MRSA communautaires
(entre autre)

Jerome Etienne
Université Claude Bernard Lyon
INSERM E023O
Initially, S. aureus virulent but susceptible to antibiotics

April 10 — Talk show queen Rosie O'Donnell has been sent home after spending five days in a New York City hospital, where she was being treated for a staph infection in her hand.
SCC\textit{mec} containing the \textit{meca} gene coding resistance to methicillin - since 1960
For 40 years, methicillin-resistant S. aureus (MRSA) infections have been in hospitals only

– After 48 hours to admission in hospital
– No specific toxins
The incidence of Hospital-MRSA is known: example for H-MRSA in blood isolates in Europe (EARSSS programme) 1999-2001
Très virulent, le nouveau staphylocoque doré est né

C’est une mutation extraordinaire d’une bactérie déjà très problématique, connue depuis plusieurs années aux États-Unis, qui a atteint l’Europe en 1999.
Présente hors des hôpitaux, cette nouvelle souche bactérienne provoque l’inquiétude du corps médical

Philippe Barraud

C’est une authentique sale bête, dont l’emergence témoigne de l’extraordinaire capacité d’adaptation des bactéries, ce que le professeur Jérôme Etienne appelle « l’intelligence bactérienne ». À la Faculté de médecine Laennec, à Lyon, ce scientifique suisse et ses collègues du Centre national des infections à staphylocoques, ont analysé les gènes de ce que le chercheur n’hésite pas à appeler un super bug, un staphylocoque doré différent de celui qui pose tant de problèmes dans les hôpitaux, en cela qu’il est à la fois plus virulent, résistant aux antibiotiques, et qu’il vit en dehors du milieu hospitalier.

« Sur 20 000 prisonniers, vous en avez 1000 d’infectés: on n’a jamais vu ça »

Jusqu’ici, les staphylocoques étaient des agents infectieux assez banals: germes ubiquitaires, ils sont présents sur l’ensemble de la planète. En fait, 20% à 50% de la population en porte, sur la peau et dans le nez. Il s’agit donc d’un micro-organisme avec lequel nous vivons tous les jours. Jusqu’ici, la problématique virulente de ces germes était en

Patrick Francoli, Division de médecine préventive hospitalière au CHUV.

« La prise en charge des malades sera plus difficile »

Le Temps: Que change l’apparition de ces nouveaux germes pour les soignants?
Patrik Francoli: La prise en charge de patients qui ont des infections à staphylocoques résistants est plus difficile car il y a davantage de risques que les traitements soient inefficaces. Ces germes finissent par faire des complications qui les amènent à l’hôpital. L’autre problème, c’est qu’en plus de gènes dits de résistance, certains se sont dotés de gènes de virulence: ils sont plus invasifs et provoquent des infections plus graves.

- Hospitaliser ces patients comporte-t-il des risques pour les hôpitaux?
- C’est une source de souci, en effet. Si ces patients nous arrivent, ces souches communautaires dangereuses pourraient s’ajouter ou se substituer aux staphylocoques déjà bien assez nombreux à l’hôpital.

- Existe-t-il une résistance ab-
The incidence of C-MRSA is not known. European countries with community-acquired MRSA (clone ST80)
Epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA)

**HOSPITAL**
- **Reservoirs**
  - hospitals
  - long-term care facilities

**COMMUNITY**
- acquired onset

**H-MRSA**
- in the community (health-care MRSA)
  - patients with risk factors
  - contact with patients with risk factors

"true" Community-MRSA
- no health care-associated risk factors
Differences between H-MRSA and C-MRSA

• H-MRSA
  – Endemic clones more specific to each country (e.g., German clone)
  – No specific toxins
  – Diversity of infections in older patients

• C-MRSA
  – Same clones endemic all over Europe
  – Specific toxins
    • Panton Valentine leukocidin (PVL) +++
    • Exfoliative toxins (+)
    • Toxic shock syndrome toxin +
  – Skin and soft tissue infections in young people

Liassine N et al JCM 2004;42:825-8
Naimi T et al JAMA. 2003;290:2976-84.
Vandenesch et al. EID, 2003;9:978-84
Panton Valentine Leukocidin in the Community
PVL has always been in the community, initially in methicillin-susceptible *S. aureus* only

- 1894: discovery by Van de Velde (1) (2) (3)
- 1932: distinguished from hemolysins by Panton and Valentine
- 1936: association of PVL with certain types of human infections
  - Stye, carbuncle, pyaemic infections, primitive suppurative cutaneous infections
- end of the 90's: Highly epidemic strains (PVL + and resistant to methicillin
Diseases associated with PVL production (in MSSA strains)

Clinical Infectious Diseases 1999;29:1128-32

Involvement of Panton-Valentine Leukocidin–Producing *Staphylococcus aureus* in Primary Skin Infections and Pneumonia

Gerard Lina,¹ Yves Piémont,² Florence Godail-Gamot,¹ Michèle Bes,¹ Marie-Odile Peter,¹ Valérie Gauduchon,¹ François Vandenesch,¹ and Jerome Etienne¹

From the ¹Centre National de Référence de Toxémies Staphylococciques, Faculté de Médecine, Lyon; ²Institut de Bactériologie, Université Louis Pasteur, Faculté de Médecine, Strasbourg; and ³Hôpital E. Muller-Moenschberg, Mulhouse, France

- PVL production associated with
  - Primary skin infections (e.g. furunculosis, 95%)
  - Community-acquired pneumonia
• **PVL production and diseases**

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>strains tested (n)</th>
<th>PVL-positive strains n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pneumonia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hospital-acquired pneumonia</td>
<td>13</td>
<td>0 (0)</td>
<td>---*</td>
</tr>
<tr>
<td>community-acquired pneumonia</td>
<td>27</td>
<td>23 (85)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td><strong>Skin infections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>superficial folliculitis</td>
<td>10</td>
<td>0 (0)</td>
<td>---</td>
</tr>
<tr>
<td>impetigo</td>
<td>4</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>finger pulp (felon)</td>
<td>15</td>
<td>2 (13)</td>
<td>NS</td>
</tr>
<tr>
<td>cutaneous abscess</td>
<td>6</td>
<td>3 (50)</td>
<td>.03</td>
</tr>
<tr>
<td>cellulitis</td>
<td>9</td>
<td>5 (55)</td>
<td>.01</td>
</tr>
<tr>
<td>furunculosis</td>
<td>30</td>
<td>28 (93)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td><strong>Other infections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>infective endocarditis</td>
<td>21</td>
<td>0 (0)</td>
<td>---a</td>
</tr>
<tr>
<td>osteomyelitis</td>
<td>13</td>
<td>3 (23)</td>
<td>NS</td>
</tr>
<tr>
<td>urinary tract infection</td>
<td>5</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>enterocolitis</td>
<td>5</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>mediastinitis</td>
<td>5</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>toxic-shock syndrome</td>
<td>9</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
</tbody>
</table>
Panton Valentine leukocidin (Luk): bi-component cytotoxins

\[ \text{lukS-PV} \quad \text{lukF-PV} \]

- Class S proteins
- Class F proteins

Synergistic action to punch the cell membranes

• Synergohymenotrophic toxin: \text{lukS-PV} and \text{lukF-PV}
Phage conversion of Panton-Valentine leukocidin in *Staphylococcus aureus*: molecular analysis of a PVL-converting phage, φSLT

Sachiko Narita, Jun Kaneko, Jun-ichi Chiba, Yves Piémont, Sophie Jarraud, Jerome Etienne, Yoshiyuki Kamio
PVL determinant is on a phage (3 ≠ phages)

Homology 15%

Homology 55%
The Panton Valentine Leukocidin

- Recognition of a specific receptor by LukS
- Incorporation of LukF and oligomerisation

Formation of a $\beta$-barrel octameric pore

Model of an octameric leukocidin pore
Miles et al. Protein Science 2002
PVL toxicity

• **PVL activities** *(Finck-Barbançon et al. 1993 Biochim. Biophys.; Baba Moussa et al. 1999 FEBS Lett.)*
  
  – Formation of pores
  
  – Opening of Ca$^{2+}$ channels

  ![Diagram showing PVL activities]

  Irreversible calcium influx

• **Lysis of host defence cells**
  
  – Polymorphonuclear cells *(release of inflammatory mediators from basophils and neutrophils)*
  
  – Monocytes and macrophages

![Image of cells, possibly showing lysis]
PVL is a pore-forming toxin: leading factor for the development of primary skin infections

• Necrotizing toxin when injected intradermally in rabbit
• Leucotoxic by pore induction
Types of infections associated with PVL+ C-MRSA

• Mainly skin and soft tissue infections, usually with no samples done for the lab (except if surgical drainage).

Community-acquired infections associated with PVL-positive *S. aureus* --> Need to ask for samplings
C-MRSA infections with PVL could be severe

Osteomyelitis

Pneumonia

These cases are rare and need specific treatment
Necrotizing pneumonia due to CA-MRSA

Hemorrhagic and necrotic lesions

Non necrotic lesions

Parenchyma

Bronchioli

Parenchyma

diffuse alveolar damage
Adhesion to human bronchi injured ex vivo

Pneumonia PVL-
Isolate 333

Necrotizing pneumonia PVL+. Isolate 557

Pieces of bronchial tissues were damaged by using a probe. Bacterial suspension of strains were added for 1 hour at 37°C. Infected bronchial tissues were then fixed and stained.
Hypothetic pathogenesis

- The initial viral infection leads to the desquamation of ciliated cells
- *S. aureus* strains adhere to basal cells, as shown in mice

PVL+ *S. aureus* strains adhere specifically on collagen I and IV and on laminin

dede Bentzman S et al JID 2004
Staphylococcus aureus necrotizing pneumonia: a well recognized entity
Association between Staphylococcus aureus strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients

- Necrotizing pneumonia associated with Panton Valentine Leukocidin (PVL) + S. aureus strains:
  - occurs in children and young adults
  - is preceded by a viral-like illness
  - is characterized by hemoptysis, leucopenia, necrotizing lesions and high lethality rate
Survival of patients

Deaths: PVL+ 75%, PVL- 47%

Median survival time
PVL+: 4 days
PVL-: 25 days
$P = 0.005$
Study of 55 patients with extensive PVL+ S. aureus pneumonia

- collected between 1988 and 2004
- 24 females/27 males
- median age: 15 years
- initial viral infection (flu virus, RSV)
- mortality rate: 47%
  - lower rate
  - median survival time: 21 days, 95% CI [0.5 - 47.5]
Survival after Pneumonia

Median: 21 days

% of survival

Days
Survival after Pneumonia

Question to investigate: factors associated with early deaths

Survival Function

Median: 21 days

Survival Function

Censored
Survival after Pneumonia

Question to investigate: factors associated with early deaths

Group I

Group II

Days

% of survival

Median: 21 days

Survival Function

Censored
Summary of factors associated with high mortality*

- Hemoptysis
- ARDS
- Low PaO2/FiO2
  - (Scarlatiniform rash)
- Low WBC
- Low platelet count
- High creatinemia

*Based on univariate analysis
How to block the effect of PVL in case of necrotizing pneumonia?

1. stop the PVL production with the used of clindamycin or linezolid,
2. stop the PVL effect by using of antibodies.
Antibodies against PVL according to the age

Treshold of positivity
Antibodies against PVL in infected patients

Infections with *PVL-* *S. aureus*  
Infections with *PVL+* *S. aureus*

P< 0.001
Commercial intravenous immunoglobulins (IVIg) contains PVL-specific antibodies

JID, 2004, 189:346-53

Graph showing the relationship between IVIg (mg/L) and anti-LukF and anti-LukS antibodies. The graph indicates that at 10,000 U/L, the levels of anti-LukF and anti-LukS increase with increasing IVIg concentration.
IVIg inhibition of PVL-induced ethidium bromide uptake by PMNs

---> no clinical trials
Back to PVL+ C-MRSA
How to measure the incidence of C-MRSA in Europe?

• From bacteriological criteria
  – For the major clones: specific antibiotic resistance patterns of European C-MRSA with:
    • PVL genes
    • tst gene
  – The antibiotic resistance pattern differs with those of H-MRSA
Typical European PVL-MRSA pattern

P: penicillin G  
OX: oxacillin  
Fox: cefoxitin  
Va: vancomycin  
L: lincomycin  
E: erythromycin  
Pt: pristinamycin  
Tet: tetracycline  
FA: fusidic ac.  
C: chloramphenico  
PEF: pefloxacin  
Sxt: cotrimoxazole  
Ft: furans  
RA: rifampicin  
TM: tobramycin  
GM: gentamicin  
FOS: fosfomycin

**Heterogeneous** resistance to methicillin (but FOX diameter <23 mm)  
**Susceptible** to fluoroquinolones, tobramycin, gentamicin  
**Resistant** to kanamycin, fusidic acid (+/- to tetracyclines)
Detection from computer database of the specific antibiotic resistance profile of the PVL-C-MRSA ONERBA Study) - 18 French hospitals

<table>
<thead>
<tr>
<th></th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>2,647</td>
<td>2,568</td>
<td>1,333</td>
</tr>
<tr>
<td>PVL pattern</td>
<td>21 (0.8%)</td>
<td>17 (0.8%)</td>
<td>9 (0.7%)</td>
</tr>
<tr>
<td>Available strains</td>
<td>11</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>PVL producing strains</td>
<td>11</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Single PGFE pattern</td>
<td>9</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>other PFGE pattern</td>
<td>2 (USA)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
### PVL producing MRSA in France
2nd ONERBA Study 2004

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total</th>
<th>Hospitals</th>
<th>Private labs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>13840</td>
<td>100</td>
<td>11126</td>
</tr>
<tr>
<td>MRSA</td>
<td>3901</td>
<td>28</td>
<td>3249</td>
</tr>
<tr>
<td>PVL pattern</td>
<td>56</td>
<td>1.4</td>
<td>55</td>
</tr>
<tr>
<td>PVL +</td>
<td>48*</td>
<td></td>
<td>47</td>
</tr>
</tbody>
</table>

* 6 strains to be tested, 2 strains not available

Patients with PVL+ C-MRSA are mainly detected in hospitals
New emerging clones are detected: with the tst gene
Characterization of the clones of the PVL+ C-MRSA
Identification and diversification of the PVL+ C-MRSA clones

• A clone is recognized by determination of:
  – sequence type
  – spa type
  – SCCmec type
  – agr type
Diversification of PFGE subtypes of PVL+ CA-MRSA of ST80
ST and *agr* types of 228 PVL+ CA-MRSA isolates from the French collection

- **203 isolates *agr3* (89%):**
  - 16 isolates ST30: Australia, New Zealand, Samoa, China, Polynesia
  - 154 isolates ST80: Europe, Algeria
  - 29 isolates ST1: USA+++
  - 4 isolates ST93: Australia
  - 1 isolates ST37: Europe
- **22 isolates *agr1* (9.6%):**
  - 14 isolates ST8: USA +++, France (one isolate), Switzerland
  - 3 isolates ST59: USA, Europe
  - 3 isolates ST377: Europe
  - 2 isolates ST22: Europe
- **3 isolates *agr2* (1.4%):**
  - 5 isolates ST5: Switzerland and Algeria
### Country or continent of detection

<table>
<thead>
<tr>
<th>ST</th>
<th>USA</th>
<th>Europe</th>
<th>Oceania</th>
<th>Africa</th>
<th>Asia</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST1</td>
<td>USA ++</td>
<td>Trump</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST8</td>
<td>USA +++</td>
<td>Trump</td>
<td>The Netherlands, France, Switzerland</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST22</td>
<td></td>
<td>Trump</td>
<td>The Netherlands, Germany</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST30</td>
<td>USA +++</td>
<td>Trump</td>
<td>The Netherlands</td>
<td>Australia, New Zealand, Tahiti, Samoa</td>
<td></td>
</tr>
<tr>
<td>ST37</td>
<td></td>
<td>The Netherlands</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST59</td>
<td>USA +</td>
<td>The Netherlands</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST72</td>
<td>USA +</td>
<td>The Netherlands</td>
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<tr>
<td>ST80</td>
<td>Europe ++</td>
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<tr>
<td>ST93</td>
<td></td>
<td>Australia</td>
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<tr>
<td>ST5</td>
<td></td>
<td>Switzerland</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td></td>
<td>The Netherlands, Switzerland, France</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The different clones of PVL+ CA-MRSA (6 STs in 2003, 11 STs in 2004)*
eBurst analysis (www.mlst.net)

Transfer of the PVL phage and/or the SCCmec type IV element
PVL+ C-MRSA are directly transmitted

- after skin to skin contact including
  - sex
  - hand-to-hand combat training
- not protected infections
- incision of the furuncle by the patient himself
- mainly between sport team members, prisoners
Transmission of C-MRSA after skin-to-skin contact during a match
PVL+ C-MRSA are indirectly transmitted

- through contact by touching objects (i.e., towels, sheets, linen, pillows, wound dressings, clothes, workout areas, sports equipment) contaminated by the infected skin of a person with MRSA.
Transmission of C-MRSA through indirect contact by touching objects

- Scanning electron microscopy of wood sample taken from the seating area of a sauna with known MRSA-positive surface culture (from Baggett HC et al J Infect Dis. 2004;189:1565-73)
Numerous outbreaks have been reported

- in social minorities (Indians, Aborigens, etc.)
- in IV drug-abusers
- in the gay community
- in athletic teams
- in military camps
- in prisons (contact with prisoners is a risk factor for C-MRSA infection)
Prisoners and the risk of transmission of C-MRSA

• TJ Dominguez JABFP 2004;17:220-6
• 10 patients in a health care clinic in San Antonio between 2002-2003
  – 5 have been incarcerated
  – 5 have been in contact with (released) prisoners
Attack rate in case of PVL+ C-MRSA outbreak

- high: 1697 prisoners out of 20 000 at the Los Angeles County Jail
  – the incidence of MRSA was 74% in 2002
- 10 cases for 100 football players
- 11 cases for 1000 soldiers in a military camp
- furuncles were thought to be "spiders bites"
Outbreaks with C-MRSA

- Very few outbreaks in Europe:
  - Example in the city of Lannion (France) Between July 2002 and February 2003: 47 cases in 11 families (total of 67 persons)

<table>
<thead>
<tr>
<th>Family</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
<th>F11</th>
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</thead>
<tbody>
<tr>
<td>Total number of persons</td>
<td>5</td>
<td>3</td>
<td>10</td>
<td>4</td>
<td>10</td>
<td>4</td>
<td>12</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Total number of infected patients</td>
<td>1</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>11</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Attack rate within a family: 59%
Nasal carriage with PVL+ C-MRSA

- Incidence low: 2-3%
- All carriers are infected patients
New emerging C-MRSA clone

with the toxic-shock syndrome toxin (tst)
Detection of toxic shock syndrome toxin positive MRSA (ST5)

<table>
<thead>
<tr>
<th></th>
<th>agr1</th>
<th>agr2</th>
<th>agr3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA-</td>
<td>1</td>
<td>5</td>
<td>69</td>
<td>75</td>
</tr>
<tr>
<td>mecA+</td>
<td>0</td>
<td>25</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>30</td>
<td>71</td>
<td>102</td>
</tr>
</tbody>
</table>

agr alleles and mecA gene in tst+ S. aureus strains (France 2002-2003)
Similar PFGE types of \textit{tst+} \textit{S. aureus} isolates

\textbf{agr 2, meca-}

\textbf{meca gene}

\textbf{agr 2, meca+}
In summary

- PVL + isolates are associated with frequent or severe infections such as necrotizing pneumonia
- PVL alone is not sufficient to induce cell damages
- PVL+ isolates adhere to damaged human airway tissue, especially on exposed basement membrane (role of previous viral infection)
In summary

• C-MRSA from Europe
  – are mainly PVL positive and correspond to the European clone ST80
  – are rarely detected among MRSA (≈ 1%)
  – are highly epidemic, but few outbreaks are reported
    – nasal carriage is unfrequent

• New strategy to develop to stop the spreading of C-MRSA
Thanks to

• INSERM E0230, Lyon, France
  – Michèle Bes, Gérard Lina, François Vandenesch
• Vincent Jarlier & Jerome Robert, ONERBA, France
• Wim Wannet, RVLM, Netherlands