

Phenotypic and genotypic analysis of antimicrobial resistance in porcine MRSA ST398 isolates from Germany

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Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates of sequence type ST398 have been identified to colonize and cause infections in animals and humans. These isolates have been identified frequently in pigs and in humans with exposure to animal husbandry, especially to swine farming. Isolates of this type were first detected in The Netherlands, but later on also in other European countries.

The aim of this study was to determine the genomic relationships of porcine MRSA ST398 isolates in Germany.

Material and Methods

In total 54 independent MRSA ST398 isolates obtained from pigs from Germany in 2004/2005 (n=5) and 2008 (n=49) on the basis of one isolate per herd were included in this study.

The isolates were subjected to *spa* typing, macrorestriction analysis with *Apal* and *SmaI*, and PCR-directed *SCCmec* typing [1, 2]. Moreover, all strains were investigated for the most relevant virulence properties by a diagnostic microarray [3].

Results

Fifty-three isolates harboured *SCCmec* type V elements while the remaining one carried *mecA* and *uggQ*, but no recombinase gene. None of the 54 isolates harboured the Panton-Valentine leukocidin genes *lukF-PV* and *lukS-PV*. The carriage of haemolysin alpha and delta genes, the protease genes *sspA*, *sspB*, and *sspP*, the *ssl* and *set* genes, as well as MSCRAMM genes was uniform among all isolates. One isolate was positive for the enterotoxin B (*seb*) gene, another three isolates for the enterotoxin K and Q (*sek*, *seq*) genes [4].

Eight different *spa* types were identified with t011 (n=39) being most predominant. The *spa* types t034, t571, t1197, t1250, t1451, t1456, and t2510 were seen in one to five isolates. Detailed analysis of these *spa* types suggested that they might have developed from t011 by recombinational events or even single basepair exchanges within one repeat (Fig. 1). Isolates of *spa* types t011 and t034 were disseminated all over Germany [4].

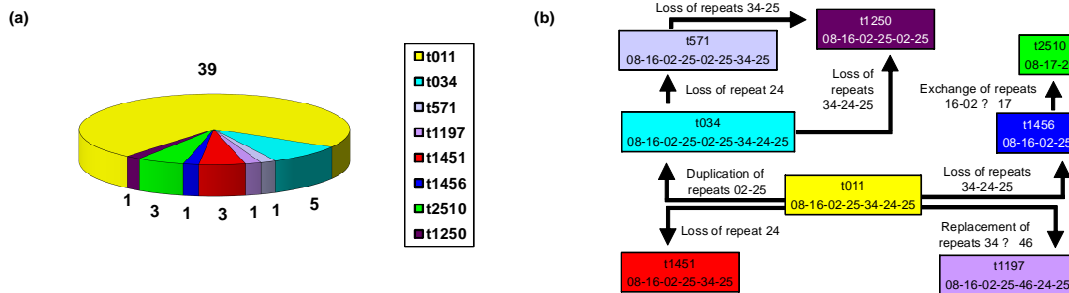


Fig. 1: (a) Distribution of the different *spa* types seen among the 54 porcine MRSA ST398 isolates.

(b) Analysis of the fine structure of the different *spa* types and a model of the development of the different *spa* types by duplication, deletion or replacement of *spa* repeats.

All 54 isolates were non-typeable by *SmaI* macrorestriction analysis, but produced a number of different fragment patterns upon *Apal* macrorestriction patterns. A total of six major patterns A – F with up to eight sub-patterns were identified upon cluster analysis using a cut-off at 80% similarity (Fig. 2). Among them, isolates assigned to the predominant cluster A were found to be distributed all over Germany whereas the isolates of cluster C were only seen in the Eastern part of Germany [4].

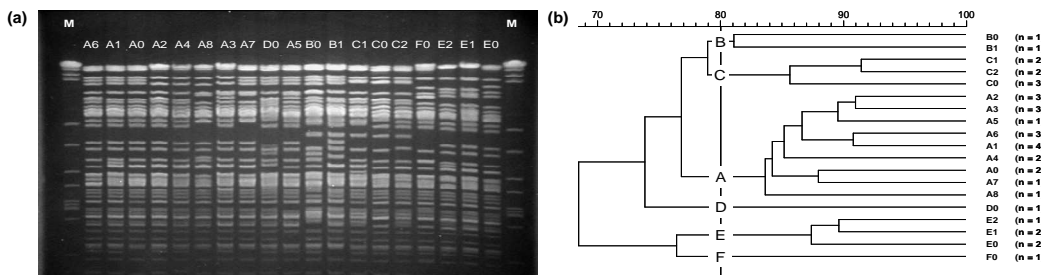


Fig. 1: (a) *Apal* fragment patterns obtained after PFGE analysis; the letters refer to the different fragment patterns in the Table, lanes M contain the *SmaI*-digested *S. aureus* strain NCTC8325.

(b) Cluster analysis of the *Apal* fragment patterns. The numbers in brackets behind the different fragment patterns indicate the numbers of isolates that exhibited the respective fragment pattern. A cut-off at 80% similarity was used to distinguish the six major clusters A-F.

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Conclusions

The results of *spa* typing and macrorestriction analysis obtained in this study revealed a high degree of diversity between the porcine MRSA ST398 isolates from Germany.

Apal proved to be a suitable and highly discriminatory restriction endonuclease for macrorestriction analysis of ST398 strains which are non-typeable by *SmaI*.

Moreover, the results obtained with the diagnostic microarray suitably supplemented the typing data.