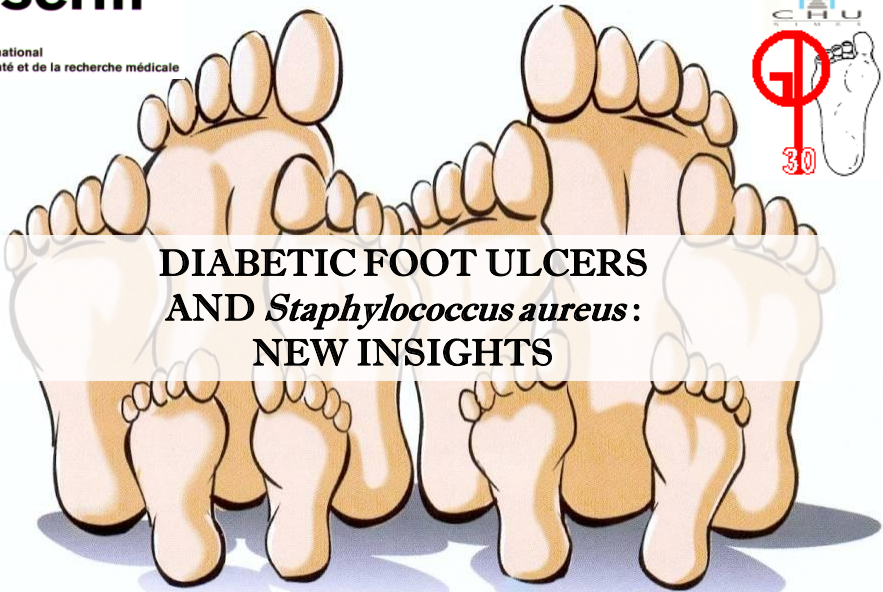



Institut national
de la santé et de la recherche médicale



DIABETIC FOOT ULCERS AND *Staphylococcus aureus* : NEW INSIGHTS

Lyon 9 décembre 2010



finovi
Fondation Innovations en Infectiologie

Some important points

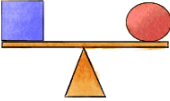
- Prevalence of diabetes mellitus in France: **3 millions** (4.5% of population, 11,2% > 65 ans)
- In 2000, cost of diabetes mellitus : **5.7 milliards €**

- Foot ulcers are common in diabetic patients with prevalence as high as **25%**

- These ulcers frequently become infected: **40 to 80% of infections**
- Spread of infections to soft tissue and to bony structures is a major causal factor for lower-limb amputation: **>13.000 amputations** in 2007 (France)
- Pronostic of amputation : **> 50%** of new amputation in 5 years and 42% of mortality

InVS, 2010; Lavery LA et al. Diabetes Care 2006

Problematic: The infection must be distinguished to bacterial colonisation



Colonisation

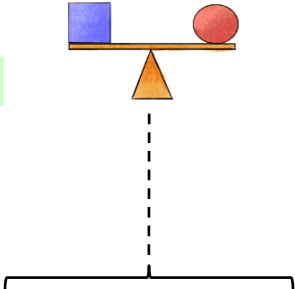
- Normal
- Low virulent bacteria
- Resident commensal flora
- Transitory bacterial flora

Infection

- Modification of flora
- Virulent bacteria
- No healing
- Extension

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Problematic: The infection must be distinguished to bacterial colonisation



Colonisation

Infection

Bacteria

- bacterial species
- potential of virulence

Host

- general status
- immunity status

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Problematic: The infection must be distinguished to bacterial colonisation

CLINICAL DIAGNOSTIC OF DIABETIC FOOT INFECTION

- Consensus of International Working Group on Diabetic Foot (2003):
Clinical criteria
4 GRADES



**COLONIZING WOUND
GRADE 1**



**INFECTING WOUND
GRADE 2**



**INFECTING WOUND
GRADE 3**



**INFECTING WOUND
GRADE 4**



BUT...

The distinction between Grade 1 and Grade 2 may be difficult

Consequences

- Misuse of antibiotic treatment
- Emerging of multidrug resistant bacteria

Bacteria n° 1:
Staphylococcus aureus and
more than 30% of Methicillin Resistant
***S. aureus* (MRSA)**

Virulence of MRSA ?

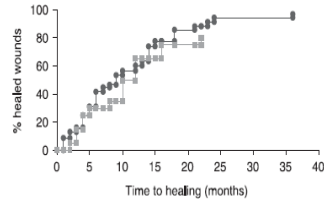


Diabet. Med. 21, 710-715 (2004)

Diabetic foot ulcer and multidrug-resistant organisms: risk factors and impact

A. Hartemann-Heurtier, J. Robert*, S. Jacqueminet, G. Ha Van, J. L. Golmard†, V. Jarlier*, and A. Grimaldi

MDRO vs non MDRO
 (p=0.71)



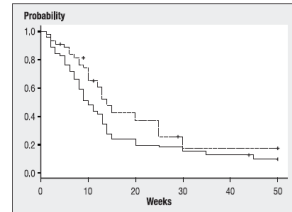
Diabetes & Metabolism 34 (2008) 363-369

Risk factors and healing impact of multidrug-resistant bacteria in diabetic foot ulcers

J.-L. Richard^a, A. Sotto^{b,c}, N. Jourdan^d, C. Combescuré^e,
 D. Vannereau^a, M. Rodier^d, J.-P. Lavigne^{b,e,f}

MRSA vs MSSA:

- p=0.036 at 6th month
- p=0.60 after 6 month



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Virulence of MRSA ?

DIABETIC/medicine

Diabet. Med. 26, 552-555 (2009)

Short Report

Are diabetic foot ulcers complicated by MRSA osteomyelitis associated with worse prognosis?

Outcomes of a surgical series

J. Aragón-Sánchez, J. L. Lázaro-Martínez*, Y. Quintana-Marrero, M. J. Hernández-Herrero,
 E. García-Morales*, J. J. Cabrera-Galván and J. V. Beneit-Montesinos*

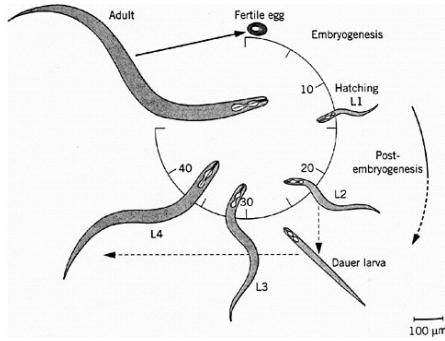
Table 3 Outcome of surgical treatment

	MSSA (n = 60)	MRSA (n = 35)	P-value
Conservative surgery, n (%)	31 (51.6)	16 (45.7)	0.57
Minor amputation, n (%)	25 (41.6)	17 (48.5)	0.51
Major amputation, n (%)	4 (6.6)	2 (5.7)	0.85
Number of surgeries performed, median (range)	1 (1-4)	1 (1-8)	0.04
Hospitalization (days), median (range)	14.5 (1-92)	20 (4-178)	0.06
Postoperative death, n (%)	1 (1.6)	0 (0)	0.45
Healing (Days), median (range)	90 (12-365)	120 (23-365)	0.05

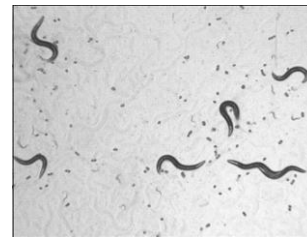
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Use of *C. elegans* model to evaluate virulence of *S. aureus* isolated from DFU



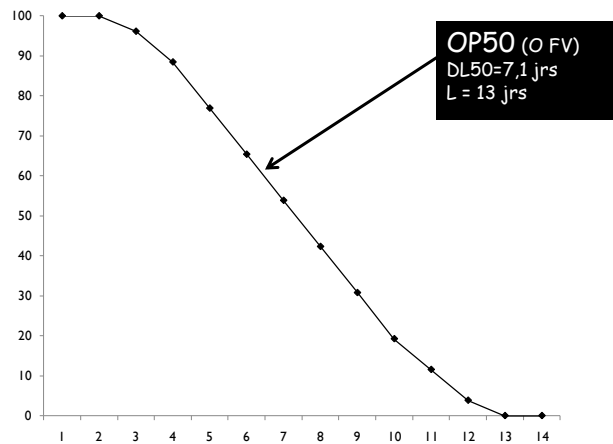
C. elegans lineage : Fer-15
 L4 stage
 Temperature: 25° C



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Virulence of MRSA ?

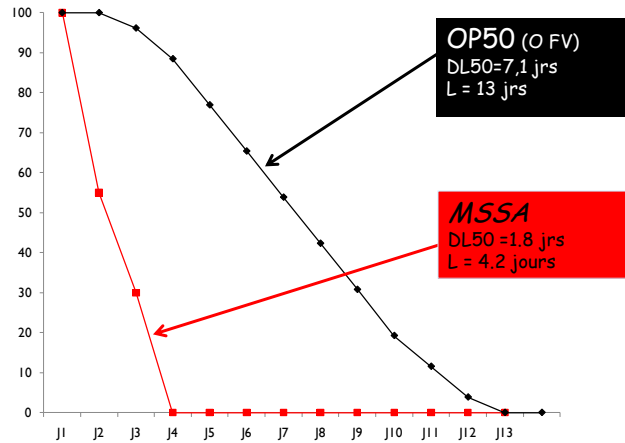


Sotto et al, Diabetes Care 2008

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Virulence of MRSA ?



Sotto et al, Diabetes Care 2008

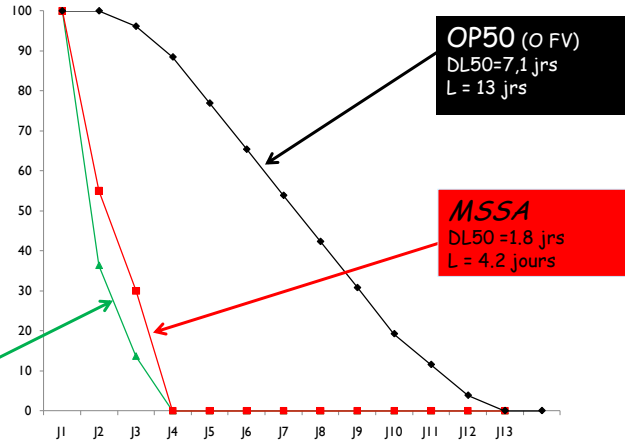
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Virulence of MRSA ?

Same virulence – No impact of meticillin resistance

MRSA
DL50 = 1.8 jrs
L = 4.1 jours



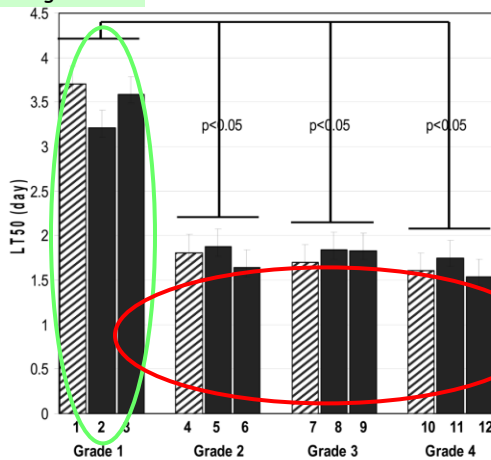
Sotto et al, Diabetes Care 2008

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Comparison of virulence of *S. aureus* isolated at different Grades

Colonising strains



The study demonstrated the co-existence of two populations of *S. aureus* strains:
 1/ colonizing strains (Grade 1) with low virulence
 2/ infecting strains (Grade 2-4) with high virulence independently of methicillin-resistance

Infected strains

Black: MSSA
 Striped: MRSA

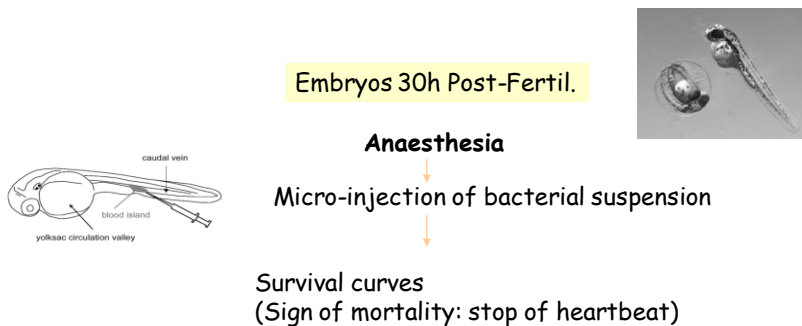
Sotto et al, Diabetes Care, 2008

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Use of zebrafish to confirm the low virulence of *S. aureus* isolated from DFU Grade 1

(Coll. S. Renshaw, Sheffield, UK)



20 embryos per strain – 1200 CFU/ embryo
 3 independent assays

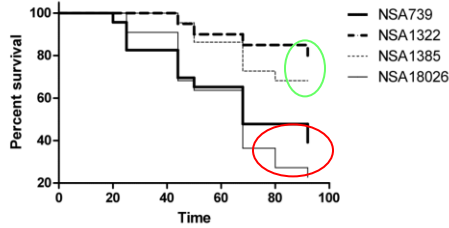
NSA1385, NSA1322: Grade 1
 NSA739, NSA18026: Grade 2-4

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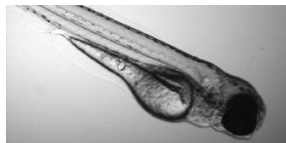
Use of zebrafish to confirm the low virulence of *S. aureus* isolated from DFU Grade 1

LWT embryos injected with 1200 CFU at 30 hpf
 01/07/10



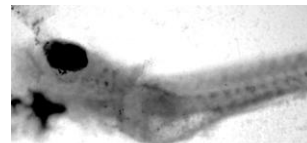
NSA1385, NSA1322: p=NS
 NSA739, NSA18026: p=NS
 NSA1385, NSA1322 vs NSA739,
 NSA18026: p < 0.001

NSA 1385 (Grade 1)



After 48h

NSA 739 (Grade 2-4)

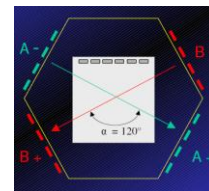
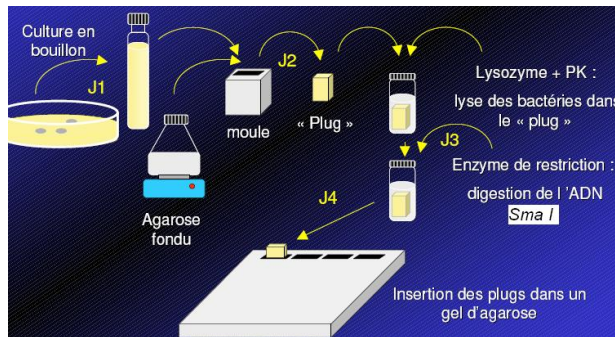


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Clonality of *S. aureus* strains isolated from Grade 1

Pulse Field Gel Electrophoresis

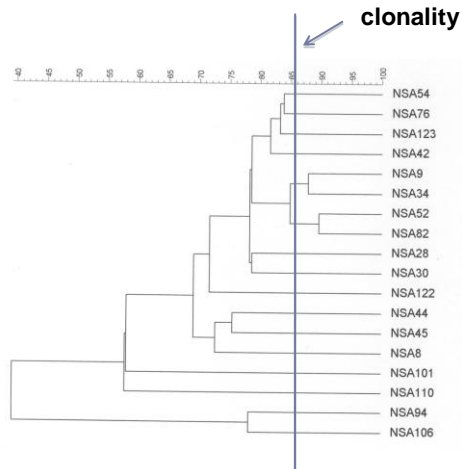


Pulse 30 to 5 sec
 for 30H
 at 6V/cm

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Clonality of *S. aureus* strains isolated from Grade 1



No evident clonality in strains isolated from Grade I

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Optical maps study

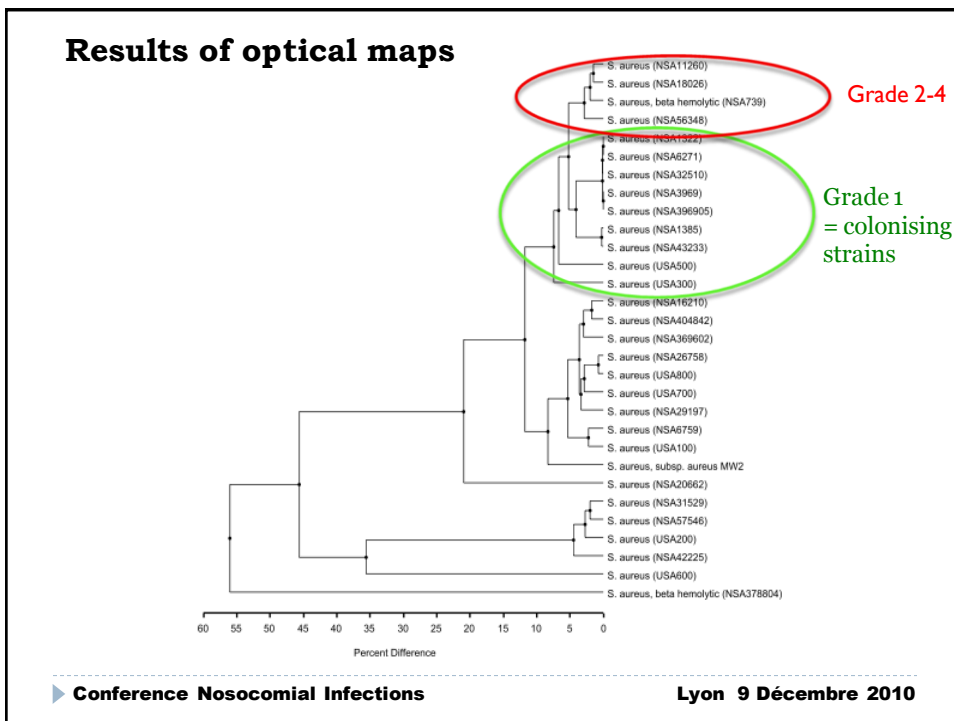
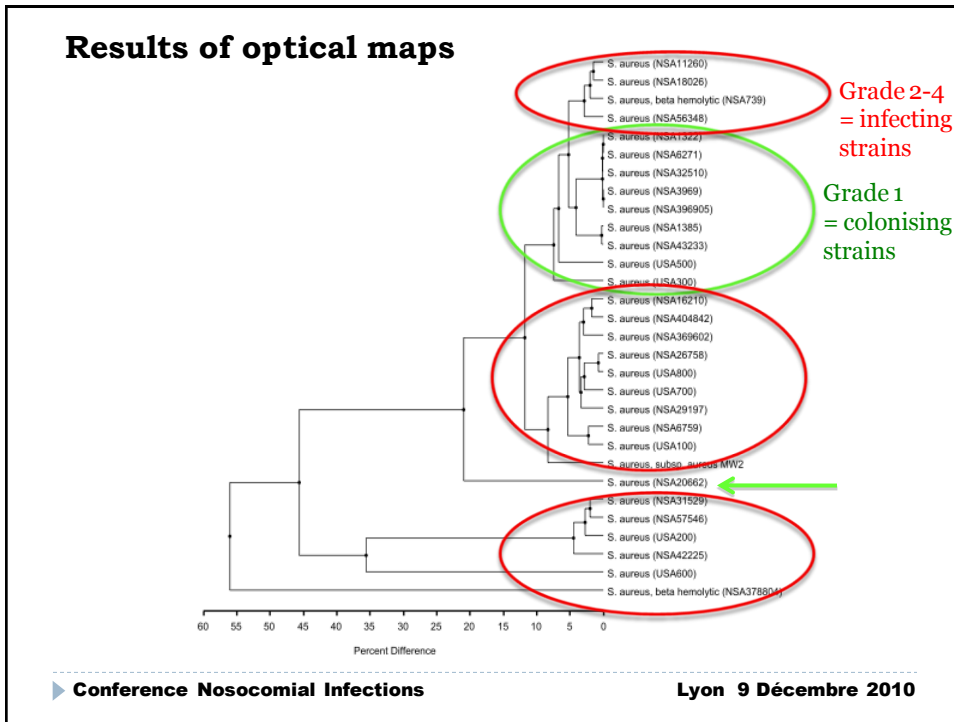
(Coll. OpGen SA, USA)

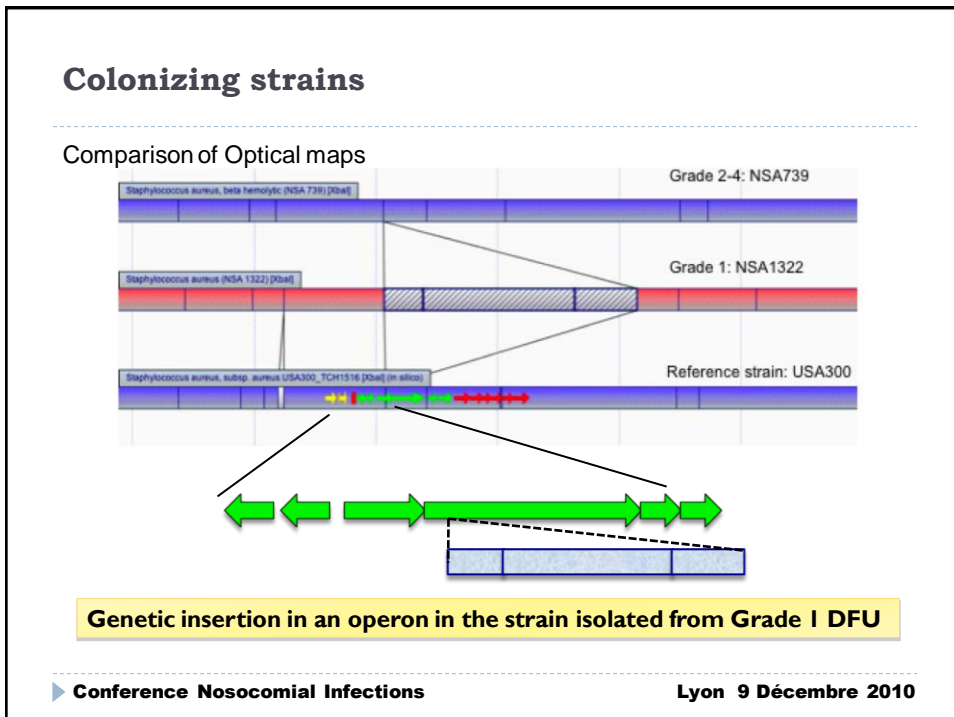
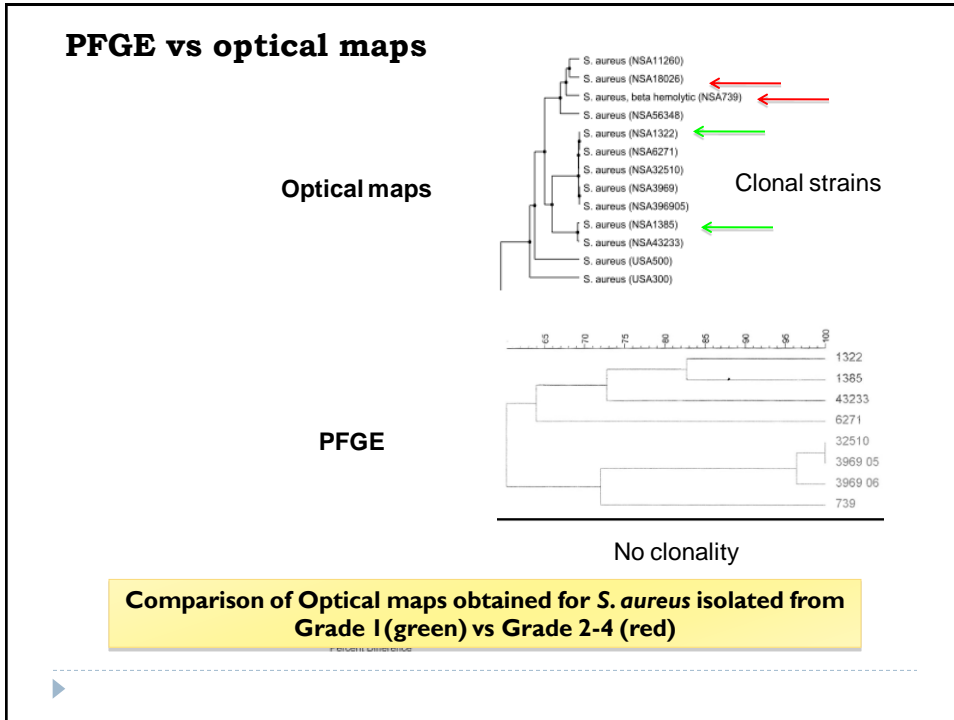
22 patients with DFU where *S. aureus* were isolated:

- 8 strains isolated from Grade 1
- 4 strains isolated from Grade 2
- 7 strains isolated from Grade 3
- 3 strains isolated from Grade 4

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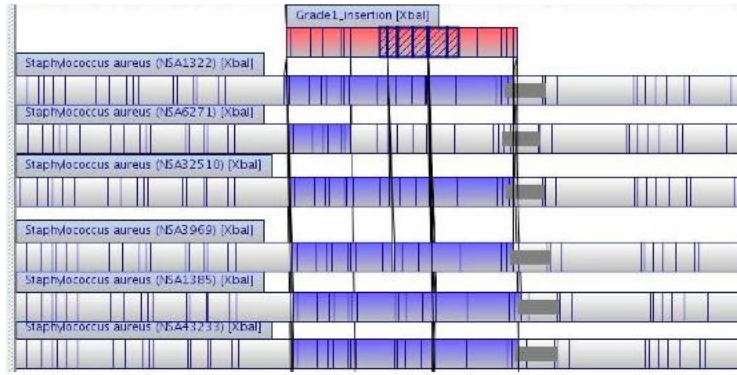
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Alignements of optical maps

All strains isolated from Grade I harbored this insertion

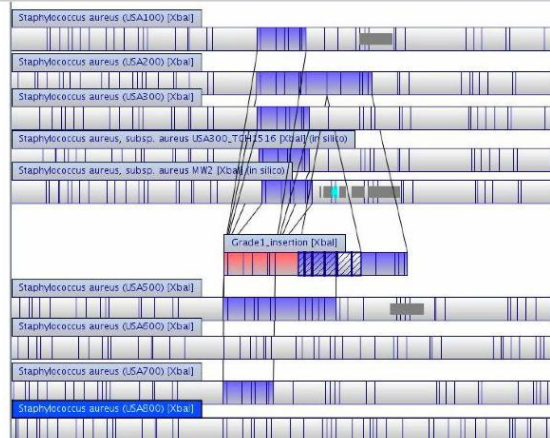


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Alignements of optical maps

No strain isolated from Grade 2-4 (and the reference strains available in PubMed) harbored this insertion



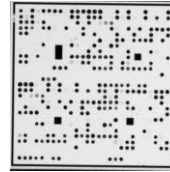
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DNA Array hybridisation kit (Clondiag - Alere)



Strips with array



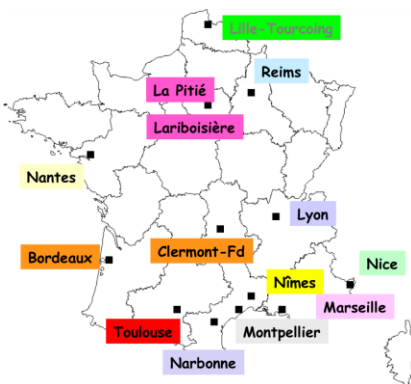
Results after hybridisation

		MLST and agr Group Assignment
Grade 1	NSA1322	agrI_CC8 (score: 92.2 %)
	NSA1385	agrII_CC5 (score: 96.3%)

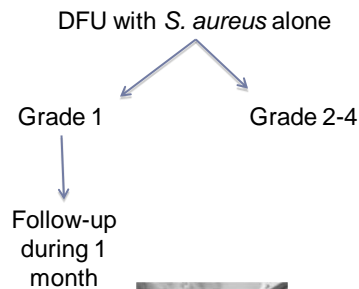
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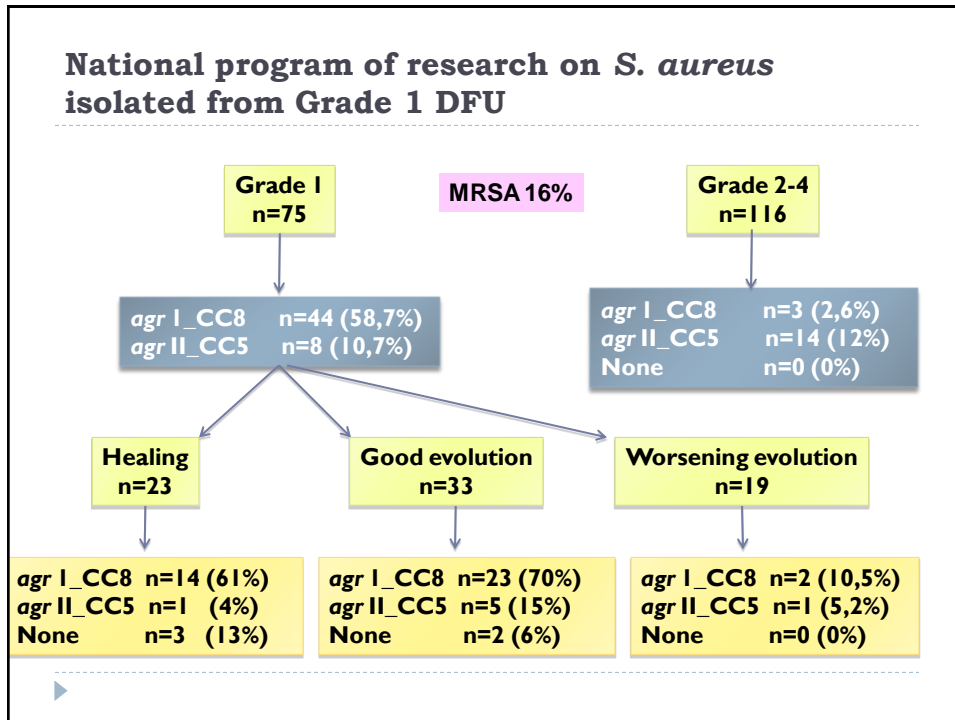
National program of research on *S. aureus* isolated from Grade 1 DFU



Recruitment: April 2008-June 2010
 13 Diabetic foot clinics



Strips with array



Conclusion on *S. aureus* and DFU

- *Characterization of two populations of *S. aureus* on DFU: a colonizing strain and an infecting strain
- *Colonizing strain: Low virulence in two models (*C. elegans* and zebrafish)
- *Differentiation between colonizing and infecting strains: Genetic insertion in colonizing strains and some strain assignments
- *No impact of methicillin resistance

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