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Short communication

High-resolution typing by MLVF unveils extensive heterogeneity of European livestock-associated methicillin-resistant *Staphylococcus aureus* isolates with the sequence type 398

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* sequence type 398 (MRSA ST398) has emerged in livestock worldwide. In particular, areas in Europe with high densities of livestock farming are affected. Consequently, the incidence of human colonization and infection with ST398 is rapidly increasing. Distinguishing different ST398 isolates with standard typing tools is problematic. The objective of this study was to examine the discriminatory power of Multiple-Locus Variable number tandem repeat Fingerprinting (MLVF) on a highly diverse ST398 collection. Our data show that MLVF combined with *spa*-typing is an attractive approach for high-resolution typing of ST398 isolates and unveiling their relatedness.

Epidemiological studies on *Staphylococcus aureus* have shown that certain lineages of this important pathogen are prevalent in specific host populations or regions of the world. Additionally, different lineages of staphylococci are encountered in healthcare facilities and the general population (Monecke et al., 2011). This complex epidemiology is particularly evident for the lineage with the Multi-Locus Sequence Type 398 (ST398), which is predominantly found in livestock, farming environments, individuals with direct or indirect livestock contacts, and further along in the food chain (Aspiroz et al., 2010; Köck et al., 2011; Wulf et al., 2011). The ST398 clone was first reported in 2005 in France, and has since been isolated in many other countries, primarily in Europe but also in North America and Asia (Armand-Lefevre et al., 2005; Bhat et al., 2009; Lewis et al., 2008; Lim et al., 2012). In Europe, the reported numbers of ST398 isolates correlate with the densities of livestock farms (Wulf et al., 2011). However, due to industrialization and globalization of the livestock industry, this lineage represents not only a potential threat for public health in agricultural regions, but also for the wider community. Consequently, it is also encountered in individuals with no apparent livestock contacts (Welinder-Olsson et al., 2008). Serious infections in humans caused by *S. aureus* ST398, especially its methicillin-resistant form (MRSA), are still rare, but the proportion of clinical cases caused by MRSA ST398 is increasing in areas with a high livestock density (Köck et al., 2011).

Identification of ST398 isolates is mostly based on Multi-Locus Sequence Typing (MLST) (Enright and Spratt, 1999). However, MLST does not reveal variations between individual ST398 isolates as recently detected by whole-genome sequencing (Price et al., 2011). While whole-genome sequencing has the highest discriminatory power, this technique is not yet sufficiently developed for day-to-day routine typing of *S. aureus* isolates (Sabat et al., 2013). Considering the increasing numbers of clinical cases related to ST398, highly discriminatory, fast and cheap typing tools are needed for effective outbreak prevention, control measures and

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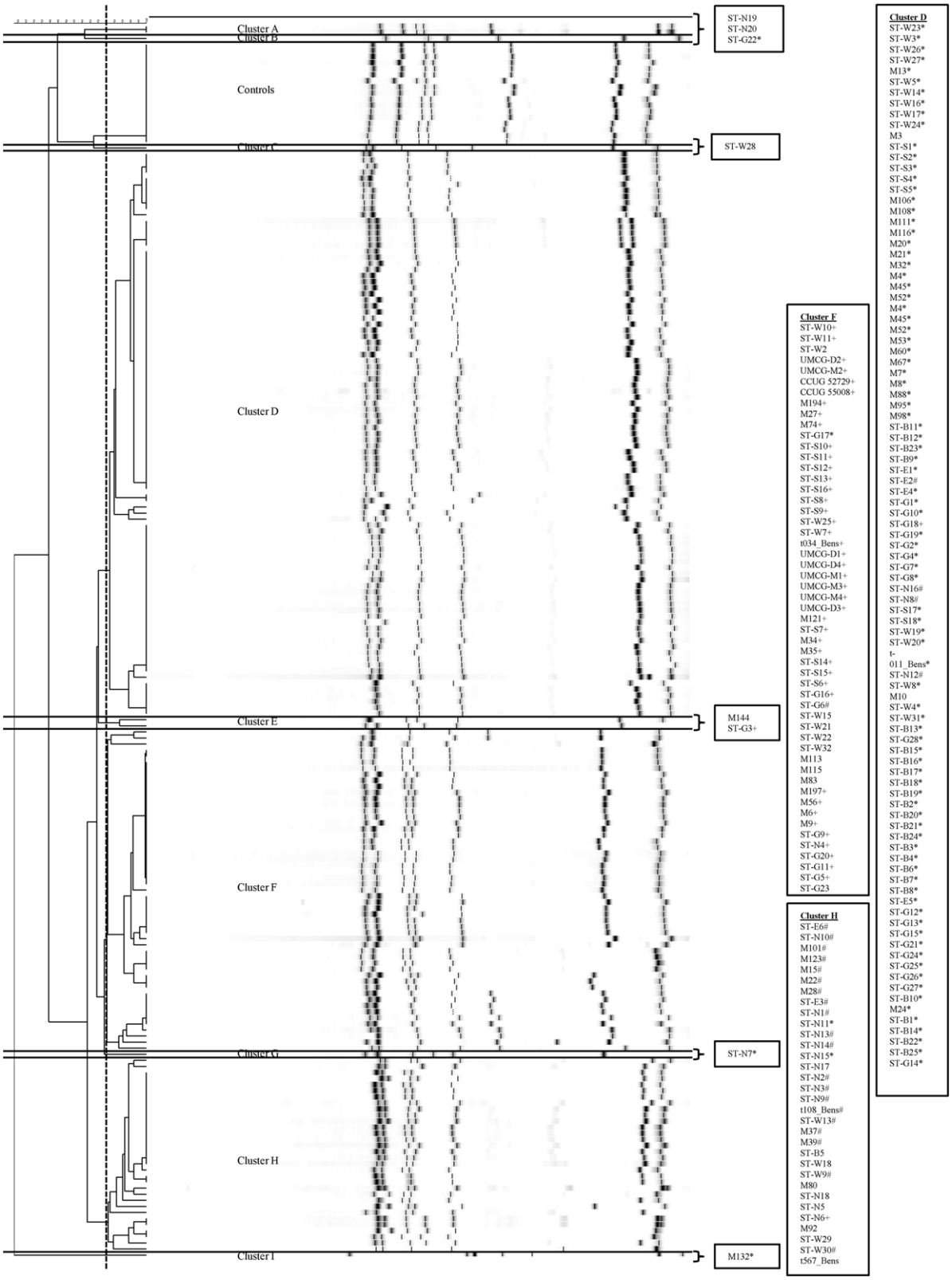

Fig. 1. MLVF dendrogram of 184 *S. aureus* ST398 isolates generated by the UPGMA algorithm. The MLVF experiments, including DNA isolation, multiplex PCR, separation of PCR products on the Bioanalyzer 2100 (Agilent Technologies), and data analyses with GelCompar II (Applied Maths, Sint-Martens-Latem) were performed as recently described (Sabat et al., 2012). The position tolerance and optimization were set to 0.9% and 0.5%, respectively. After visual inspection of the MLVF dendrogram, six different cut-off values (89%, 80%, 78%, 75% and 68%) were chosen for testing the concordance between MLVF and *spa*-types. On the cluster level, the highest (and very good) concordance between MLVF and *spa*-typing was found at 75% (Adjusted Rand's Coefficient 0.712). Therefore, the cut-off value was set to 75%. Additionally to the 184 studied ST398 isolates, 2 control isolates with sequence type 9, and 17 control samples of the isolate M2 were included in this delineation. The names of clusters and individual *S. aureus* isolates are indicated in the dendrogram. Specific information on the investigated *S. aureus* isolates is presented in the Supporting Information Table 1. The three most predominant *spa*-types are indicated in the dendrogram: *, t011; +, t034; and #, t108.