler S, Feßler AT *et al*. (2023). *Microbiol Spectr*. 11:e0077023.

[3] Clinical and Laboratory Standards Institute (CLSI) (2022): CLSI supplement M100, 32nd Ed.