



**HAL**  
open science

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

S Dahyot, J Lebeurre, F Laumay, X. Argemi, C. Dubos, L. Lemée, G. Prevost, P. François, M. Pestel-Caron

► **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

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



# 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.

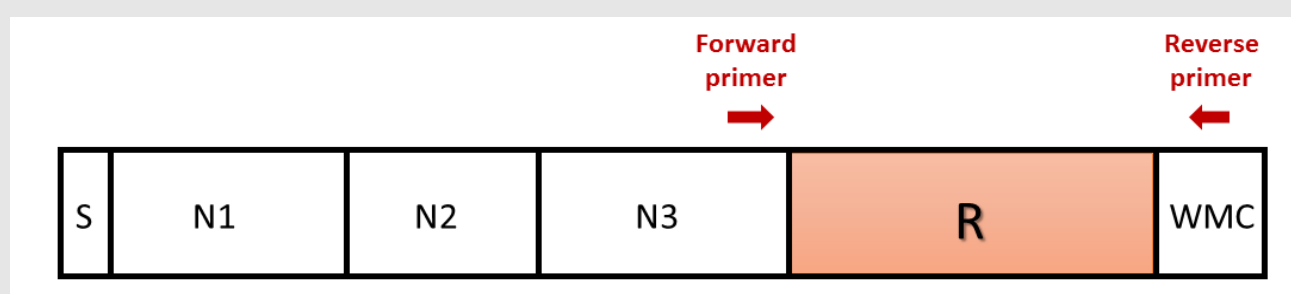


## Introduction

- 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

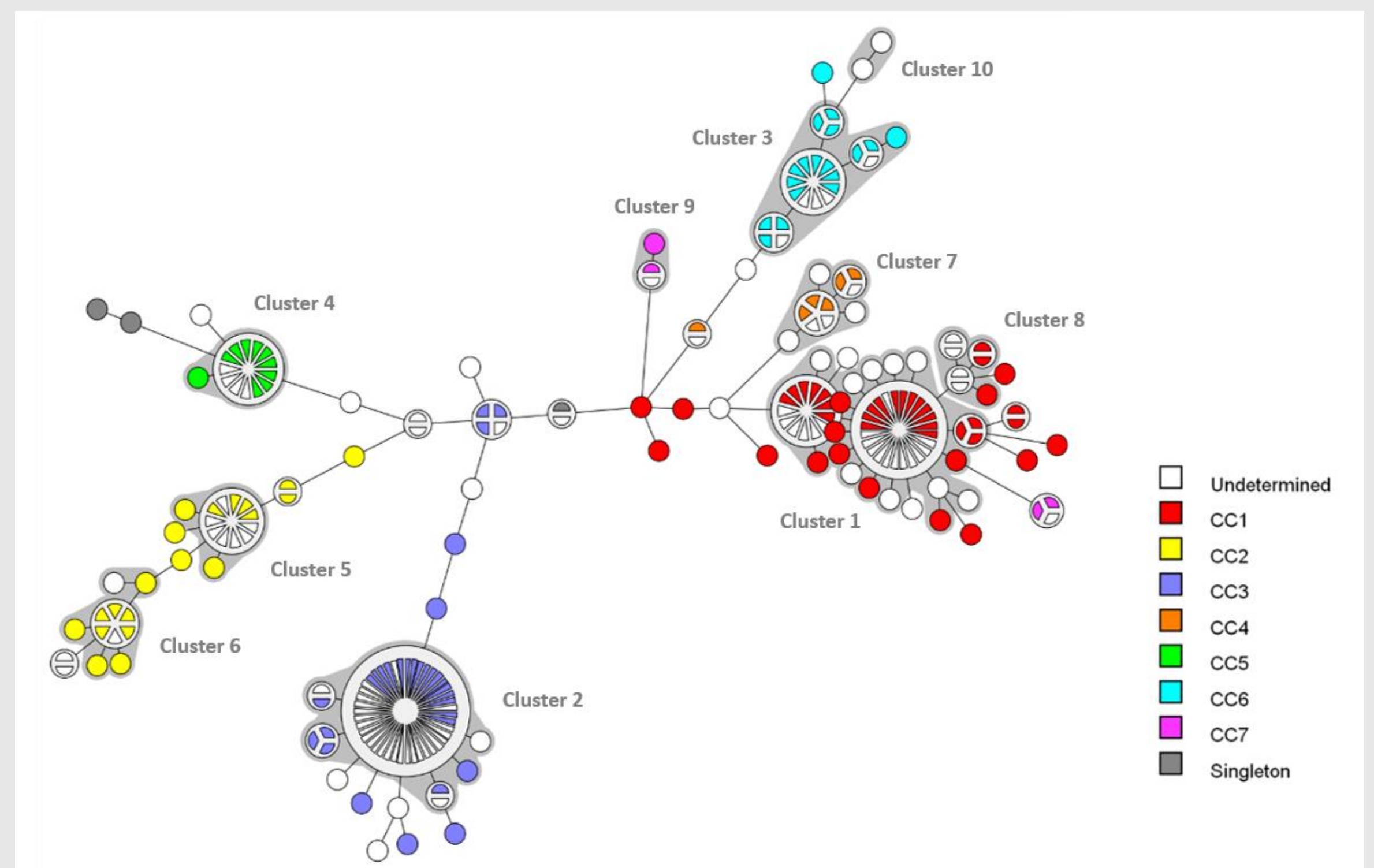
- Comparison of *fbl*-typing clustering with MLST and TRST data for **128 isolates**
- Discriminatory power** of *fbl*-typing ( $DI_{fbl} = 0.964$ ) (Table 1):
  - higher than MLST ( $DI_{MLST} = 0.899$ )
  - equivalent to TRST ( $DI_{TRST} = 0.943$ )
- Clustering results** of *fbl*-typing:
  - congruent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

Typing method	All isolates ( <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.

<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.*