Multiple-Locus Variable Number Tandem Repeat (VNTR) Analysis (MLVA) : a helpful tool for subtyping Staphylococcus lugdunensis

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Multiple-Locus Variable Number Tandem Repeat (VNTR) Analysis (MLVA): a helpful tool for subtyping *Staphylococcus lugdunensis*

P2050 session « *Staph is not dead* »

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Introduction

Staphylococcus lugdunensis (SLU) is an emergent virulent coagulase-negative *Staphylococcus* that is increasingly responsible for severe infections resembling those caused by *S. aureus,* such as skin and soft tissue infections, infective endocarditis and bone and joint infections.

In an attempt to generate informative sequence data for subtyping SLU, we developed both a classical length-based **MLVA** scheme and a **sequence-based MLVA scheme** (Tandem Repeat Sequence Typing, **TRST**), and assessed their performances compared to MultiLocus Sequence Typing (**MLST**)[1] and Multivirulence Locus Sequence Typing (**MVLST**)[2].

Methods

MLVA and TRST assays development

- SLU genomes **N920143**[3] and **HKU09-01**[4] were screened *in silico* for the presence of tandem repeats (TRs) with Tandem Repeats Finder software (TRF).
- Selected VNTRs loci were tested on a panel of :
  - **30 genetically diverse SLU strains** representative of the main clonal groups defined by MLST (including 10 closely related isolates).
  - a collection of **98 clinical isolates** (82 pathogenic isolates from diverse clinical settings and 16 carriage isolates) collected from November 2013 to March 2016 at the University Hospital of Strasbourg in France [5], to evaluate overall performances of the new methods.
- **Number** and **sequences** of TRs were identified for each VNTR with **BioNumerics software 7.6**, and each unique combination was converted into distinct **MLVA types (MTs)** and **TRST types (TRTs).**

MLST and MVLST assays

- MLST and trilocus-MVLST genotyping were performed for the **128** isolates by sequencing 7 housekeeping[1] and 3 virulence-associated genes[2], respectively.
- Allelic profiles and corresponding **sequence types (STs)** as well as **trilocus virulence types (VT^{T}s)** were determined with BioNumerics. STs were clustered into **clonal complexes (CCs)** with eBURST v3.

Data analysis

- All typing data were uploaded into BioNumerics to generate **minimum spanning trees** (**MST**).
- Discriminatory ability of the methods were evaluated by the **Simpson's index of diversity (DI)** and associated **confidence intervals (CI)**.

[1]Chassain B. *et al.* Multilocus sequence typing analysis of *S. lugdunensis* implies a clonal population structure. 2012. *J. Clin. Microbiol.*
[2]Didi J. *et al.*. 2014. Multi-virulence-locus sequence typing of *S. lugdunensis* generates results consistent with a clonal population structure and is reliable for epidemiological typing. *J. Clin. Microbiol.*
[3]Heilbronner S. *et al.* 2011. Genome sequence of *S. lugdunensis* N920143 allows identification of putative colonization and virulence factors. *FEMS Microbiology Letters.*
[4]Tse H. *et al.* 2010. Complete Genome Sequence of *Staphylococcus lugdunensis* Strain HKU09-01. *Journal of Bacteriology.*
[5]Argemi X. *et al.* 2017. VISLISI trial, a prospective clinical study allowing identification of a new metalloprotease and putative virulence factor from *S. lugdunensis*. *Clin Microbiol Infect.*

Results

MLST and MVLST genotyping

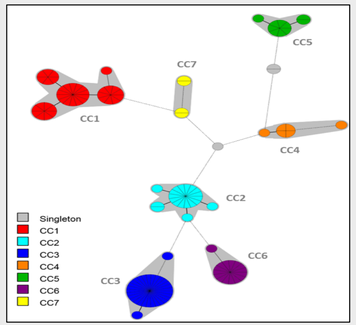

Figure 1. MST of the 128 SLU isolates typed by MLST.
The colors used are based on CCs. Each circle represents an ST and its size is proportional to the number of strains. Thick, short lines connecting two types denote types differing in a single locus; thin, longer lines connect double-locus variants; and dashed lines indicate the most likely connection between two types differing in more than two loci. Gray zones around circles delineate CCs.

- **MLST** :
  - **25 STs** distributed into **7 CCs (Figure 1)**
  - Main CCs: **CC1** (n=38) and **CC3** (n=31)
  - DI_{MLST} = **0,898**
- **MVLST** :
  - **21 VT^{T}s**
  - Clustering similar to that of MLST
  - DI_{MVLST} = **0,845**

Characteristics of the 7 VNTRs *loci*

- Identification of **28** VNTRs with TRF → **7** selected: **SLU1 to SLU7**
- **Heterogeneity** of TR copy ranges and DI **(Table 1).**

VNTR name	Putative function	Repeat size (bp)	Copy no. range	MLVA scheme		TRST scheme		
				DI	CI^c 95%	No. of allelles	DI	CI 95%
SLU1	Non-coding	57	1-3	0.531	0.500-0.562	6	0.656	0.609-0.702
SLU2	Hypothetical protein	58	1-4	0.595	0.523-0.667	21	0.801	0.745-0.856
SLU3	Hypothetical protein	48	3-11	0.679	0.615-0.743	27	0.889	0.859-0.918
SLU4	Non-coding	57	1-6	0.666	0.617-0.715	14	0.757	0.706-0.808
SLU5	Non-coding	57	2-5	0.479	0.393-0.566	19	0.849	0.819-0.879
SLU6	Hypothetical protein	58	1-5	0.566	0.503-0.629	18	0.850	0.819-0.880
SLU7	AraC family transcriptional regulator	24	2-3	0.090	0.022-0.158	4	0.120	0.043-0.197

Table 1. Characteristics of the 7 VNTRs *loci*.

MLVA and TRST genotyping and clustering

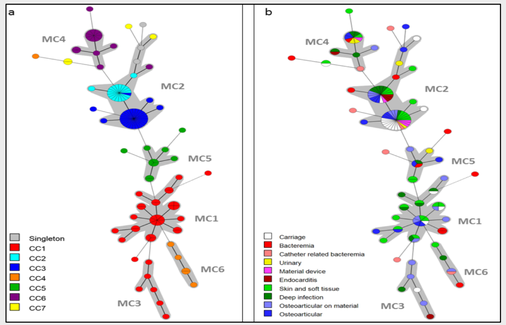

Figure 2. MST of the 128 SLU isolates typed by MLVA.
The colors used are based on (a) CCs defined by MLST and (b) clinical contexts. Each circle represents a MT and its size is proportional to the number of strains. Gray zones around circles delineate MCs.

- **MLVA** :
  - **55 MTs** distributed into **6 MCs (Figure 2a)**
  - Main MCs: **MC1** (n=30) and **MC2** (n=54)
  - DI_{MLVA} = **0,933**
    → **more discriminant than MLST/MVLST**
  - No cluster specific to clinical presentation (**Figure 2b**)

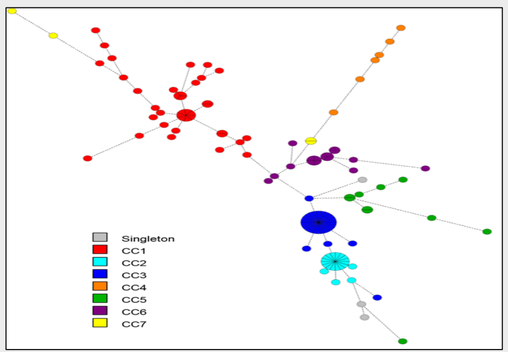

Figure 3. MST of the 128 SLU isolates typed by TRST.
The colors used are based on CCs defined by MLST. Each circle represents a TRT and its size is proportional to the number of strains.

- **TRST :**
  - **69 TRTs (Figure 3)**
  - DI_{TRST} = **0,943**
  → **highly discriminant**

MLVA and TRST clustering : distribution similar to that obtained by MLST, with efficient subdivision of some CCs (especially the CC1)

Conclusion

- We describe here the first **VNTRs-based schemes** for *Staphylococcus lugdunensis* subtyping.
- MLVA and TRST were **more discriminant** compared to MLST and MVLST, and the clustering achieved by the four typing methods **was highly congruent.**
- In conclusion, **MLVA and TRST represent very promising tools** to distinguish between strains of homogenous lineages in this clonal species, and provide valuable information for molecular epidemiological studies of *Staphylococcus lugdunensis*.