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

Infection

Normal
Low virulent bacteria
Resident commensal flora
Transitory bacterial flora

Modification of flora
Virulent bacteria
No healing
Extension

Problematic: The infection must be distinguished to bacterial colonisation

Colonisation

Infection

Bacteria
- bacterial species
- potential of virulence

Host
- general status
- immunity status
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
GRADE 3 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)
MDRO vs non MDRO (p=0.71)

MRSA vs MSSA:
- p=0.036 at 6th month
- p=0.60 after 6 month

Virulence of MRSA?

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

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

MRSA vs MSSA:
- p=0.036 at 6th month
- p=0.60 after 6 month

Virulence of MRSA?

Short Report
Are diabetic foot ulcers complicated by MRSA osteomyelitis associated with worse prognosis?
Outcomes of a surgical series

Table 1: Outcome of surgical treatment

<table>
<thead>
<tr>
<th></th>
<th>MSSA (n = 60)</th>
<th>MRSA (n = 35)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conservative supply, (%)</td>
<td>81 (81.4)</td>
<td>16 (45.7)</td>
<td>0.057</td>
</tr>
<tr>
<td>Wound infection, (%)</td>
<td>29 (48.3)</td>
<td>17 (48.6)</td>
<td>0.91</td>
</tr>
<tr>
<td>Muscle infection, (%)</td>
<td>4 (6.6)</td>
<td>2 (5.7)</td>
<td>0.83</td>
</tr>
<tr>
<td>Number of operative procedures, median (range)</td>
<td>1 (1-4)</td>
<td>1 (1-4)</td>
<td>0.94</td>
</tr>
<tr>
<td>Hospitalization days, median (range)</td>
<td>54.1 (1-192)</td>
<td>24 (1-176)</td>
<td>0.06</td>
</tr>
<tr>
<td>Transfusion, (%)</td>
<td>1 (1.4)</td>
<td>3 (8.6)</td>
<td>0.48</td>
</tr>
<tr>
<td>Healing (days), median (range)</td>
<td>90 (12-305)</td>
<td>129 (23-360)</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Use of *C. elegans* model to evaluate virulence of *S. aureus* isolated from DFU

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

Virulence of MRSA?

**OP50 (O FV)**  
DL50 = 7.1 jrs  
L = 13 jrs

Sotto et al, Diabetes Care 2008
Virulence of MRSA?

**MSSA**
- DL50 = 1.8 jrs
- L = 4.2 jours

**MRSA**
- DL50 = 1.8 jrs
- L = 4.1 jours

Same virulence – No impact of meticillin resistance

*Sotto et al, Diabetes Care 2008*

**Conference Nosocomial Infections**

Lyon 9 Décembre 2010
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

**Comparaison 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

**Infecting strains**

Sotto et al, Diabetes Care, 2008

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

(Coll. S. Renshaw, Sheffield, UK)

**Embryos 30h Post-Fertil.**

**Anaesthesia**

**Micro-injection of bacterial suspension**

**Survival curves**

(Sign of mortality: stop of heartbeat)

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

**NSA1385, NSA 1322: Grade 1**
**NSA739, NSA18026: Grade 2-4**
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

[Graph showing percent survival over time]

- NSA739
- NSA1322
- NSA1385
- NSA18026

**NSA1385, NSA 1322: p=NS**

**NSA739, NSA18026: p=NS**

**NSA1385, NSA 1322 vs NSA739, NSA18026: p <0.001**

**After 48h**

NSA 1385 (Grade 1)  
NSA 739 (Grade 2-4)

---

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

**Pulse Field Gel Electrophoresis**

Culture en bouillon

J1

Lysozyme + PK

J2

lyse des bactéries dans le "plug"

J3

Enzyme de restriction:
digestion de l’ADN

Sma I

J4

Insertion des plugs dans un gel d’agarose

Pulse 30 to 5 sec for 30H at 6V/cm
Clonality of *S. aureus* strains isolated from Grade 1

No evident clonality in strains isolated from Grade 1

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
Results of optical maps

Grade 1 = colonising strains
Grade 2-4 = infecting strains
**PFGE vs optical maps**

**Optical maps**

**Clonal strains**

**PFGE**

No clonality

**Comparison of Optical maps obtained for *S. aureus* isolated from Grade 1 (green) vs Grade 2-4 (red)**

---

**Colonizing strains**

**Comparison of Optical maps**

**Genetic insertion in an operon in the strain isolated from Grade 1 DFU**
All strains isolated from Grade 1 harbored this insertion.

No strain isolated from Grade 2-4 (and the reference strains available in PubMed) harbored this insertion.
DNA Array hybridisation kit (Clondiag - Alere)

| Strips with array | Results after hybridisation |

<table>
<thead>
<tr>
<th>Grade 1</th>
<th>MLST and agr Group Assignment</th>
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</thead>
<tbody>
<tr>
<td>NSA1322</td>
<td>agrI_CC8 (score: 92.2 %)</td>
</tr>
<tr>
<td>NSA1385</td>
<td>agrII_CC5 (score: 96.3%)</td>
</tr>
</tbody>
</table>

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

Recruitment: April 2008-June 2010
13 Diabetic foot clinics
National program of research on S. aureus isolated from Grade 1 DFU

Grade 1
n=75
agr I_CC8  n=44 (58,7%)
agr II_CC5  n=8 (10,7%)

MRSA 16%

Grade 2-4
n=116
agr I_CC8  n=3 (2,6%)
agr II_CC5  n=14 (12%)
None         n=0 (0%)

Healing
n=23
agr I_CC8  n=14 (61%)
agr II_CC5  n=1 (4%)
None         n=3 (13%)

Good evolution
n=33
agr I_CC8  n=23 (70%)
agr II_CC5  n=5 (15%)
None         n=2 (6%)

Worsening evolution
n=19
agr I_CC8  n=2 (10,5%)
agr II_CC5  n=1 (5,2%)
None         n=0 (0%)

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)

*Differenciation between colonizing and infecting strains: Genetic insertion in colonizing strains and some strain assignments

*No impact of methicillin resistance
# Acknowledgements

<table>
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