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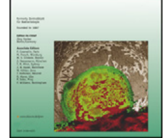

Short communication

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.

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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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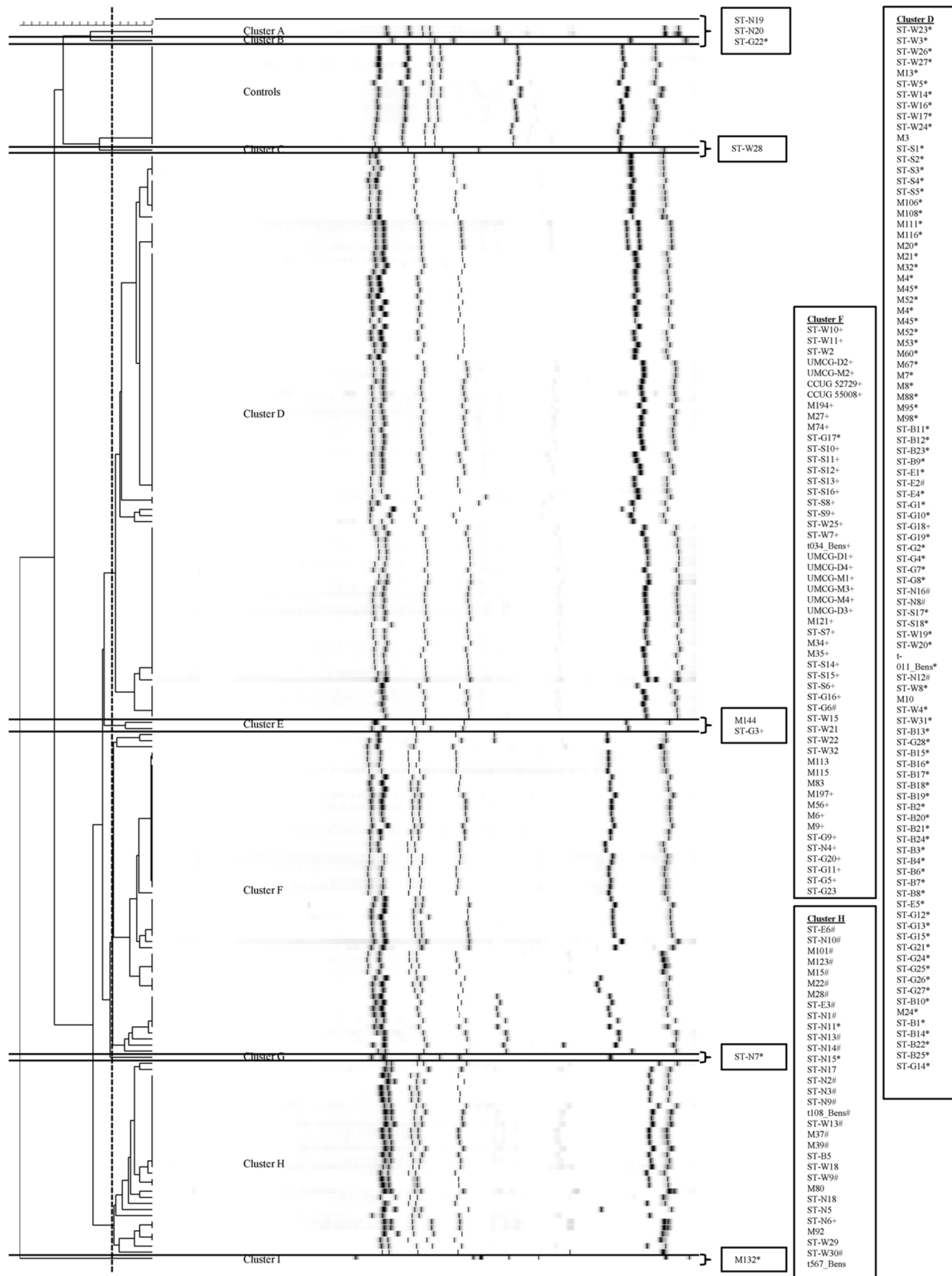

Fig. 1. MLVF dendrogram of 184 *S. aureus* ST398 isolates generated by the UPGMA algorithm. The MLVF experiments, including DNA isolation, multiplex PCR, separation of PCR products on the Bioanalyzer 2100 (Agilent Technologies), and data analyses with GelCompar II (Applied Maths, Sint-Martens-Latem) 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.

traceability studies. Unfortunately, typing with the 'gold standard' method pulsed-field gel electrophoresis (PFGE) is problematic, since chromosomal DNA of ST398 isolates is resistant to macro-restriction with *Sma*I due to methylation of the respective cleavage site (Bens et al., 2006). Also, PFGE is relatively slow, labor-intensive, and expensive. A more generally used method to cluster ST398 isolates is *spa*-typing, which is based on sequencing of the protein A gene (Koreen et al., 2004). To date, several closely related *spa*-types have been identified among ST398, the most prevalent being t011, t034 and t108 (Smith and Pearson, 2011).

The present study was aimed at exploring the applicability of a Variable-Number Tandem-Repeat (VNTR) typing method for distinguishing ST398 isolates and determining their relatedness. Such methods have a high discriminatory power and are rapid, cheap and easy-to-use (Sabat et al., 2012). Specifically, Multiple-Locus VNTR Fingerprinting (MLVF) was applied to distinguish 184 different ST398 isolates collected from 7 European countries between 2003 and 2010 (Supporting Information Table 1). These isolates included 10 different *spa*-types of which seven were detected in more than one isolate. The majority of isolates belonged to *spa*-types t011 (n = 92), t034 (n = 45) and t108 (n = 25). In addition, two ST9 control isolates with the *spa*-type t899 were used for MLVF. The MLVF analysis was performed as recently described (Sabat et al., 2012), and the results plus details on the relevant parameters and settings for generation of the dendrogram and subsequent clustering are provided in Fig. 1. This analysis yielded 49 different MLVF banding patterns (Fig. 1); eighteen were derived from two or more isolates (155 isolates) and 31 from single isolates. MLVF grouped the 186 isolates into 9 clusters denoted as A (n = 2), B (n = 1), C (n = 1), D (n = 93), E (n = 2), F (n = 53), G (n = 1), H (n = 32) and I (n = 1). Notably, the banding pattern of the ST9 control isolates was grouped in the entirely unrelated cluster A. In general, MLVF grouped isolates with the same *spa*-type into the same cluster. Importantly, it revealed sub-clusters of isolates with the same *spa*-type. However, certain isolates with different *spa*-types were also grouped into one cluster or sub-cluster. Specifically, cluster D included mostly isolates with *spa*-type t011, cluster F isolates with *spa*-type t034, and cluster H isolates with *spa*-type t108.

The clusters generated by MLVF could not be linked to general epidemiological features, such as the country of origin, year of isolation or source. However, detailed evaluation of the dendrogram revealed an interesting alignment of six isolates from a Spanish farmers' family (Aspiroz et al., 2010). Although most isolates from this family were ST398 with closely related *spa*-types, MLVF grouped them into different clusters. Isolates ST-E1, ST-E2 and ST-E4 showed identical banding patterns, whereas isolates ST-E3, ST-E5 and ST-E6 yielded different patterns. Interestingly, the MLVF pattern of isolates ST-E1/E2/E4 was also observed for isolates from Belgium, Germany, the Netherlands and Switzerland (Fig. 1). The MLVF pattern of the ST-E3 isolate was also observed in 15 isolates from the Netherlands, whereas that of the closely related ST-E6 isolate was unique. The family members were thus exposed to at least three different ST398 MLVF types.

The fact that MRSA ST398 has become a frequent clone in livestock in Europe and is spreading in humans might imply that it is likely to cause treatment problems in the future, not only in the community but also in nosocomial settings. The view that multi-resistant MRSA ST398 is likely to become a nosocomial problem is underscored by the fact that ~25% of the MRSA isolates recovered from patients entering the University Medical Center Groningen between 2003 and 2010 belonged to this lineage (Sabat et al., 2012). These 46 non-replicate MRSA isolates yielded 15 distinct MLVF banding patterns that were evenly distributed over the entire dendrogram, being grouped in 5 of the 8 clusters (Fig. 1).

The here presented high-resolution typing of ST398 isolates has until now not been performed for such a large and diverse collection of ST398 isolates. Our data show that MLVF can be applied to follow lines of transmission of ST398 in order to prevent or control possible outbreaks (Sabat et al., 2012). This approach is complementary to *spa*-typing and, together, MLVF and *spa*-typing could replace MLST, which is so far used as sole criterion in epidemiological analyses of ST398. Moreover, MLVF is capable of unveiling the relationships between different ST398 isolates. These are crucial features for molecular typing of *S. aureus*, epidemiological surveillance and effective infection control measures within hospitals. Lastly, the identification of specific MLVF clusters and sub-clusters represents an excellent starting point for tracking the evolution of *S. aureus* ST398.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijmm.2013.02.015.

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