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# *Rapid characterisation of methicillin-resistant Staphylococcus aureus clinical isolates for an im proved drug prescription*

**Objectives** Until recently, *Staphylococcus aureus* was considered a nosocomial pathogen, but recent reports have shown that *S. aureus* is also responsible for infections acquired in the community, affecting healthy and young people having no documented contact with medical care centres. The ability to rapidly and reliably identify relatedness between clinical isolates is crucial for the investigation of outbreaks and also for the epidemiological surveillance of strain dissemination.

**Conclusions** A new genotyping assay method based on variable numbers of tandem repeats (MLVA) was developed. The assay permits automated real-time epidemiology, allowing not only the genotyping of MRSA (methicillin-resistant *S. aureus*) but also the characterisation of MSSA (methicillin-susceptible *S. aureus*). All steps were optimised to ensure rapid turnaround time (4 hours, ›100 strains/day) and moderate costs (‹4-5 CHF/strain). The performance, discriminatory power and reproducibility of this method (turnaround time 4 hours) was estimated at least equivalent to PFGE (which requires 24 hours). The results will be reported on the Web by the use of a shared database, which is currently under construction. Furthermore, a multiplex quantitative PCR assay relying on the determination of the *agr* type was developed in order to complete characterisation of *S. aureus* isolates.
To conclude, in contrast to other European countries showing a limited number of clonetypes, our geographical area appears to be exposed to numerous importation events easily identifiable using MLVA.

## *Main results and findings*

**Development of MLVA** Development of a new automated genotyping assay allowing real-time epidemiology based on variable number of tandem repeats – that is, a "multi locus variable number of tandem repeats analysis assay" (MLVA). The main features of the assay are:

- A multiplex PCR using 10 primer pairs targeting gene regions with variable numbers of tandem repeats (the first assay used 8 primers; 2 primer pairs were added recently to increase discrimination power).
- The PCR amplification products are analysed using microcapillary electrophoresis (the development of the peak detection algorithm was challenging).
- Each strain is automatically assessed by cluster analysis using software developed in-house.
- All steps were optimised to ensure rapid turnaround time (4 hours, >100 strains/day) and moderate costs (<4-5 CHF/strain).
- Web reporting of the results is possible by the use of a shared database. The reference database of *S. aureus* strains includes epidemic clones of MRSA (EMRSA) with detailed epidemiological characteristics. Therefore, relying on this database, typing of the profile of unknown or newly identified MRSA (whatever their origin) will be possible.
- MLVA was validated (discriminatory power and reproducibility) on a large collection of *S. aureus* clinical isolates (from either long term carriers or defined nosocomial outbreaks) and a panel of control strains previously characterised using standard methods. The performance of this method (turnaround time 4 hours) was estimated at least equivalent to PFGE (which requires 24 hours). Comparison of MLVA to several genotyping methods including comparative genome hybridisation on microarrays covering the whole *S. aureus* genome revealed that MLVA clearly discriminates clusters of hospital acquired MRSA from community acquired MRSA.
- Furthermore, a multiplex quantitative PCR assay relying on the determination of the *agr* type was developed in order to complete the characterisation of *S. aureus* isolates. This work showed that each different *agr* type was found in limited numbers of genetic backgrounds.

**Implementation of the MLVA**

- In collaboration with Dr. S. Harbarth and Prof. D. Pittet, MLVA was performed on a collection of community acquired MRSA (CA-MRSA) clinical isolates. Based on the assay, it could be documented that CA-MRSA recovered from patients at admission displayed a large genetic diversity in the area under study.

- In collaboration with Dr. H. Sax, MLVA was used to investigate outbreaks in the neonatology ward. MLVA revealed that 3 different strains were responsible for infections in 17 different patients, after transmission event.
- In collaboration with Prof. S. Lacroix, MLVA made it possible to show that patients suffering from chronic rhinosinusitis carried the same strain of *S. aureus* for years.
- In collaboration with Dr. S. Harbarth and Prof. D. Pittet, MLVA was used to characterise MRSA strains of community origin over a period of 13 years, strains identified as non-multiresistant to antibiotics. These strains showed a wide diversity of molecular profiles.
- Collaboration with Dr. D. Melles and Pr. A van Belkum (Erasmus University, Rotterdam) is in progress for the evaluation of the correlation between strain patterns and types of infections (clinical or colonising strains collected from patients with well characterised medical follow-up).

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