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# fbl-typing of *Staphylococcus lugdunensis*: a frontline tool for epidemiological studies

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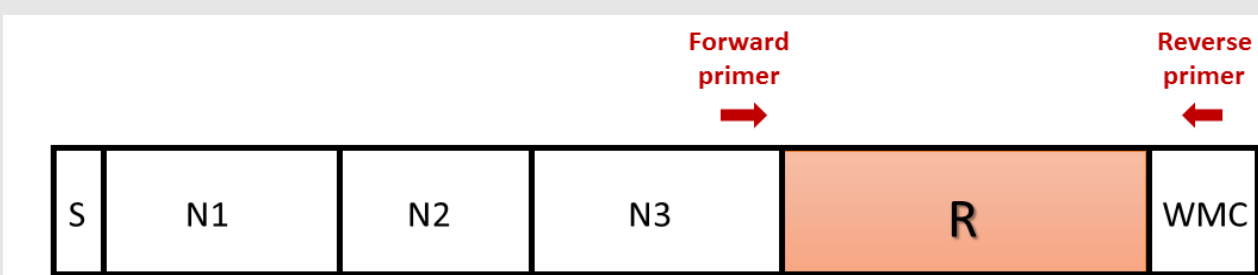
## Introduction

- Staphylococcus lugdunensis* is increasingly recognized as a virulent pathogen, responsible for severe infections with an outcome resembling that of *Staphylococcus aureus* rather than that caused by coagulase-negative *staphylococci*
- Molecular typing methods** based on Sanger sequencing have been developed to characterize *S. lugdunensis* genetic diversity:
  - MultiLocus Sequence Typing (**MLST**)<sup>1</sup> : **7** housekeeping genes
  - Tandem Repeat Sequence Typing (**TRST**)<sup>2</sup> : **7** Variable Number of Tandem Repeats (VNTRs)
- However, MLST and TRST daily use is **time consuming** and **expensive**

→ We developed a single locus typing scheme for *S. lugdnensis*, based on the DNA sequence analysis of the R-domain within the *fbl* gene encoding the fibrinogen-binding protein Fbl

## Strains & Methods

- Analysis of **240 *S. lugdunensis*** isolates recovered from 230 patients (various clinical and geographical origins):
  - 128** isolates previously characterized by **MLST** and **TRST**, collected from five French regions and Sweden <sup>2,3</sup>
  - 106** isolates collected at University Hospital of Rouen in 2016
- fbl*-typing:
  - amplification and sequencing of the ***fbl* R-domain** (18-bp repeats region) (**Figure 1**)
  - determination of the **numbers** as well as the **sequences** of each *fbl* repeats (BioNumerics 7.6)
  - each unique combination of repeats = a ***fbl*-type**
- Deduction of genetic relationships by **minimum spanning tree** analysis
- Evaluation of the discriminatory power by calculation of Simpson's Index of diversity (**DI**) and associated Confidence Intervals (**CI**)



**Figure 1. Schematic representation of the *fbl* gene of *S. lugdunensis*.** Boxes indicate segments of the gene: S, signal peptide, N1-, N2-, and N3-domains, R, repeat domain, WMC: W, wall spanning; M, membrane spanning; C, cytoplasmic positively charge tail. The locations of the forward and reverse primers used to amplify and sequence the R-domain are shown at the top.

<sup>1</sup>Chassain B. *et al.* Multilocus sequence typing analysis of *S. lugdunensis* implies a clonal population structure. 2012. *J. Clin. Microbiol.*

<sup>2</sup>Dahyot S. *et al.* Multiple-Locus Variable Number Tandem Repeat Analysis (MLVA) and Tandem Repeat Sequence Typing (TRST), helpful tools for subtyping *S. lugdunensis*. 2018. *Sci. Rep.*

<sup>3</sup>Argemi X. *et al.* VISLISI trial, a prospective clinical study allowing identification of a new metalloprotease and putative virulence factor from *S. lugdunensis*. 2017. *Clin. Microbiol Infect.*

## Results

### ✓ *fbl*-typing results

- Sequencing and assembling of *fbl* repeats obtained for the 240 isolates:
  - length of the *fbl* R-domain : **9 to 52 repeats**
  - number of sequences of 18-bp repeat : **54 individual sequences**  
→ number of unique combination : **92 *fbl*-types** (fbl9a to fbl52a)
- Most common *fbl*-types : **fbl47b** (*n* = 43), **fbl45f** (*n* = 24) and **fbl41a** (*n* = 13)
- fbl*-typing clustering (**Figure 2**):
  - 10 clusters** identified (1 to 10)
  - main clusters: **cluster 1** (57 isolates, 20 *fbl*-types) and **cluster 2** (52 isolates, 6 *fbl*-types)

### ✓ Concordance between typing methods

- Comparison of *fbl*-typing clustering with MLST and TRST data for 128 isolates
- Discriminatory power** of *fbl*-typing ( $DI_{fbl} = 0.964$ ) (**Table 1**) :
  - higher than MLST** ( $DI_{MLST} = 0.899$ )
  - equivalent to TRST ( $DI_{TRST} = 0.943$ )
- Clustering results** of *fbl*-typing :
  - congruent with MLST
  - fbl*-types predict **MLST clonal complexes (CCs)** with **100%** of probability (**Figure 2**)

Typing method	All isolates ( <i>n</i> = 230)			TRST panel ( <i>n</i> = 123)		
	No. of genotypes	DI <sup>a</sup>	CI <sup>b</sup> 95%	No. of genotypes	DI <sup>a</sup>	CI <sup>b</sup> 95%
<i>fbl</i> -typing	92	0.946	0.929-0.964	60	0.964	0.949-0.979
MLST	ND <sup>c</sup>	ND <sup>c</sup>	ND <sup>c</sup>	25	0.899	0.872-0.926
TRST	ND <sup>c</sup>	ND <sup>c</sup>	ND <sup>c</sup>	69	0.943	0.915-0.971

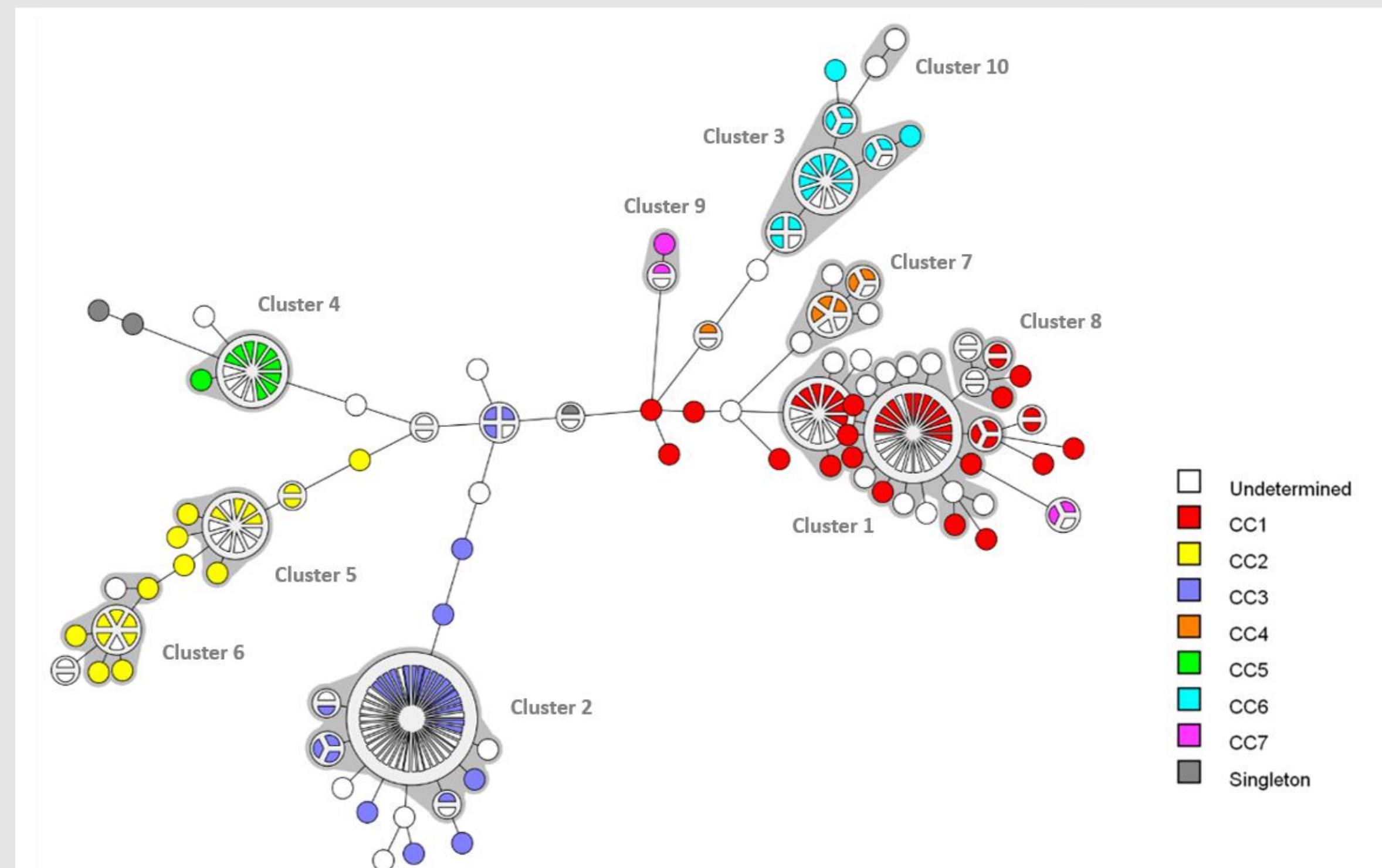
**Table 1. Discriminatory power of the three typing methods for unrelated isolates.**

Only one genotype by patient was included to avoid any bias.

<sup>a</sup>DI: Simpson's Diversity index. <sup>b</sup>CI: Confidence Interval. <sup>c</sup>ND: Not Determined.

### ✓ Development of « *fbl*-typing Server »

- Development of a **Web tool** publicly available: <http://fbl-typing.univ-rouen.fr>
- Allows **identification of *fbl*-types** from sequencing data (**FASTA** format)



**Figure 2. Minimum spanning tree analysis of the 240 *S. lugdunensis* isolates based on *fbl*-types.**

Cluster analysis was performed using the polymorphic VNTR typing plugin of BioNumerics. *fbl*-types separated by a minimum spanning tree distance of ≤ 2 (i.e., if they were ≥ 97% similar) were considered closely related and assigned to the same cluster. Each circle represents a *fbl*-type and its size is proportional to the number of isolates. The length of the branches expressed the minimum spanning tree distance between two *fbl*-types. Gray zones around circles delineate *fbl* clusters. The colors used are based on clonal complexes (CCs) defined by MLST, undetermined = unknown CC (isolates non characterized by MLST).

## Conclusion

- ✓ *fbl* R-domain is a reliable target as a **frontline tool** for the **single locus** genotyping of clinical isolates of *S. lugdunensis*.
- ✓ *fbl*-typing is an **easy** to use, **cost-effective**, **rapid** and **portable** method, suitable for local and international **epidemiological studies**.
- ✓ Impact of *fbl* polymorphisms on the **structure of the protein** and in **virulence** remains to be determined.