

O549

Abstract (oral session)

**Staphylococcus aureus USA300: how to discriminate between the bad, the worse and the ugly**

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**Objectives:** Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a serious threat for public health worldwide. Of particular concern is the MRSA clone USA300. This lineage is predominant in the United States but has disseminated to other countries in recent years. Noteworthy, Pulsed-field gel electrophoresis (PFGE), which is the 'gold standard' for typing *S. aureus*, cannot be used to distinguish between the 'classical' community-acquired USA300 clone with the spa-type t008 (ST8-IVa, Panton-Valentine leukocidin positive) and a related nosocomial clone with the spa-type t024 (ST8-IVa, PVL negative). Therefore, the objective of this study was to determine the applicability of Multiple-Locus Variable number tandem repeat Fingerprinting (MLVF) in the discrimination of these two related clones. In addition, we compared the exoproteomes of the respective strains, because the secreted proteins represent a major reservoir of *S. aureus* virulence factors.

**Methods:** 78 USA300(-like) isolates were collected at the Statens Serum Institute in Denmark. Multiplex PCR with subsequent electrophoretic separation with an Agilent 2100 Bioanalyzer was performed as described by Sabat et al. 2003. Secreted proteins were collected and analyzed by SDS-PAGE.

**Results:** MLVF grouped the 78 isolates into two distinct clusters: A (n = 40) and B (n = 38). Importantly, all isolates of cluster A had spa-type t024, whereas all isolates of cluster B had spa-type t008. Furthermore, both clusters were composed of several patterns, but within each cluster most of the strains yielded the same pattern. Thus, the majority of the strains within each cluster are genetically closely related. The exoproteome analyses complemented the MLVF data, since differences in the secreted proteins of *S. aureus* isolates in clusters A and B were clearly detectable.

**Conclusion:** MLVF has the discriminatory power needed to distinguish between the community-acquired USA300 clone and closely related lineages with the same PFGE profile. Additionally, MLVF allows a differentiation of isolates with the same spa-type into sub-clusters, which will be relevant for further epidemiological studies. In addition, exoproteome analyses on the community-acquired USA300 clone and related nosocomial lineages will provide better insights into their virulence potential. Ultimately, this will lead to a better understanding of critical determinants for staphylococcal fitness and pathogenesis.