

# Plasmid Localisation Of Resistance Genes In Porcine MRSA ST398 Isolates

Kadlec K<sup>1,\*</sup>, Ehrlich R<sup>2</sup>, Monecke S<sup>3</sup>, Steinacker U<sup>4</sup>, Kaspar H<sup>4</sup>, Mankertz J<sup>4</sup>, Schwarz S<sup>1</sup>

<sup>1</sup> Institute of Farm Animal Genetics, Friedrich-Loeffler-Institute (FLI), Neustadt-Mariensee, Germany; <sup>2</sup> CLONDIAG GmbH, Jena, Germany; <sup>3</sup> Institute for Medical Microbiology and Hygiene, Dresden University of Technology, Dresden, Germany; <sup>4</sup> Federal Office of Consumer Protection and Food Safety (BVL), Berlin, Germany

## Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates of the sequence type ST398 have been identified to colonize and cause infections in animals and humans [1].

In addition to the resistance to  $\beta$ -lactam antibiotics, MRSA isolates have been described to show resistance to other antimicrobial agents [2].

This study aimed at identifying the genes responsible for selected resistance properties and their location.

## Material and Methods

A total of 54 independent MRSA ST398 isolates collected in Germany from pigs with acute infections were investigated for their susceptibility to 31 different antimicrobial agents by broth micro-dilution according to the CLSI [3].

The corresponding resistance genes were identified by microchip analysis [4] or by PCR.

Selected resistance genes were tested for their plasmid localisation by Southern blotting and/or transformation experiments.

## Conclusions

This study revealed that large plasmids harbouring two or three resistance genes are frequently identified in porcine MRSA ST398 isolates.

The co-localisation of resistance genes on the same plasmid allows the acquisition and the maintenance of such plasmids under the selective pressure of different antimicrobial agents.

## Acknowledgements

We thank Vera Nöding and Kerstin Meyer for excellent technical assistance.

## References

- [1] van Belkum A, Melles DC, Peeters JK, et al. (2008) Methicillin-resistant and -susceptible *Staphylococcus aureus* sequence type 398 in pigs and humans. *Emerg Infect Dis.* 14: 479-83.
- [2] Schwarz S, Kadlec K, and Strommenger B. (2008) Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* detected in the BFT-GermVet monitoring programme 2004-2006 in Germany. *J Antimicrob Chemother.* 61:282-5.
- [3] CLSI document M31-A3, (2008) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard - Third edition, ISSN 0273-3099.
- [4] Monecke S, Slickers P, and Ehrlich R. (2008) Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. *FEMS Immunol Med Microbiol.* 53:237-51.

## Results

The distribution of MIC values is shown in Table 1.

**Table 1: Distribution of MIC values among the MRSA isolates.**

Antimicrobial agent(s)	No. of isolates with MIC of ... [mg/L]													Resistant*		MIC <sub>50</sub>	MIC <sub>90</sub>			
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256			512	no.	%
Oxacillin+2%NaCl	-	-	-	-	-	-	-	-	1 <sup>c</sup>	16	37	-	-	-	-	-	54	100.0	≥16	≥16
Ampicillin	-	-	-	-	-	-	-	-	2	9	26	15	1	1	-	54	100.0	16	32	
Amoxicillin/clavulanic acid <sup>b</sup>	-	-	-	-	-	-	-	2	25	23	4	-	-	-	-	27	50.0	4	8	
Erythromycin	-	-	-	10	20	-	-	-	-	-	-	-	24	-	-	24	44.4	0.5	≥64	
Clindamycin	-	-	3	18	6	3	-	-	-	-	1	-	-	23	-	-	-	0.25	≥128	
Chloramphenicol	-	-	-	-	1	-	-	2	34	15	-	1	1	-	2	3.7	8	16		
Florfenicol	-	-	-	-	-	-	-	35	17	-	-	2	-	-	-	-	4	8		
Tetracycline	-	-	-	-	-	-	-	-	-	-	1	7	45	1	-	54	100.0	128	128	
Sulfamethoxazole/trimethoprim <sup>b</sup>	-	3	18	9	2	7	9	5	1	-	-	-	-	-	1	1.9	0.12	2		
Trimethoprim	-	-	-	2	6	13	5	-	-	-	-	-	-	28	-	-	≥256	≥256		
Nalidixic acid	-	-	-	-	-	-	-	-	-	-	-	24	21	4	5	-	64	128		
Enrofloxacin	-	-	-	20	21	2	6	1	2	2	-	-	-	-	-	-	0.25	1		
Gentamicin	-	-	-	6	30	7	-	2	1	-	3	3	1	-	-	7	13.0	0.5	32	
Tiamulin	-	-	-	-	33	11	-	1	2	-	3	-	4	-	-	-	0.5	32		

\* only applied, if CLSI-approved breakpoints were available in document M31-A3 [4]; <sup>b</sup> the MIC to amoxicillin/clavulanic acid or to sulfamethoxazole/trimethoprim are given only as MIC to amoxicillin or to trimethoprim, respectively; <sup>c</sup> the resistant isolates are written in red.

Macrolide/lincosamide resistance was seen in 23 isolates; the genes *erm(A)*, *erm(B)* and/or *erm(C)* were identified alone or in different combinations (Table 2, Fig. 1): Three isolates harboured *erm(A)* alone, six isolates *erm(B)* alone, 11 isolates *erm(C)* alone, two isolates *erm(A)* and *erm(C)*, a single isolate harboured *erm(A)* and *erm(B)* and in one isolate none of the three genes was identified.

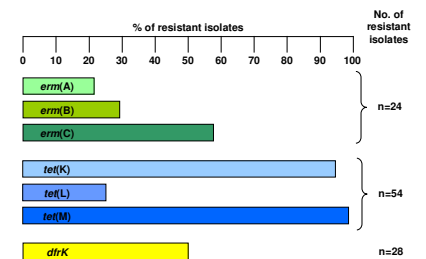
All 54 isolates were tetracycline-resistant; the genes *tet(M)*, *tet(K)* and *tet(L)* were detected (Table 2, Fig. 1). Most of the isolates (n=41) harboured *tet(M)* and *tet(K)*, 11 isolates harboured all three genes [*tet(M)*, *tet(K)*, and *tet(L)*], a single isolate harboured only *tet(M)*, and one isolate harboured solely *tet(L)*.

Trimethoprim resistance was identified in 28 isolates; the gene *dhfrK* was detected in 14 isolates.

The two chloramphenicol/florfenicol resistant isolates harboured *fexA*.

**Table 2: Resistance genes identified among the isolates.**

Antimicrobial agent	Resistance gene(s)
Oxacillin	<i>mecA</i>
Ampicillin	<i>mecA</i> , <i>blaZ</i>
Erythromycin/clindamycin	<i>erm(A)</i> , <i>erm(B)</i> , <i>erm(C)</i>
Chloramphenicol/florfenicol	<i>fexA</i>
Tetracycline	<i>tet(M)</i> , <i>tet(K)</i> , <i>tet(L)</i>
Trimethoprim	<i>dhfrK</i>



**Fig. 1: Prevalence of selected resistance genes.**

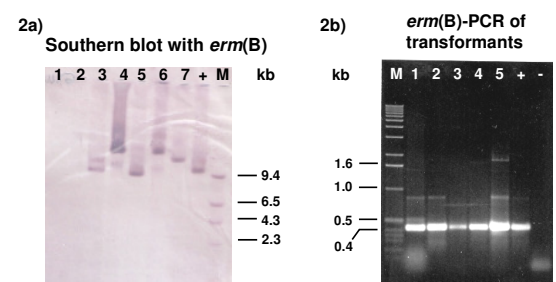
The genes *fexA*, *tet(M)*, and *tet(K)* were located on the chromosome, while *tet(L)* was located on plasmids (ca. 14 - 40 kb) in 13 isolates (Table 3).

The gene *dhfrK* was located on the same plasmid as *tet(L)* in all 13 cases or on a smaller plasmid (ca. 8 kb) in the remaining case. Five of the *tet(L)/dhfrK*-harbouring plasmids carried also *erm(B)* (Fig. 2).

The gene *erm(A)* was not shown to be plasmid-located, whereas the gene *erm(C)* was located on a small plasmid (ca. 2.5 kb) in all 13 isolates.

**Table 3: Plasmid location of transferable resistance genes.**

No. of isolates	Resistance gene	Plasmid located	Plasmid size
7	<i>erm(B)</i>	5	~ 40, 30 kb
14	<i>erm(C)</i>	13	~ 2.5 kb
13	<i>tet(L)</i>	13	~ 40, 30, 18, 14 kb
14	<i>dhfrK</i>	14	~ 40, 30, 18, 14, 8 kb



**Fig. 2: Plasmid location of *erm(B)*: a) Southern Blot of plasmid DNA and b) PCR of macrolide resistant transformants.**