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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**)[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. 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 [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.

[1]Chassain B. *et al.* Multilocus sequence typing analysis of *S. lugdunensis* implies a clonal population structure. 2012. *J. Clin. Microbiol.*
[2] 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.*
[3]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 (*n* = 230)			TRST panel (*n* = 123)		
	No. of genotypes	DI[a]	CI[b] 95%	No. of genotypes	DI[a]	CI[b] 95%
fbl-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.
[a]DI: Simpson's Diversity index. [b]CI: Confidence Interval. [c]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)

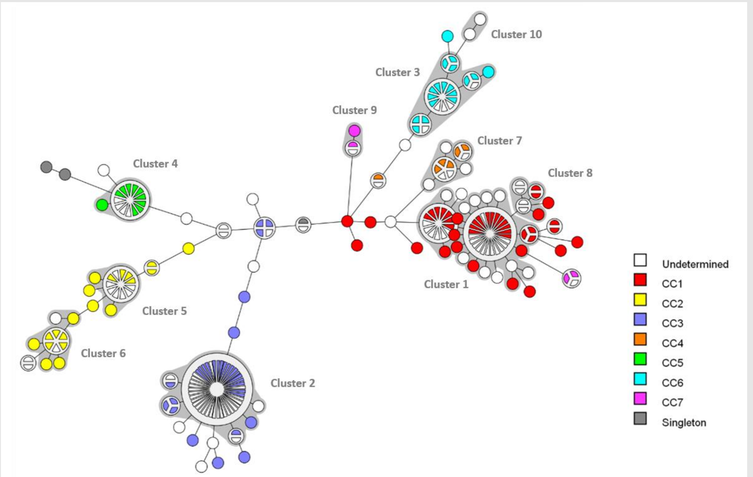

Figure 2. Minimum spanning tree analysis of the 240 *S. lugdunensis* isolates based on *fbl*-types.
Cluster analysis was performed using the polymorphic VNTR typing plugin of BioNumerics. *fbl*-types separated by a minimum spanning tree distance of ≤ 2 (i.e., if they were ≥ 97% similar) were considered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