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► **To cite this version:**

S Dahyot, J Lebeurre, F Laumay, X. Argemi, C. Dubos, et al.. fbl-typing of *Staphylococcus lugdunensis*: a frontline tool for epidemiological studies. ECCMID, Apr 2019, Amsterdam, Netherlands. hal-02264266

**HAL Id: hal-02264266**

**<https://normandie-univ.hal.science/hal-02264266>**

Submitted on 6 Aug 2019

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

S. Dahyot<sup>1</sup>, J. Lebeurre<sup>2</sup>, F. Laumay<sup>3</sup>, X. Argemi<sup>4,5</sup>, C. Dubos<sup>1</sup>, L. Lemée<sup>1</sup>, G. Prévost<sup>4</sup>, P. François<sup>3</sup>, M. Pestel-Caron<sup>1</sup>

<sup>1</sup>Normandie Univ, UNIROUEN, GRAM EA2656, Rouen University Hospital, F-76000 Rouen, France; <sup>2</sup>Normandie Univ, UNIROUEN, GRAM EA2656, F-76000 Rouen, France; <sup>3</sup>University of Geneva Hospitals, Genomic Research Laboratory, Service of Infectious Diseases, Geneva, Switzerland; <sup>4</sup>Université de Strasbourg, CHRU de Strasbourg, VBP EA7290, Fédération de Médecine Translationnelle de Strasbourg, Institut de Bactériologie, F-67000 Strasbourg, France; <sup>5</sup>Hôpitaux Universitaires, Maladies Infectieuses et Tropicales, F-67000 Strasbourg, France.

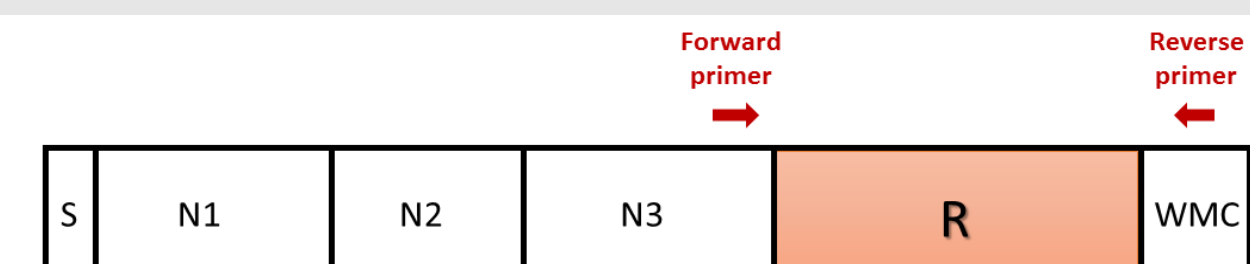
## Introduction

- 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. lugdunensis*, 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.

## 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 ( $n = 230$ ) |                 |                     | TRST panel ( $n = 123$ ) |                 |                     |
|--------------------|----------------------------|-----------------|---------------------|--------------------------|-----------------|---------------------|
|                    | 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         |

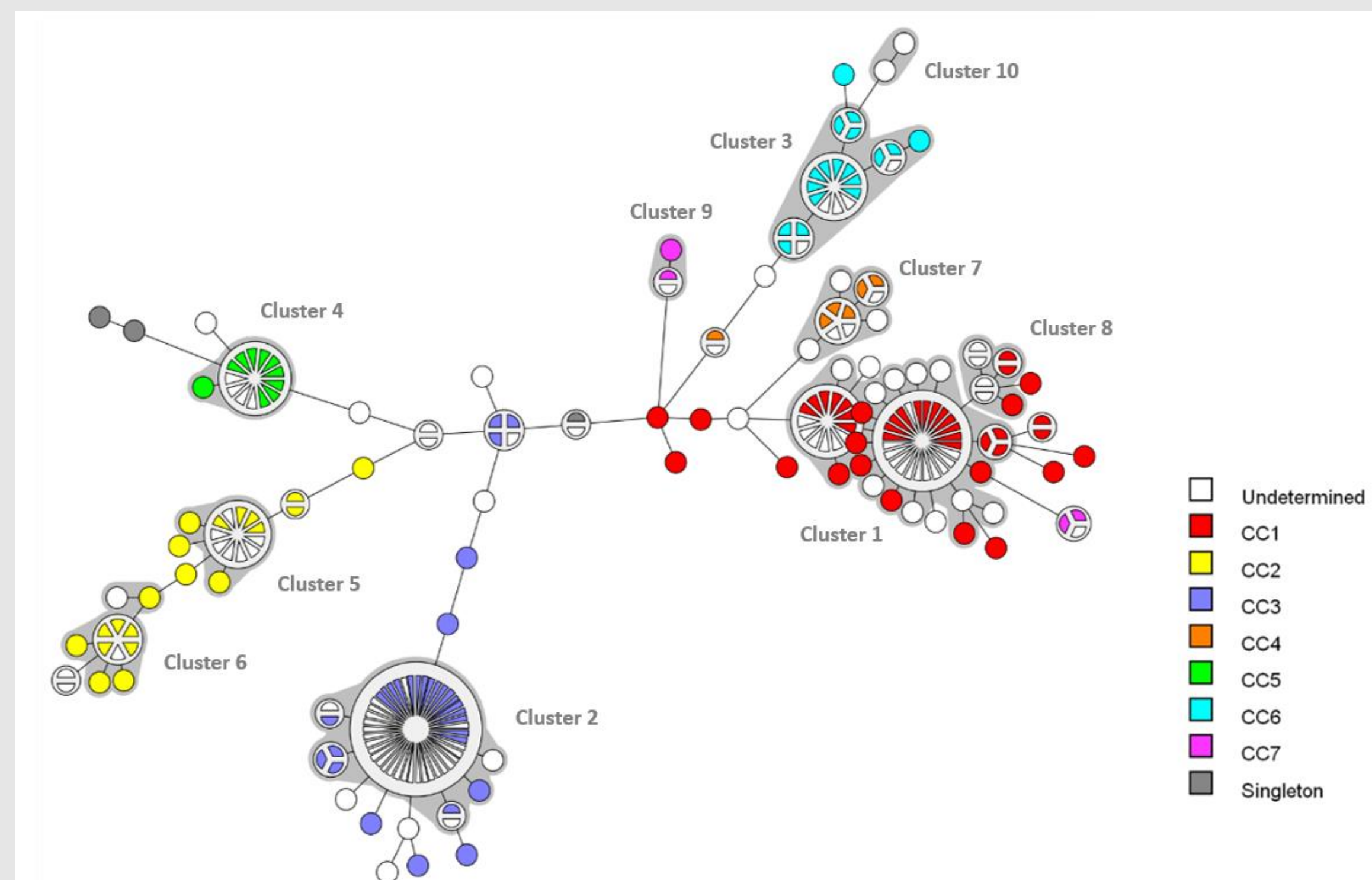
**Table 1.** Discriminatory power of the three typing methods for unrelated isolates.

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  $\leq 2$  (i.e., if they were  $\geq 97\%$  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.

<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.*