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

S. Dahyot¹, J. Lebeurre², F. Laumay³, X. Argemi^{4,5}, C. Dubos¹, L. Lemée¹, G. Prévost⁴, P. François³, M. Pestel-Caron¹

¹Normandie Univ, UNIROUEN, GRAM EA2656, Rouen University Hospital, F-76000 Rouen, France; ²Normandie Univ, UNIROUEN, GRAM EA2656, F-76000 Rouen, France; ³University of Geneva Hospitals, Genomic Research Laboratory, Service of Infectious Diseases, Geneva, Switzerland; ⁴Université de Strasbourg, CHRU de Strasbourg, VBP EA7290, Fédération de Médecine Translationnelle de Strasbourg, Institut de Bactériologie, F-67000 Strasbourg, France; ⁵Hôpitaux 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)¹: **7** housekeeping genes
 - Tandem Repeat Sequence Typing (TRST)²: **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^{2,3}
 - **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**)

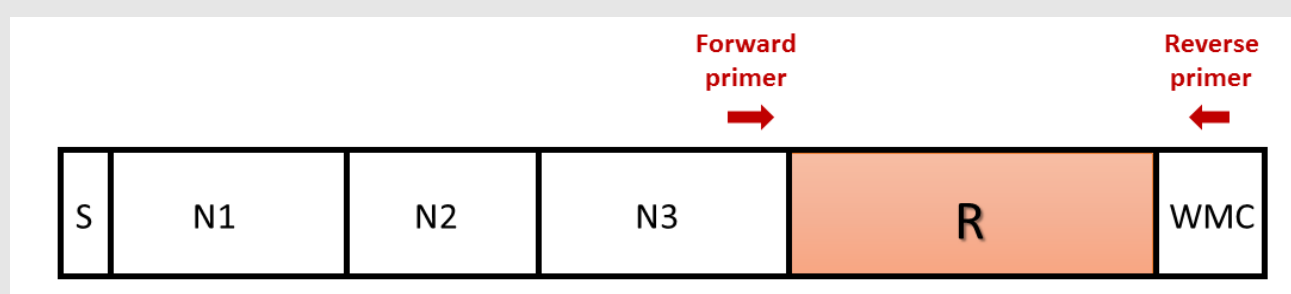


Figure 1. Schematic representation of the *fbl* gene of *S. lugdunensis*. Boxes indicate segments of the gene: S, signal peptide, N1-, N2-, and N3-domains, R, repeat domain, WMC: W, wall spanning; M, membrane spanning; C, cytoplasmic positively charge tail. The locations of the forward and reverse primers 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 ^a | CI ^b 95% | No. of genotypes | DI ^a | CI ^b 95% |
| <i>fbl</i> -typing | 92 | 0.946 | 0.929-0.964 | 60 | 0.964 | 0.949-0.979 |
| MLST | ND ^c | ND ^c | ND ^c | 25 | 0.899 | 0.872-0.926 |
| TRST | ND ^c | ND ^c | ND ^c | 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. ^aDI: Simpson's Diversity index. ^bCI: Confidence Interval. ^cND: 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)

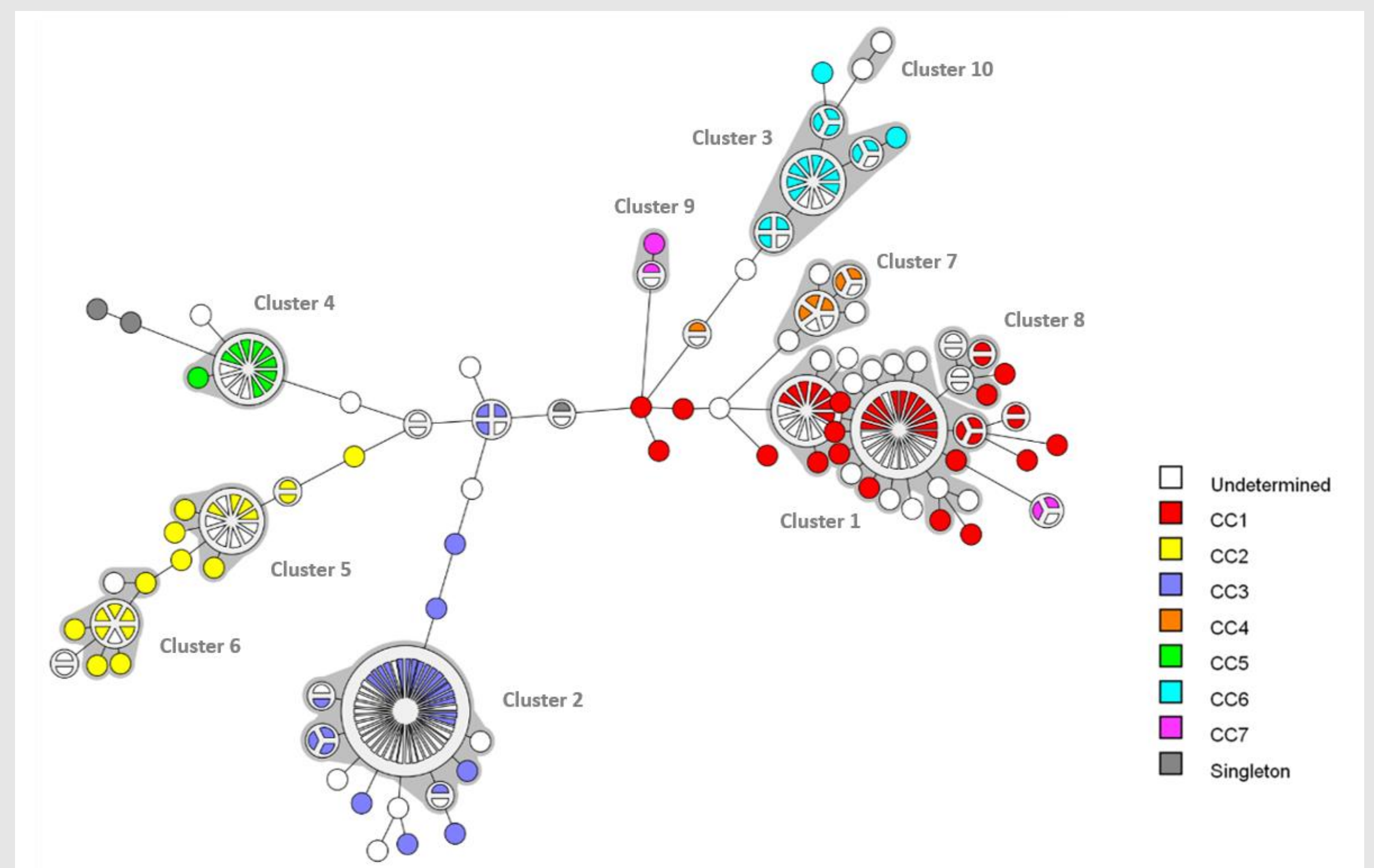


Figure 2. Minimum spanning tree analysis of the 240 *S. lugdunensis* isolates based on *fbl*-types. Cluster analysis was performed using the polymorphic VNTR typing plugin of BioNumerics. *fbl*-types separated by a minimum spanning tree distance of ≤ 2 (i.e., if they were ≥ 97% similar) were considered 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.

¹Chassain B. et al. Multilocus sequence typing analysis of *S. lugdunensis* implies a clonal population structure. 2012. *J. Clin. Microbiol.*
²Dahyot 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.*
³Argemi 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.*