MRSA communautaires *(entre autre)*

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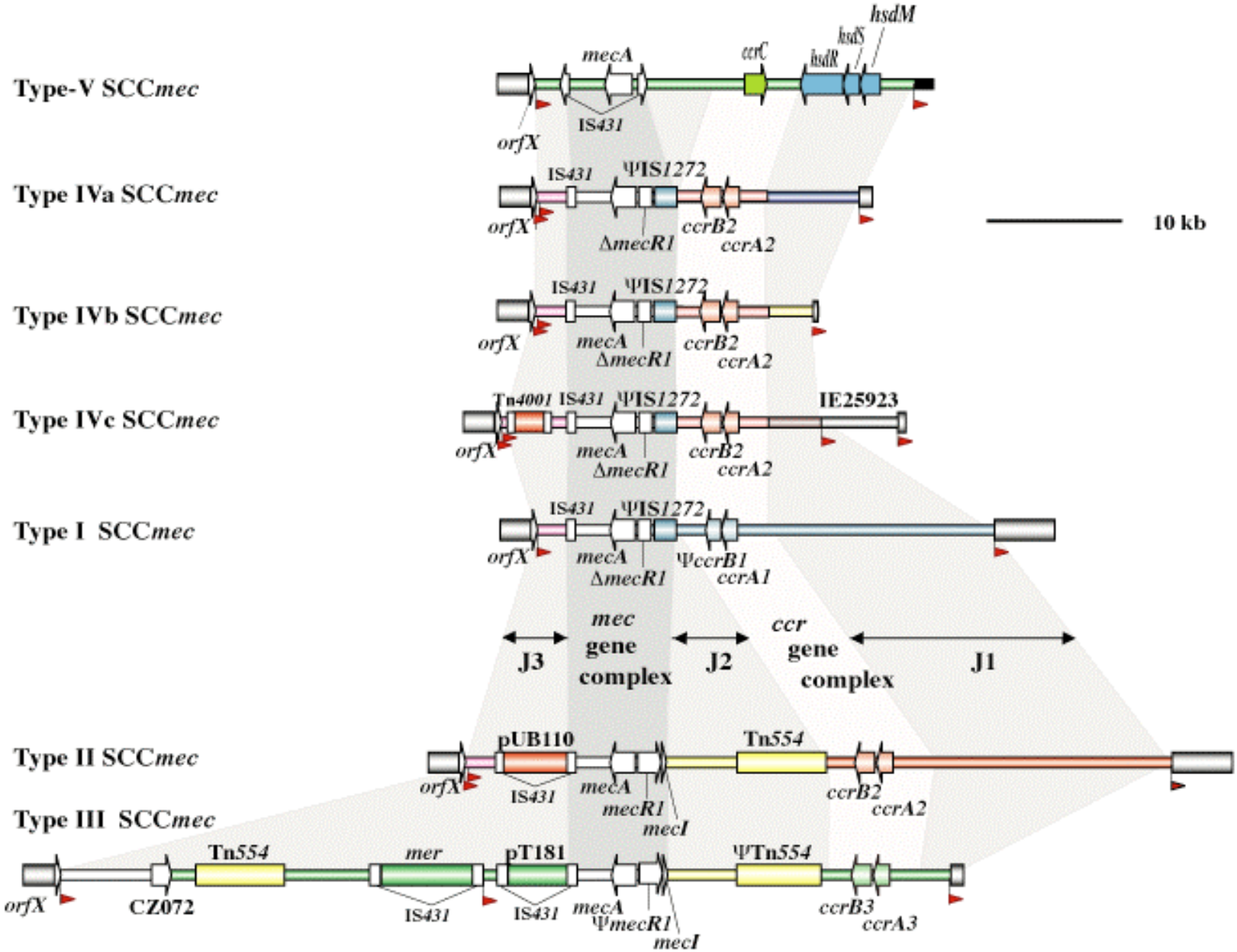
INSERM E023O

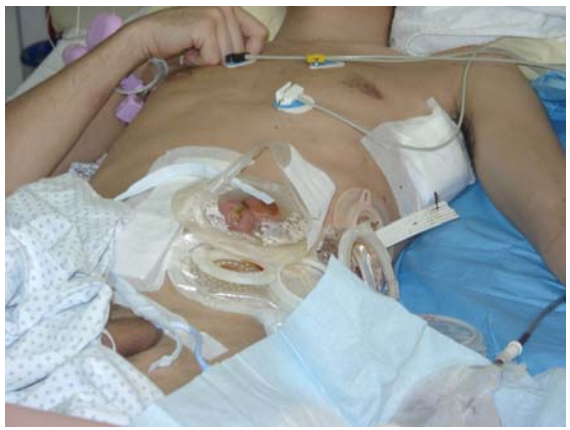
Initially, S. aureus virulent but susceptible to antibiotics

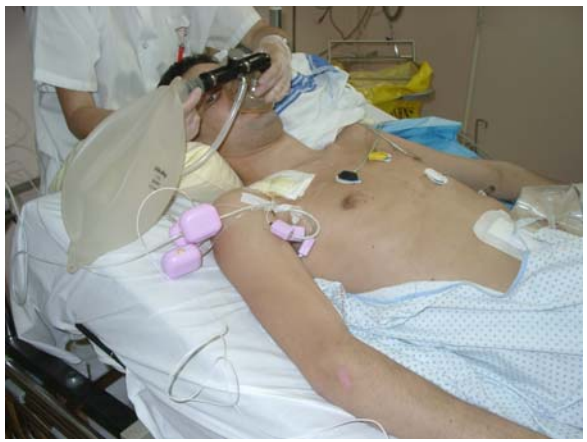
April 10 — Talk show queen Rosie O'Donnell has been sent home after spending five days in a New York City hospital, where she was being treated for a staph infection in her hand

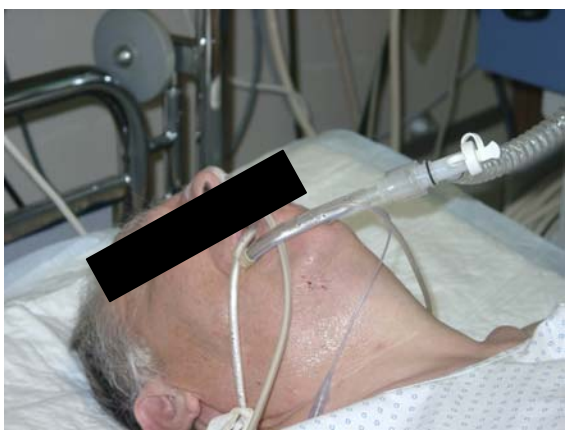
SCC*mec* containing the *mecA* gene coding resistance to methicillin - since 1960

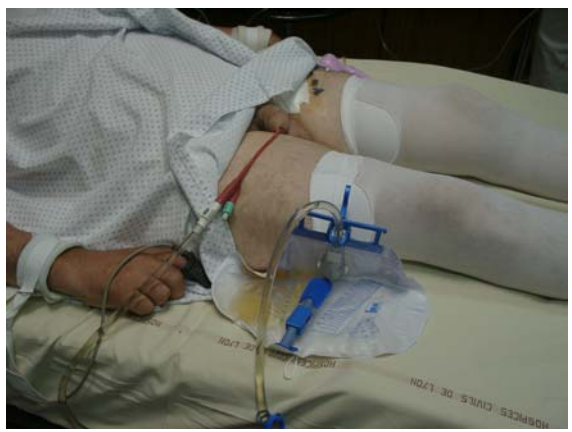


For 40 years, methicillin-resistant *S. aureus* (MRSA) infections have been in hospitals only



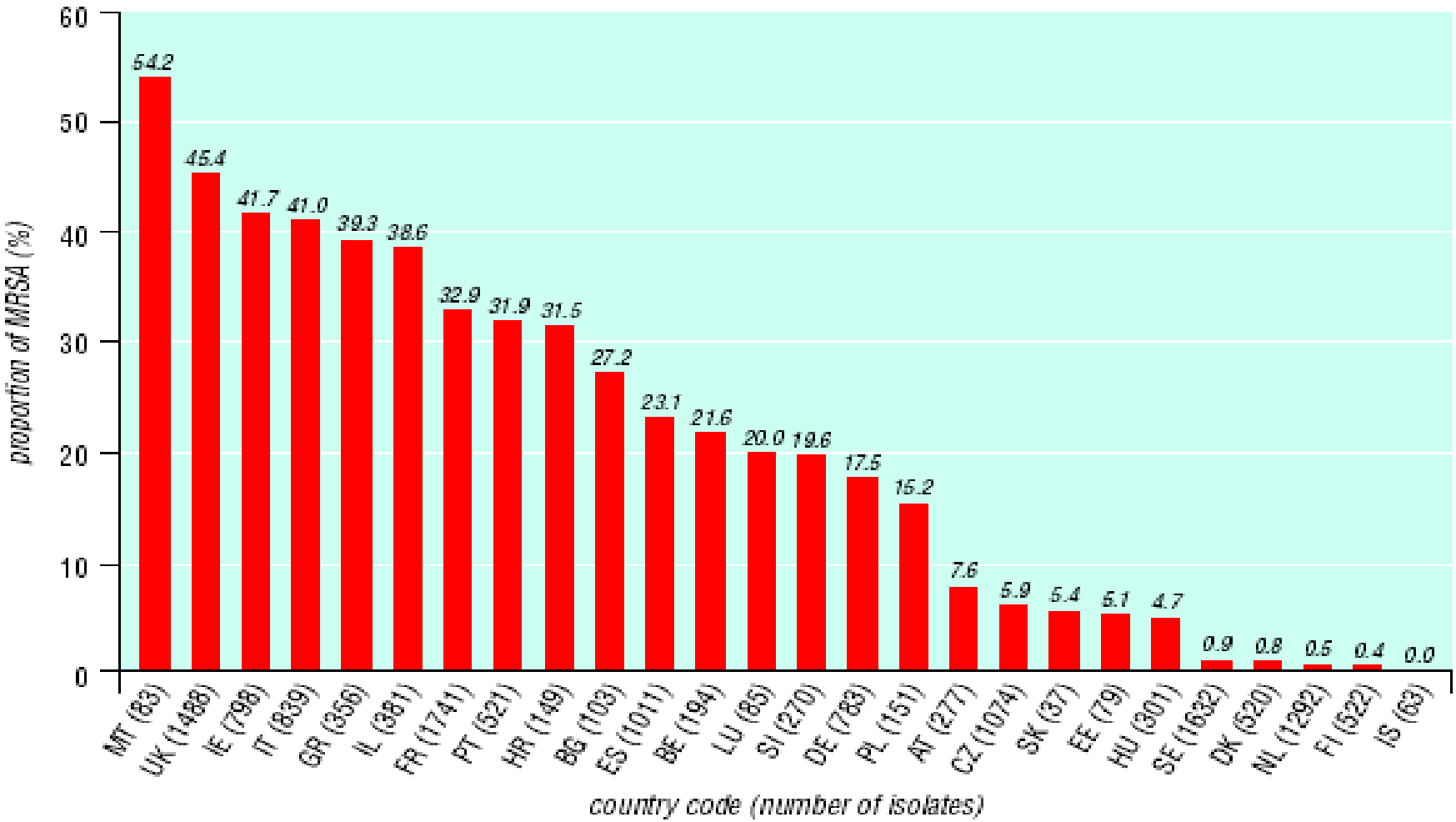






- After 48 hours to admission in hospital
- no specific toxins

The incidence of Hospital-MRSA is known: example for H-MRSA in blood isolates in Europe (EARSS programme) 1999-2001



In 1999, the emergence of community-MRSA

Très virulent, le nouveau staphylocoque doré est né

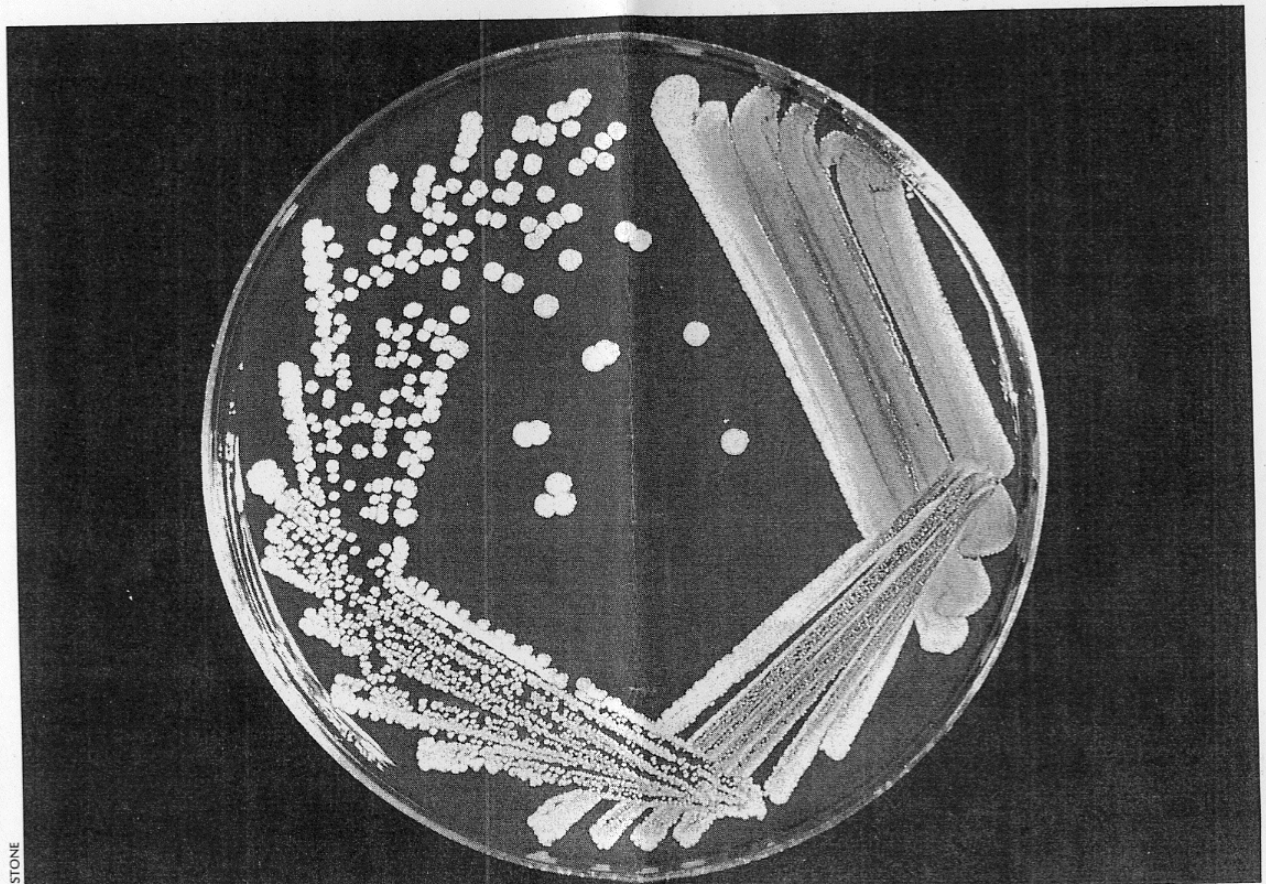
C'est une mutation extraordinaire d'une bactérie déjà très problématique, connue depuis plusieurs années aux Etats-Unis, qui a atteint l'Europe en 1999. Présente hors des hôpitaux, cette nouvelle souche bactérienne provoque l'inquiétude du corps médical

Philippe Barraud

C'est une authentique sale bête, dont l'émergence témoigne de l'extraordinaire capacité d'adaptation des bactéries, ce que le professeur Jérôme Etienne appelle «l'intelligence bactérienne». A 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 principale de ces germes était que



KEYSTONE

Le Staphylococcus aureus. *«Ce qui est vraiment nouveau, explique le professeur Etienne de la Faculté de médecine Laennec, à Lyon,* ARCHIVES

QUESTIONS À

Patrick Francioli, Division de médecine préventive hospitalière au CHUV.

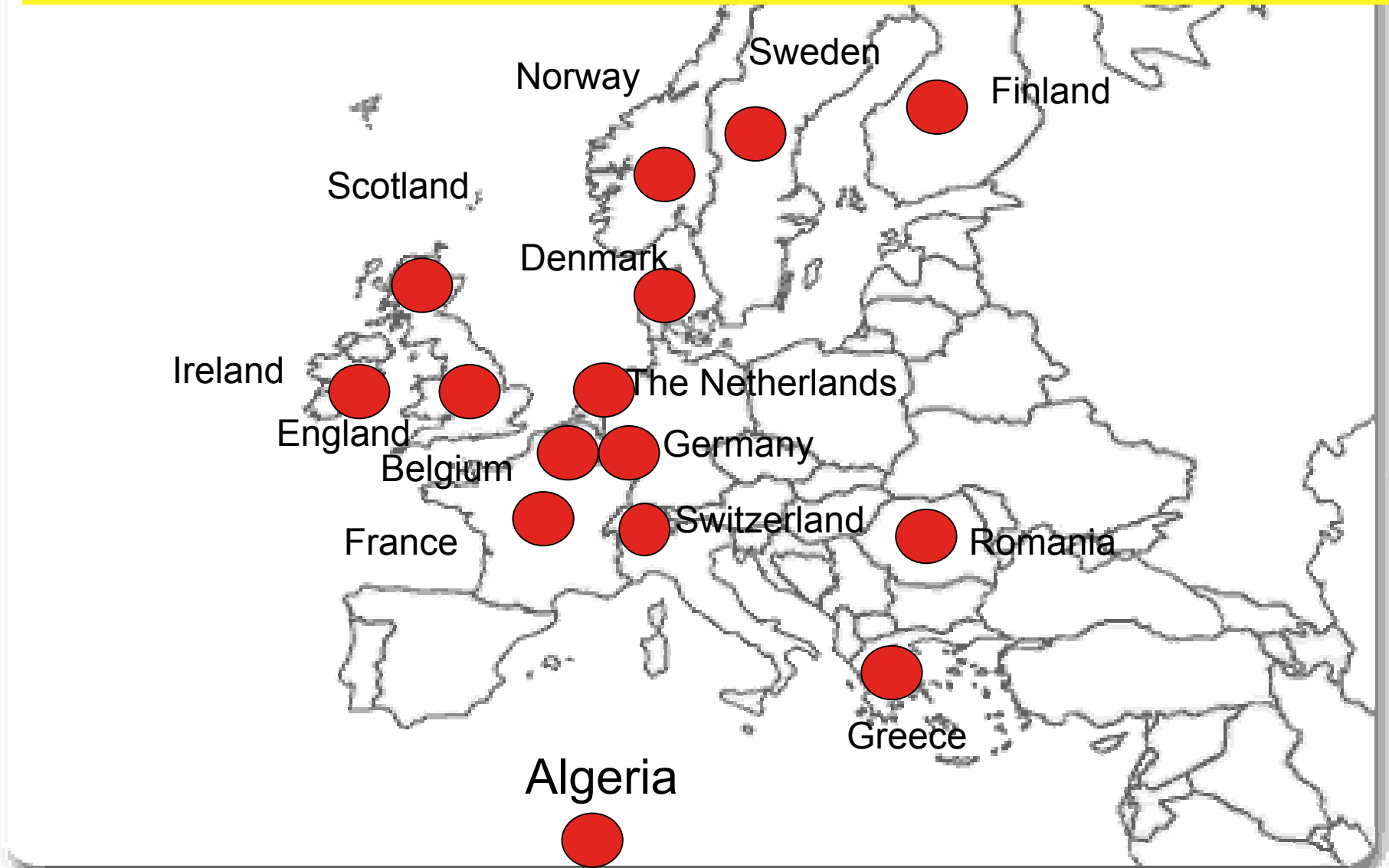
«La prise en charge des malades sera plus difficile»

Le Temps: Que change l'apparition de ces nouveaux germes pour les soignants?
Patrick Francioli: 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 gens 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.

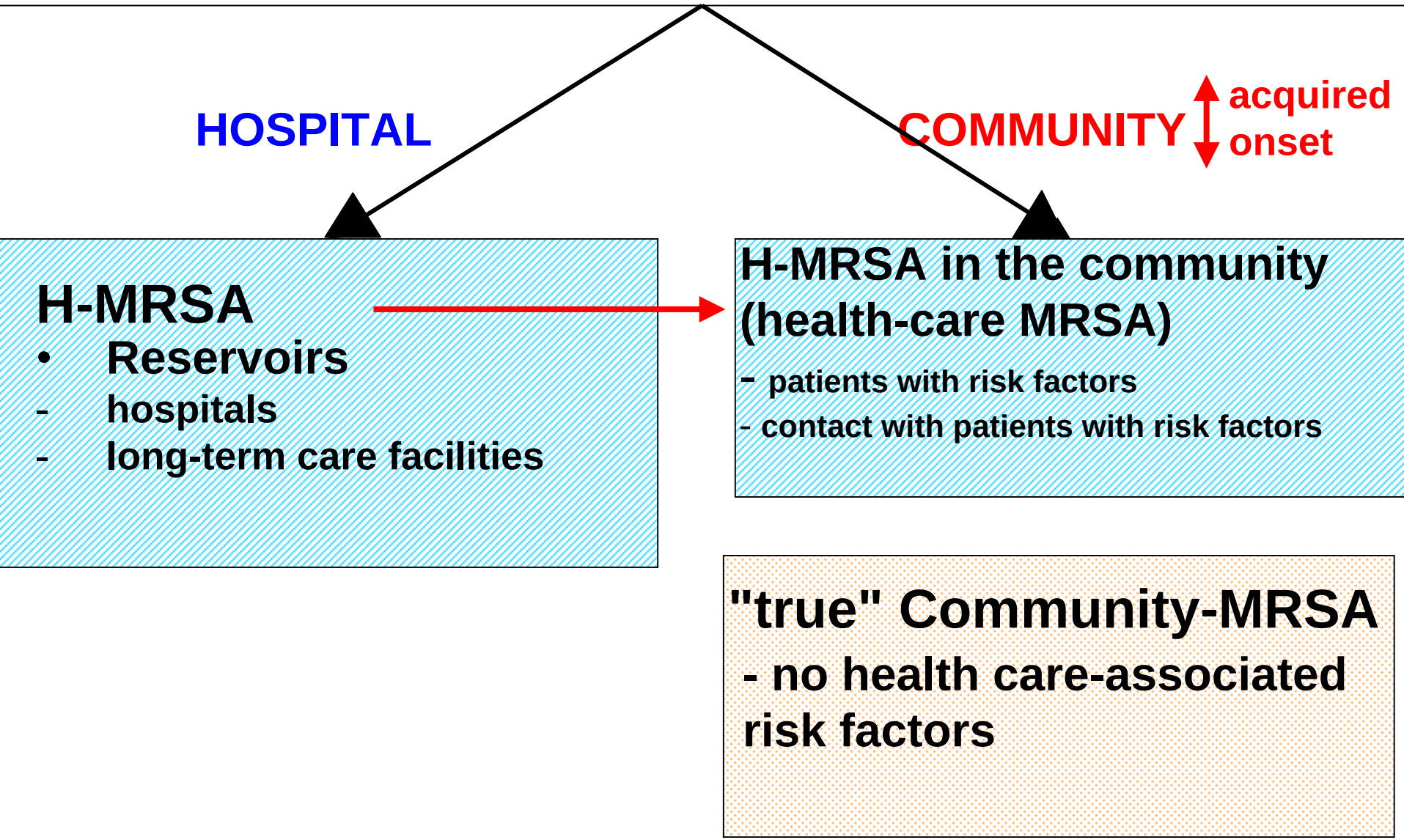
– Hospitaliser ces patients comporte-t-il des risques pour les hôpitaux?
– C'est une source de souci, en effet. Si ces patients nous arrivent, ces souches communautaires dangereuses pourraient s'ajouter ou se substituer aux staphylocoques déjà bien assez nombreux à l'hôpital.

– 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)



Differences between H-MRSA and C-MRSA

- H-MRSA
  - Endemic clones more specific to each country (eg 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,[3] Valérie Gauduchon,[1] François Vandenesch,[1] and Jerome Etienne[1]

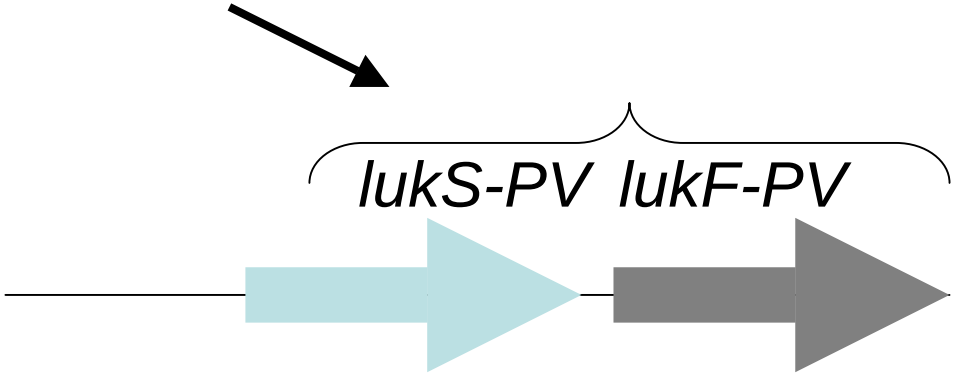
From the [1]Centre National de Référence de Toxémies Staphylococciques, Faculté de Médecine, Lyon; [2]Institut de Bactériologie, Université Louis Pasteur, Faculté de Médecine, Strasbourg; and [3]Hôpital E. Muller-Moenschberg, Mulhouse, France

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

- PVL production and diseases

Type of infection	strains tested (n)	PVL-positive strains n	(%)	*P* value
Pneumonia				
hospital-acquired pneumonia	13	0	(0)	---*
community-acquired pneumonia	27	23	(85)	< .001
Skin infections				
superficial folliculitis	10	0	(0)	---
impetigo	4	0	(0)	NS
finger pulp (felon)	15	2	(13)	NS
cutaneous abscess	6	3	(50)	.03
cellulitis	9	5	(55)	.01
furunculosis	30	28	(93)	< .001
Other infections				
infective endocarditis	21	0	(0)	---ą
osteomyelitis	13	3	(23)	NS
urinary tract infection	5	0	(0)	NS
enterocolitis	5	0	(0)	NS
mediastinitis	5	0	(0)	NS
toxic-shock syndrome	9	0	(0)	NS

Panton Valentine leukocidin (Luk): bi-component cytotoxins



- Class S proteins
- Class F proteins

synergistic action to punch the cell membranes

•Synergohymenotropic toxin: *lukS*-PV and *lukF*-PV

ELSEVIER

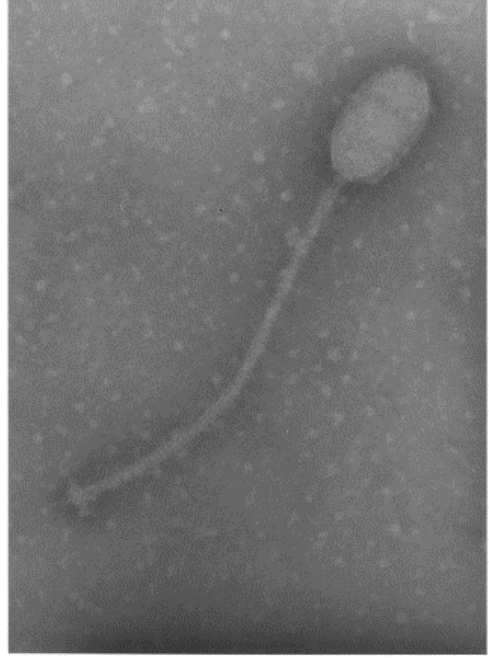
Gene 268 (2001) 195–206

GENE
AN INTERNATIONAL JOURNAL ON GENES AND GENOMES

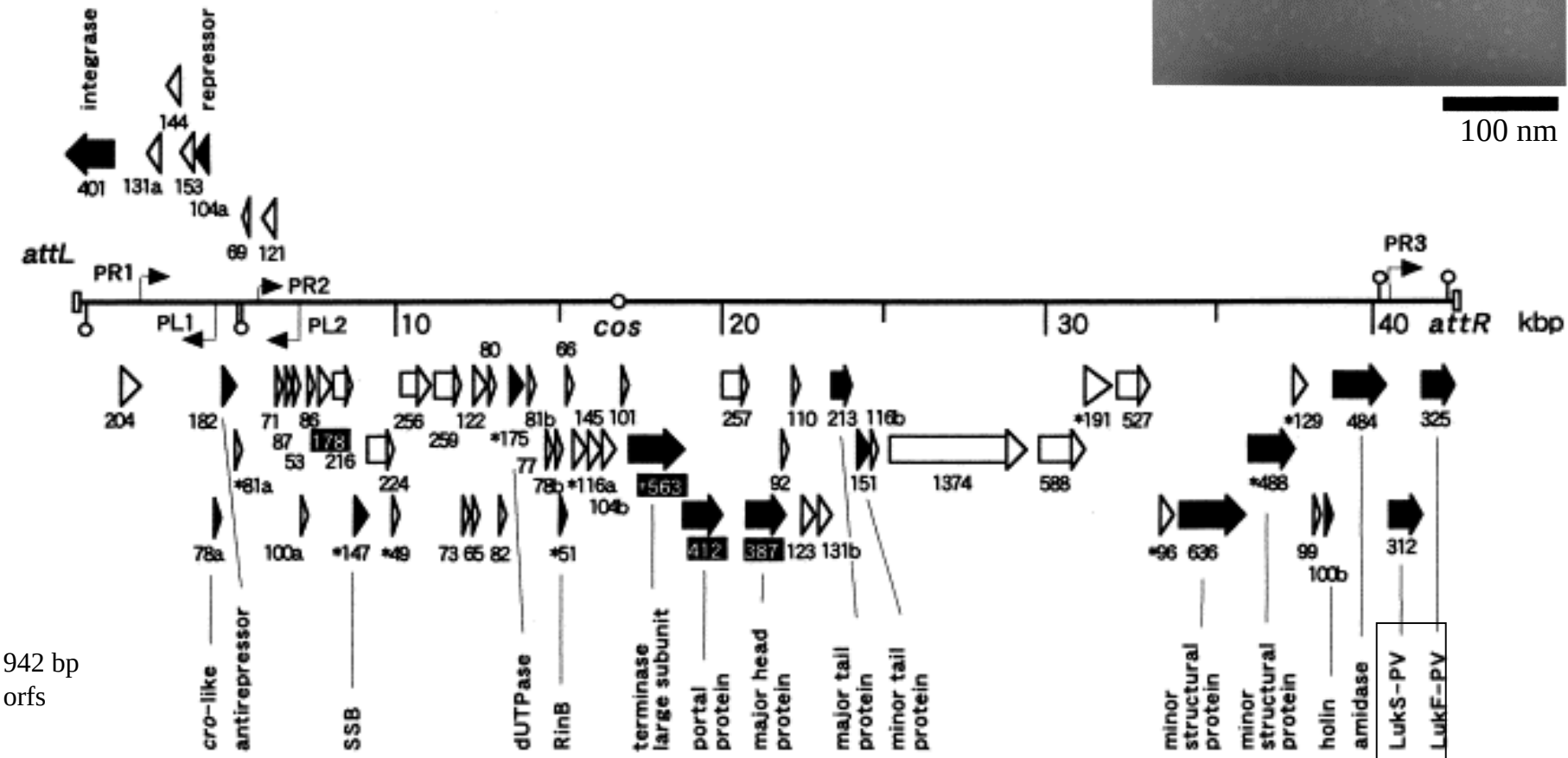
www.elsevier.com/locate/gene

Phage conversion of Panton-Valentine leukocidin in *Staphylococcus aureus*: molecular analysis of a PVL-converting phage, ϕSLT

Sachiko Narita[a], Jun Kaneko[a], Jun-ichi Chiba[a], Yves Piémont[b], Sophie Jarraud[c], Jerome Etienne[c], Yoshiyuki Kamio[a,*]

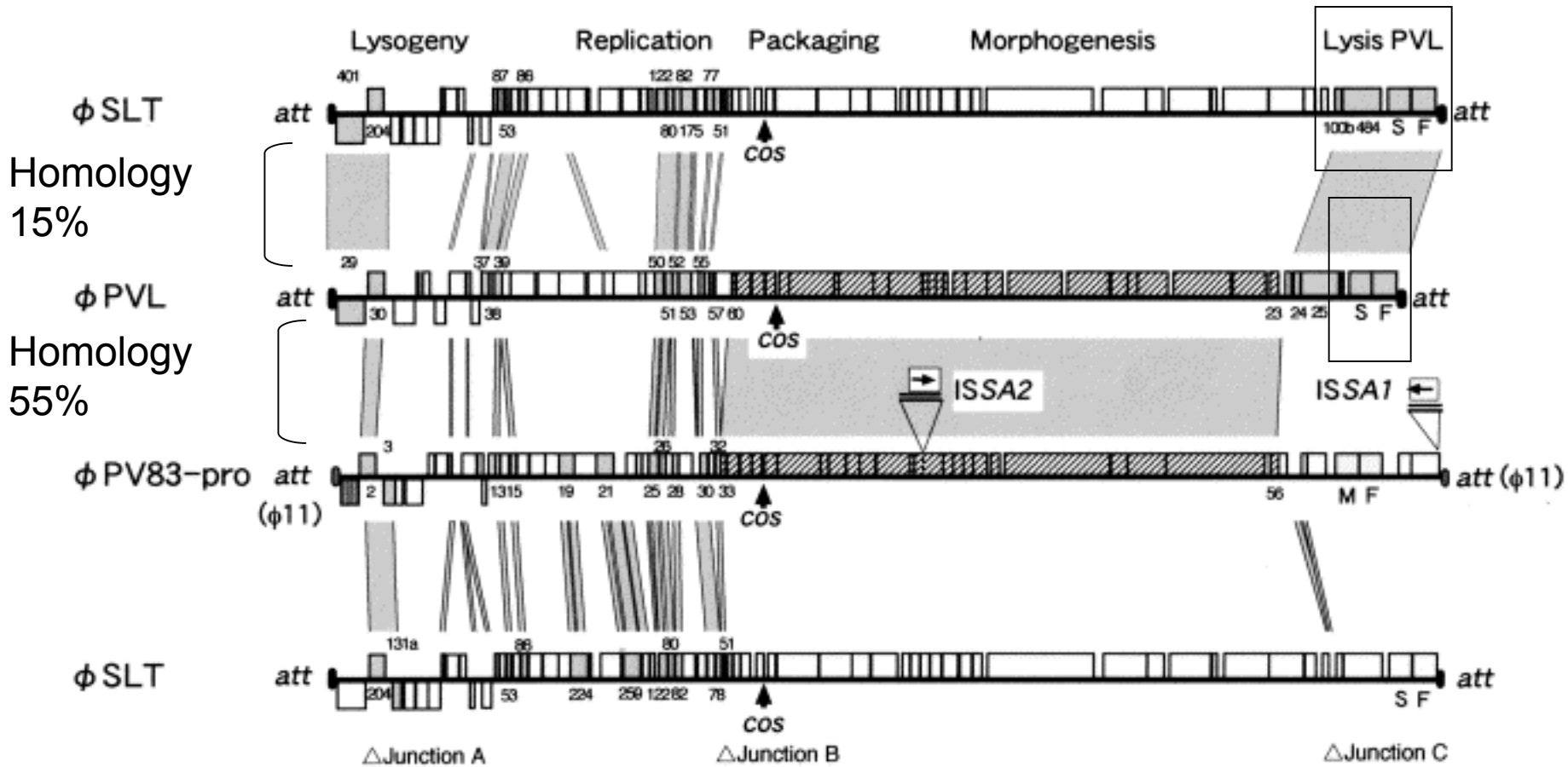


100 nm



42 942 bp
62 orfs

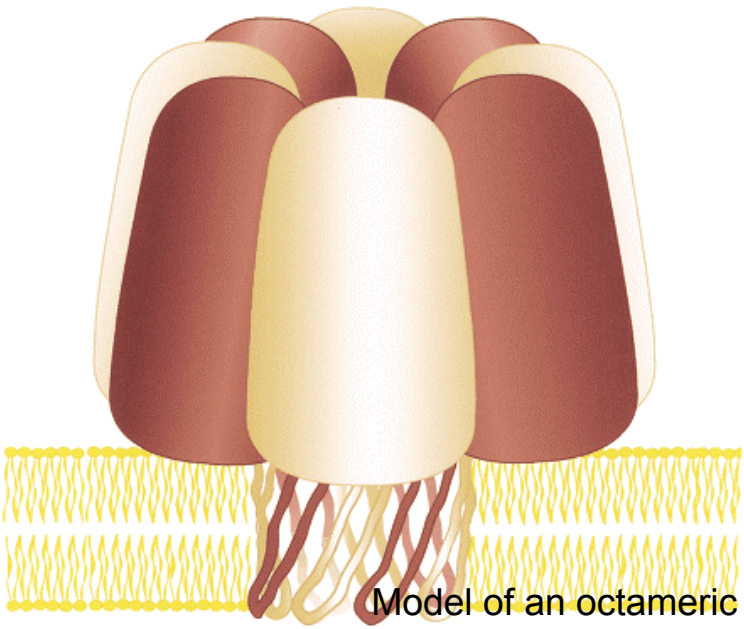
PVL determinant is on a phage (3 ≠ phages)



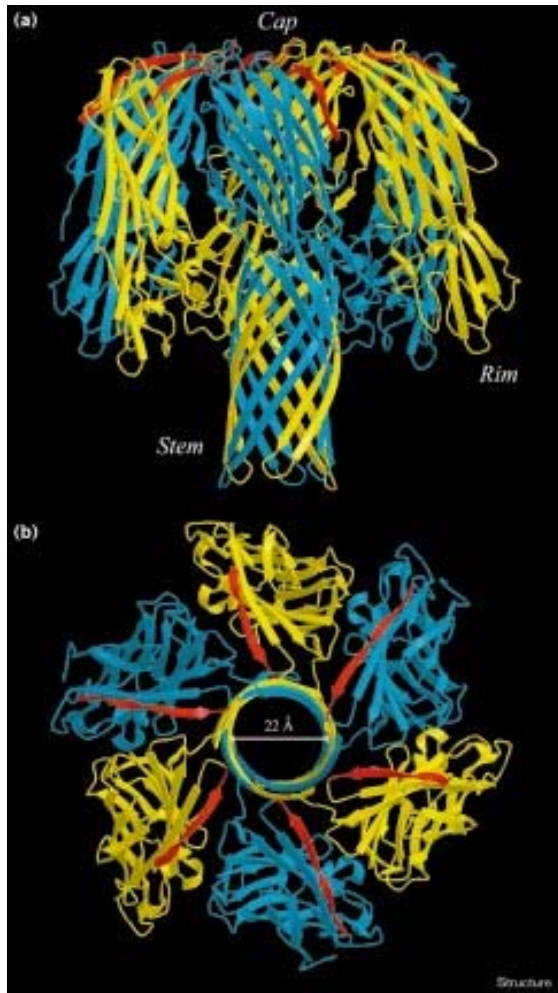
The Panton Valentine Leukocidin

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

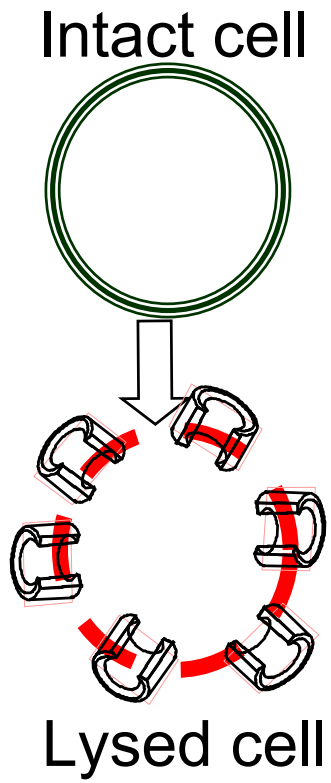
➡ Formation of a β-barrel octameric pore



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



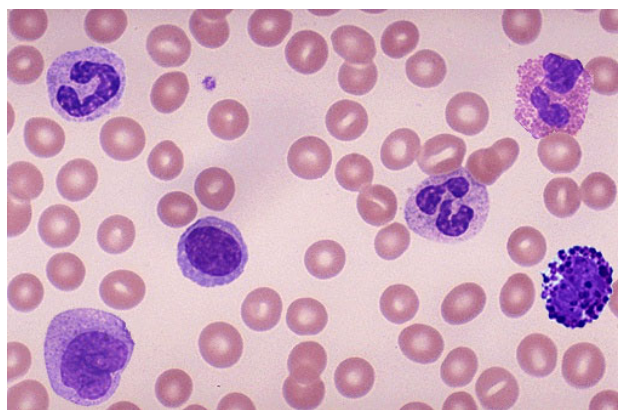
PVL toxicity



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

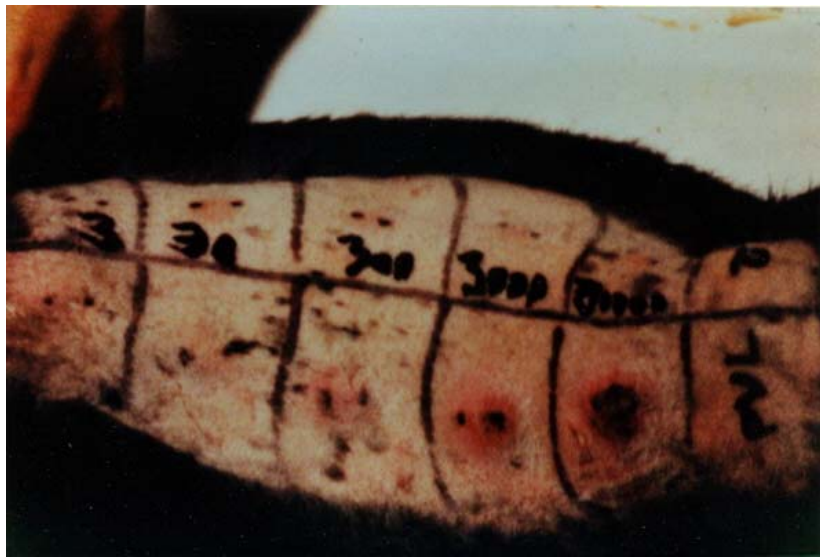
  ➡ Irreversible calcium influx

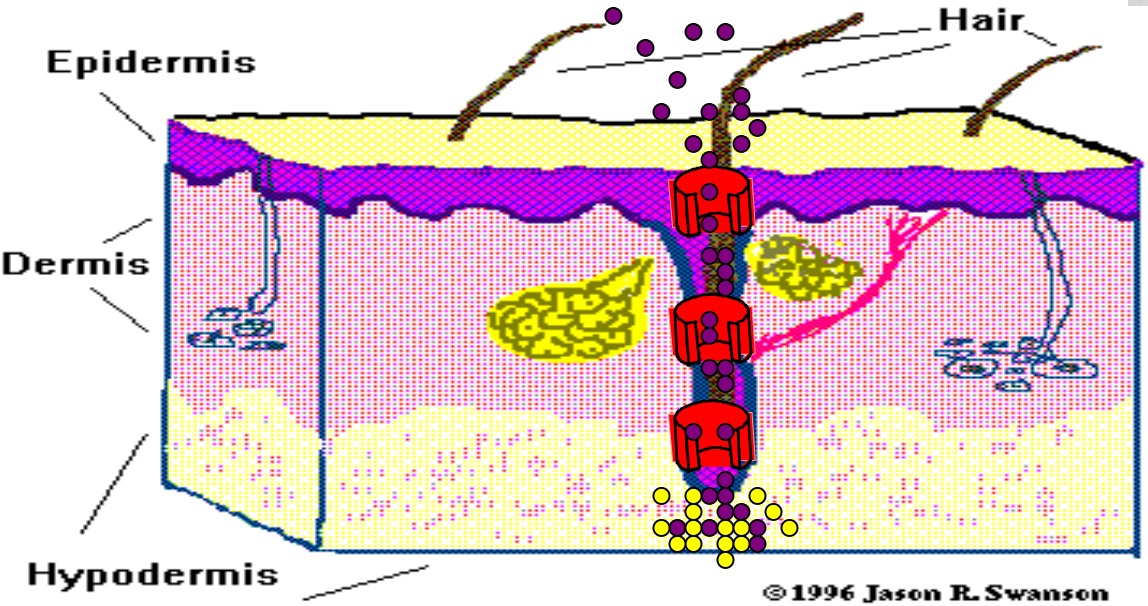
- Lysis of host defence cells
  - Polymorphonuclear cells (release of inflammatory mediators from basophils and neutrophils)
  - Monocytes and macrophages

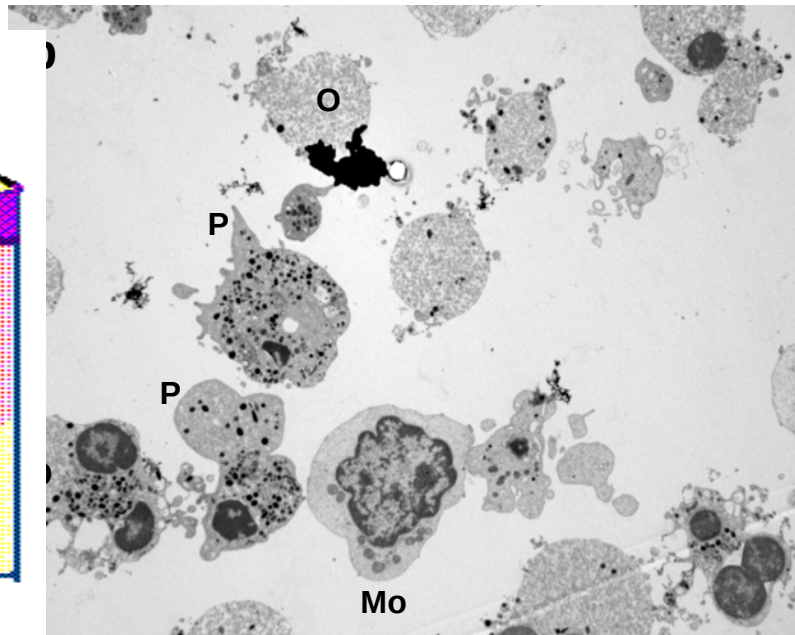


PVL is a pore-forming toxin : ---> leading factor for the development of primary skin infections

- Necrotizing toxin when injected intradermally in rabbit
- Leucotoxic by pore induction

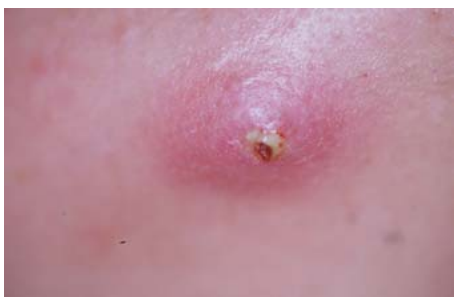
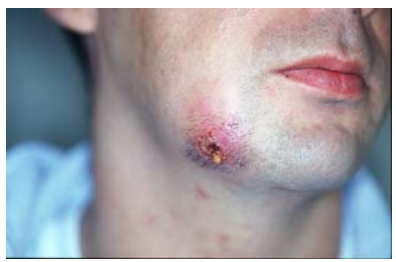
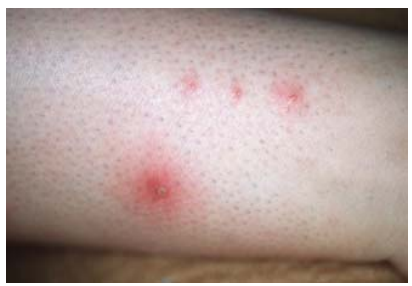
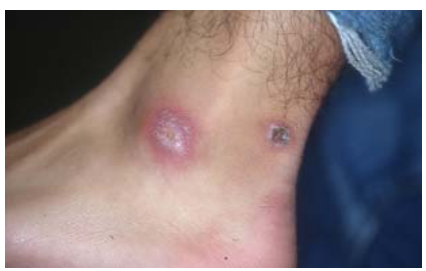
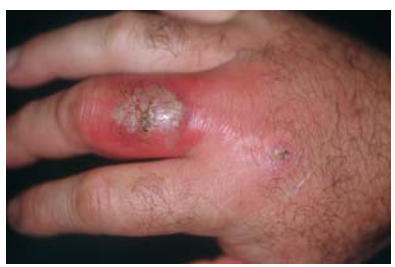






Types of infections associated with PVL+ C-MRSA

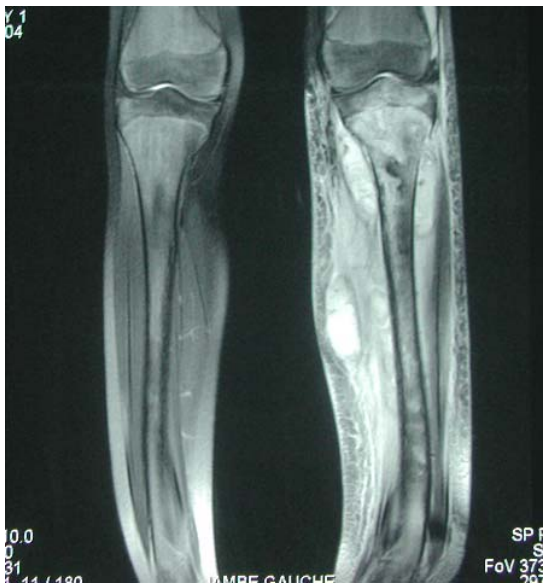
- Mainly skin and soft tissue infections, usually with no samples done for the lab (except if surgical drainage).

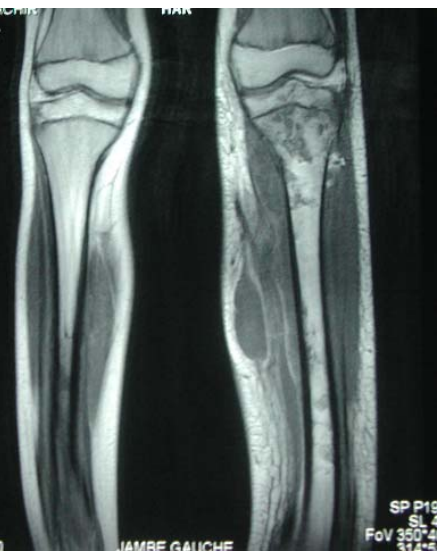






Community-acquired infections associated with PVL-positive *S. aureus*
--> Need to ask for samplings

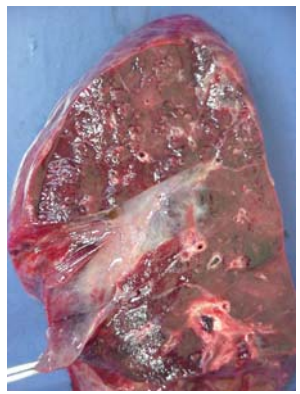
C-MRSA infections with PVL could be severe

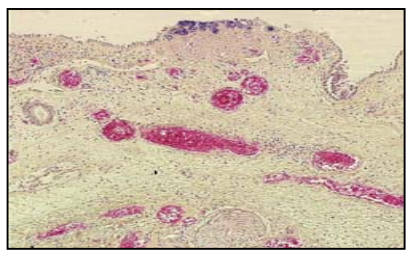
Osteomyelitis

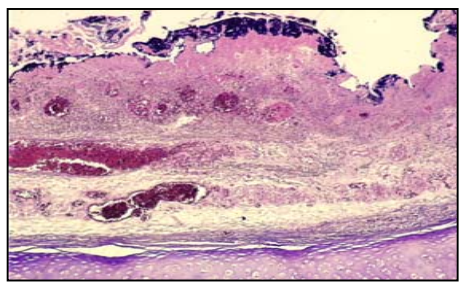


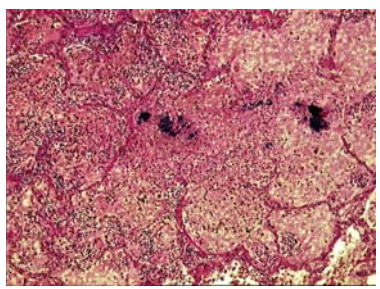


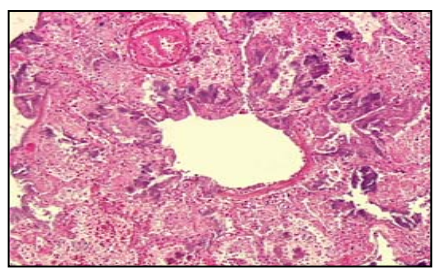
Pneumonia





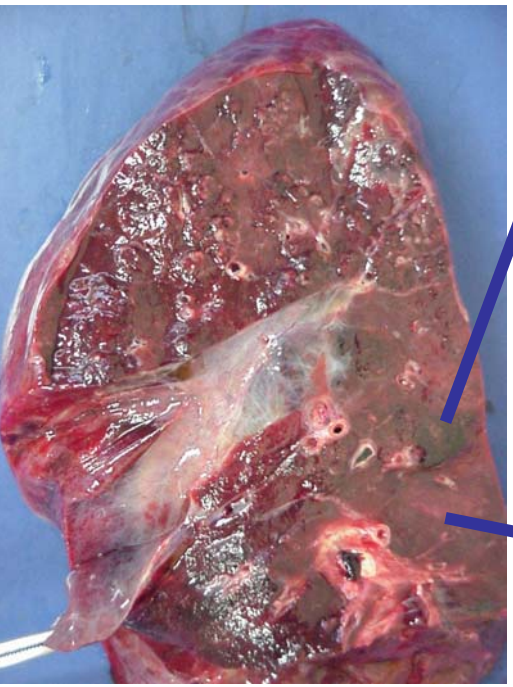






These cases are rare and need specific treatment

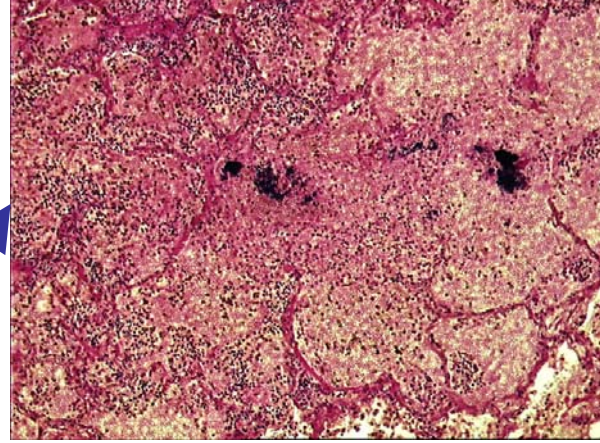
Necrotizing pneumonia due to CA-MRSA



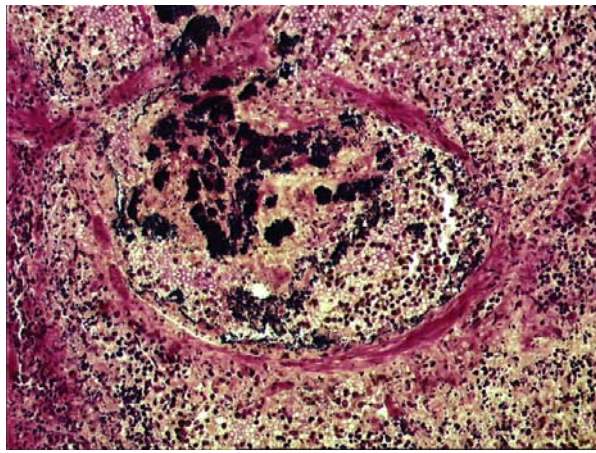
Hemorrhagic and necrotic lesions

Non necrotic lesions

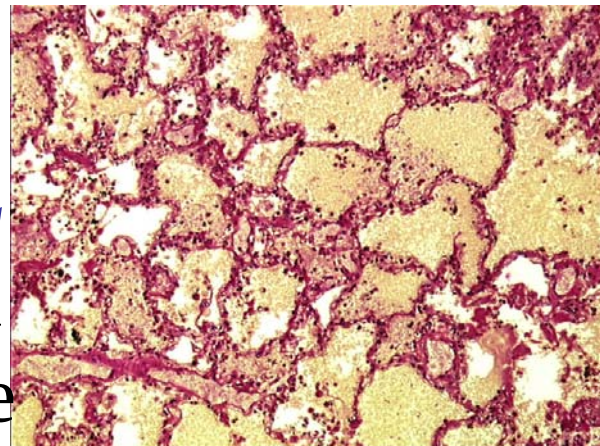
Parenchyma



Bronchioli

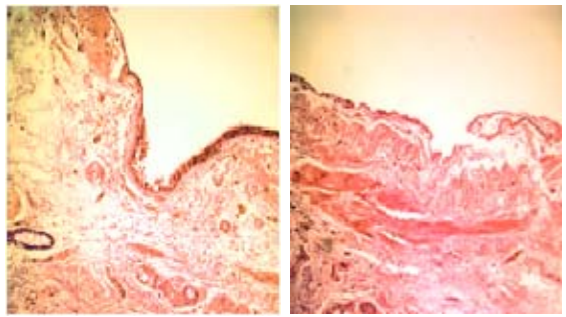


Parenchyma diffuse alveolar damage

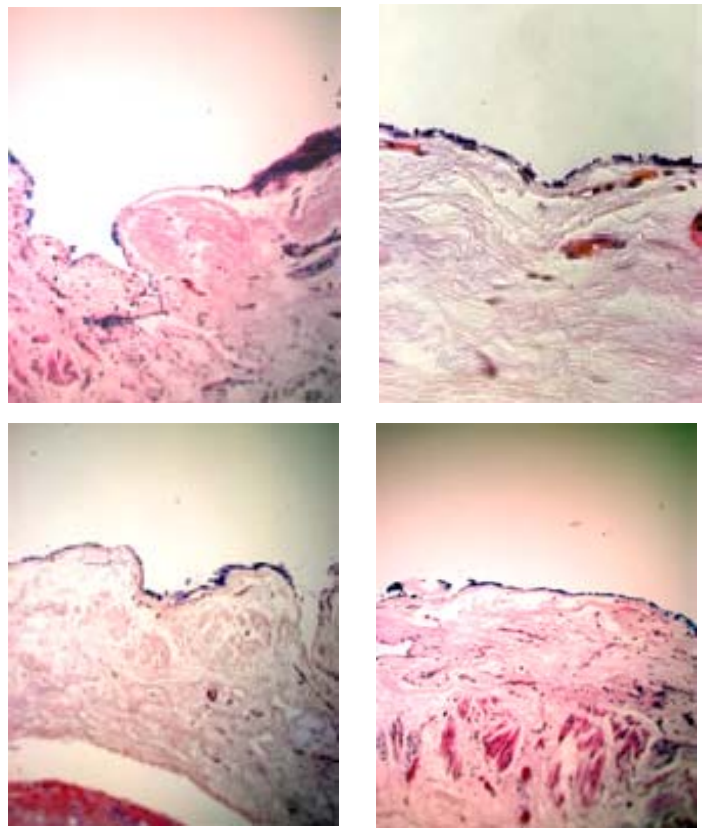


Adhesion to human bronchi injured *ex vivo*

Pneumonia
PVL-
Isolate 333



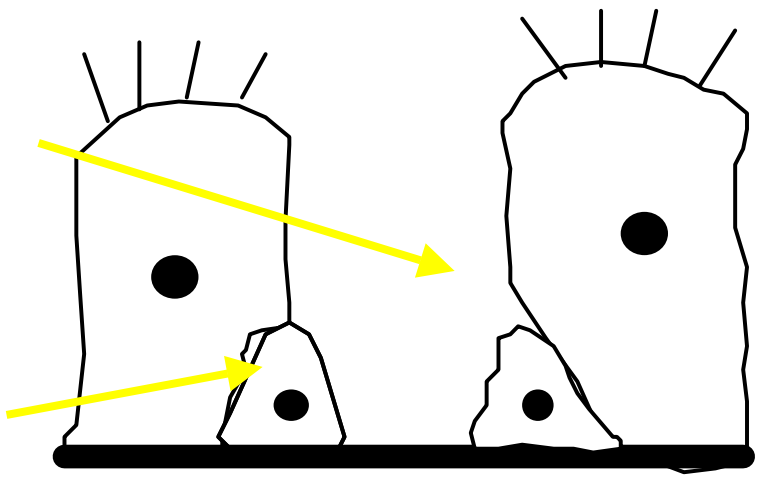
Necrotizing pneumonia
PVL+. Isolate 557



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

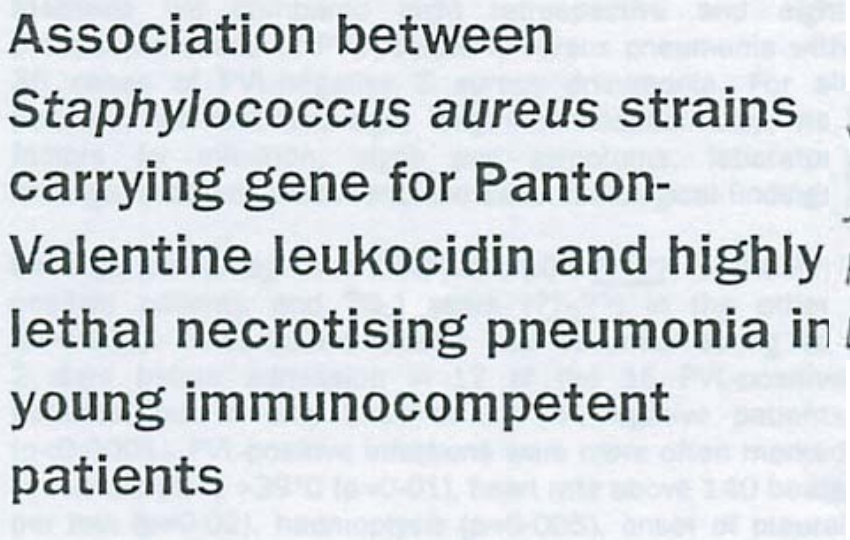
Hypothetic pathogenesis

- 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
de Bentzman S et al JID 2004

Staphylococcus aureus necrotizing pneumonia: a well recognized entity



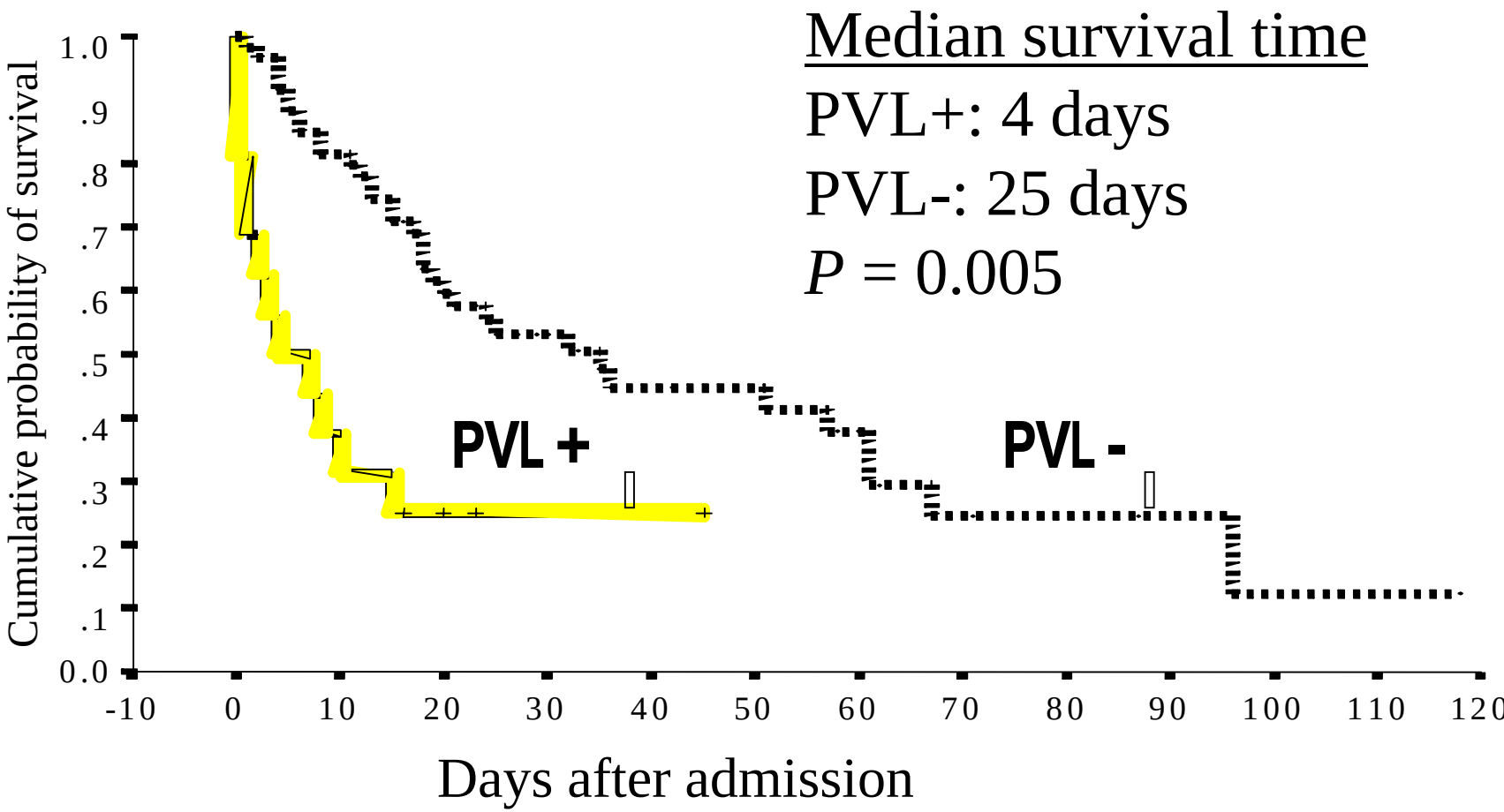
Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients

Lancet, 2002,9308:753

Yves Gillet, Bertrand Issartel, Philippe Vanhems, Jean-Christophe Fournet, Gerard Lina, Michèle Bes, François Vandenesch, Yves Piémont, Nicole Brousse, Daniel Floret, Jerome Etienne

- 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

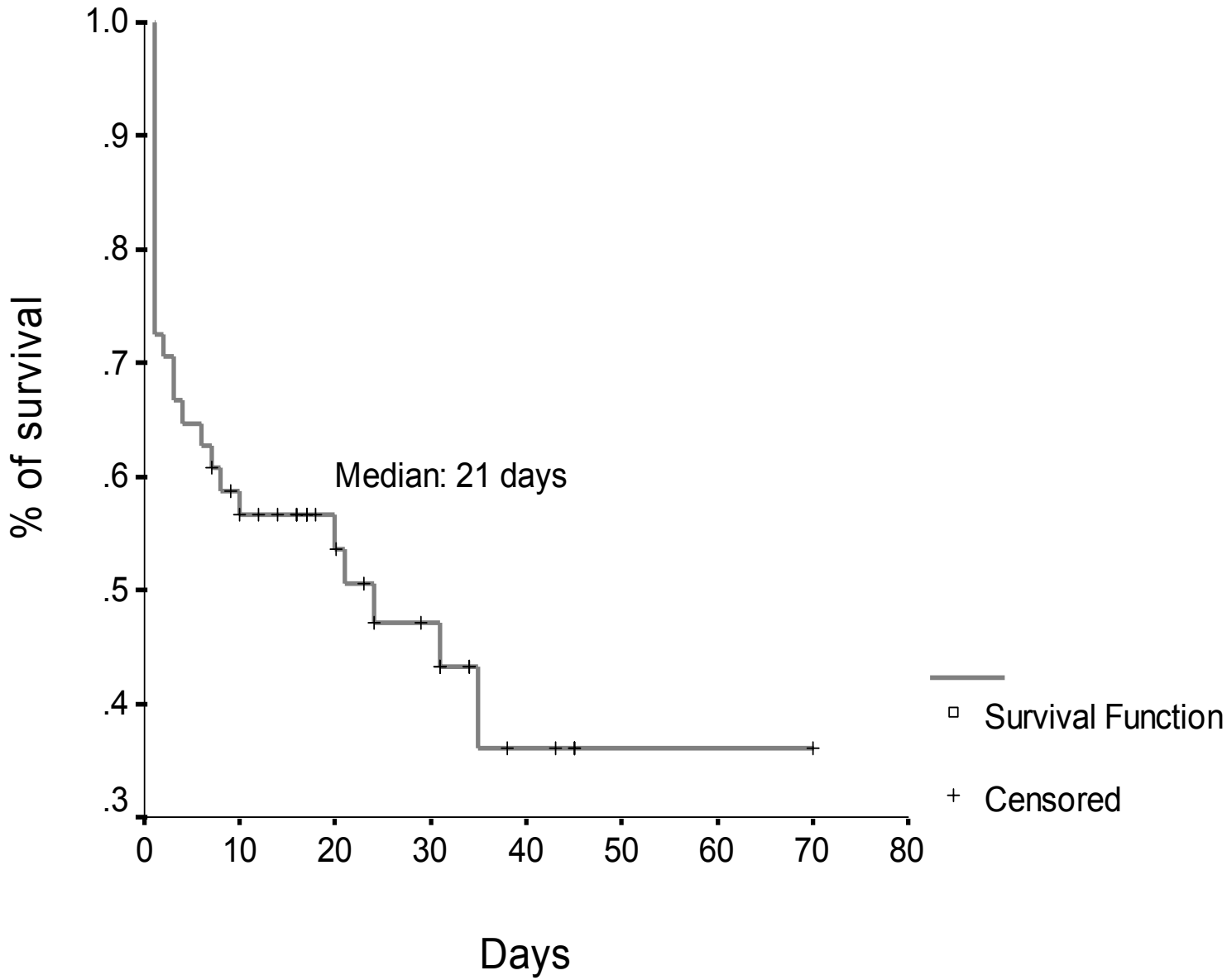
Survival of patients
Deaths : PVL+ 75%, PVL- 47%



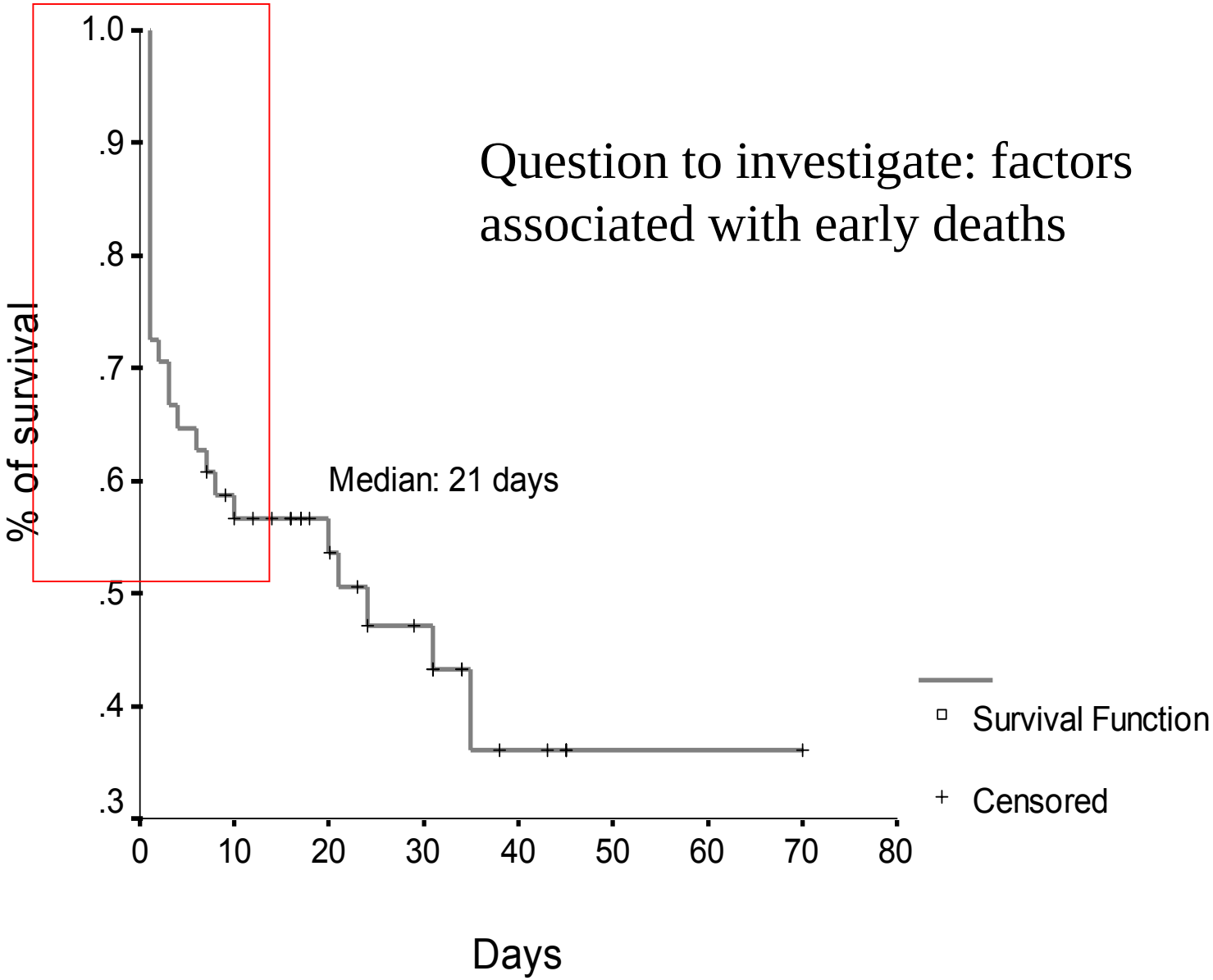
Study of 55 patients with extensive PVL+ *S. aureus* pneumonia

- 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



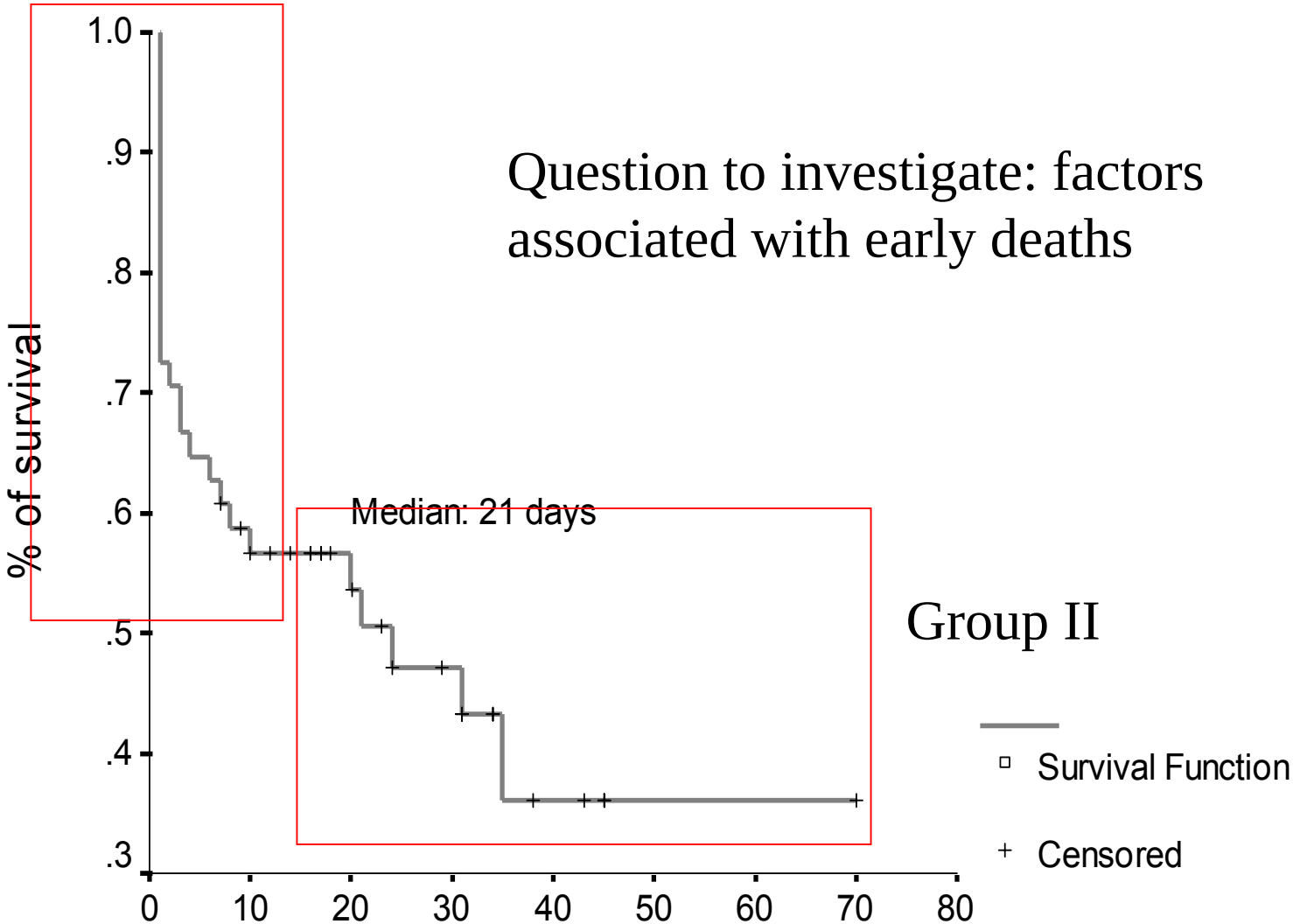
Survival after Pneumonia



Question to investigate: factors associated with early deaths

Survival after Pneumonia

Group I



Question to investigate: factors associated with early deaths

Group II

Summary of factors associated with high mortality*

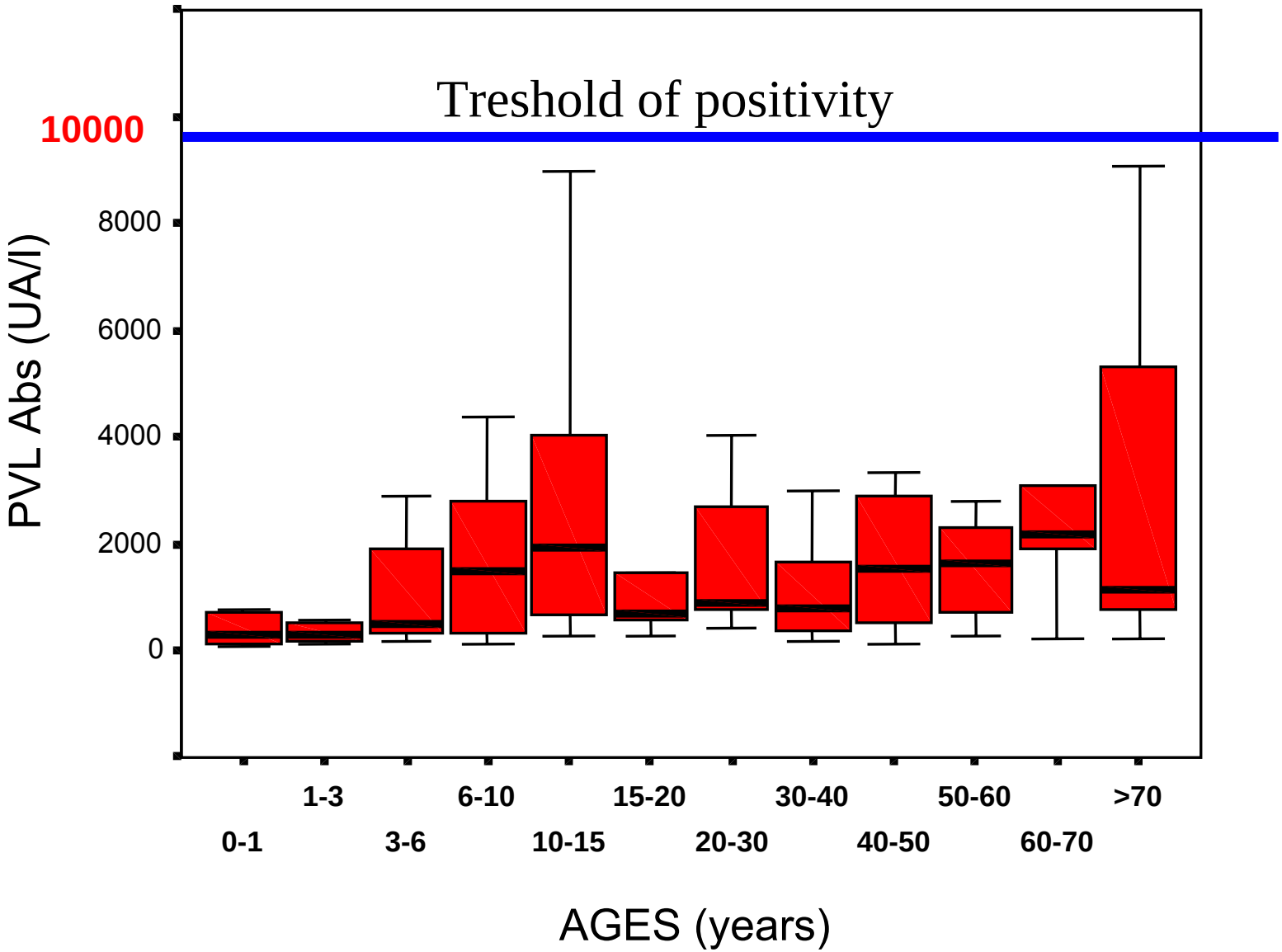
- Hemoptysis
- ARDS
- Low PaO2/FiO2
- (Scarlatiniform rash)
- Low WBC
- Low platelet count
- High creatinemia

*Based on univariate analysis

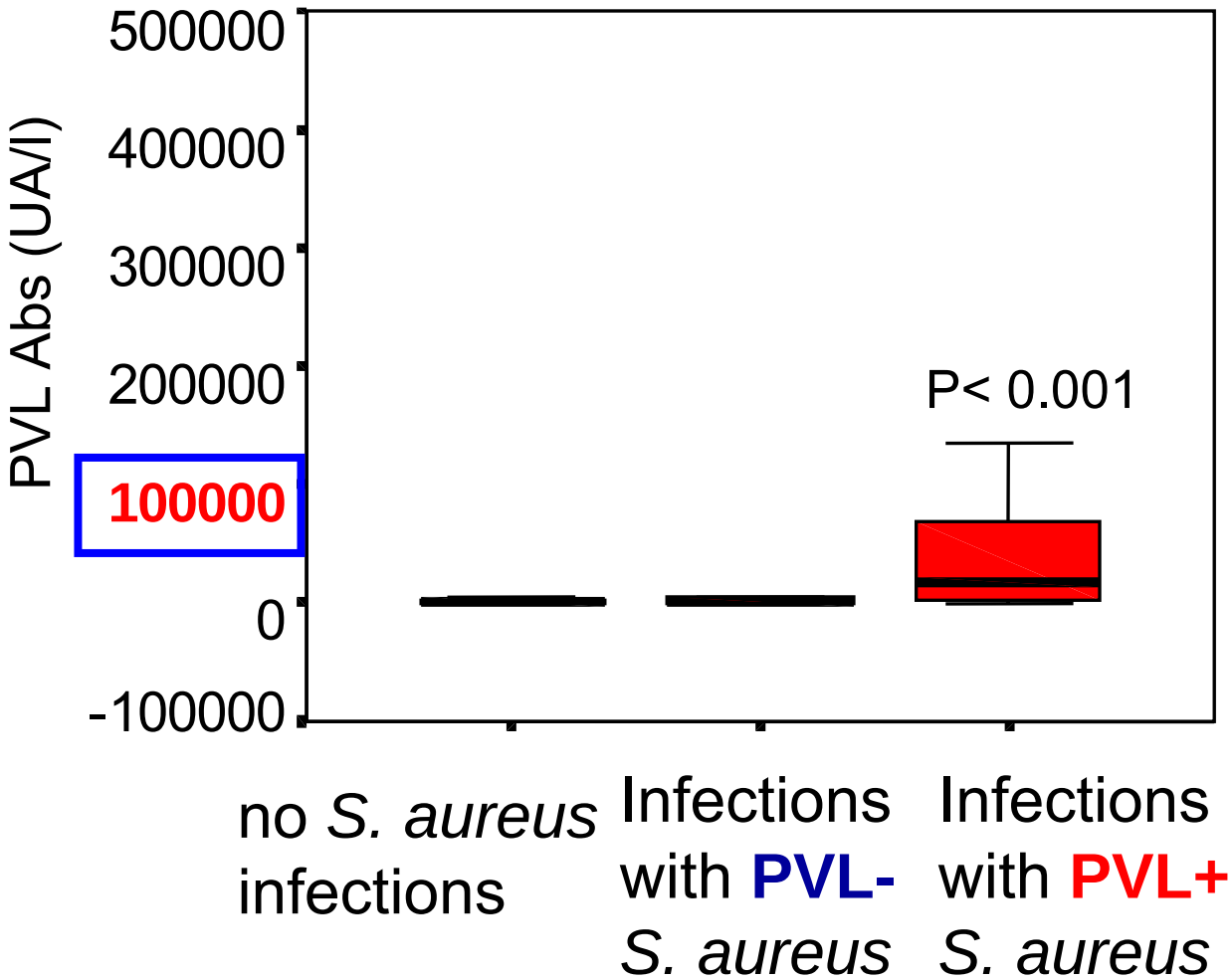
How to block the effect of PVL in case of necrotizing pneumonia ?

1. stop the PVL production with the used of clindamycin or linezolid,
2. stop the PVL effect by using of antibodies.

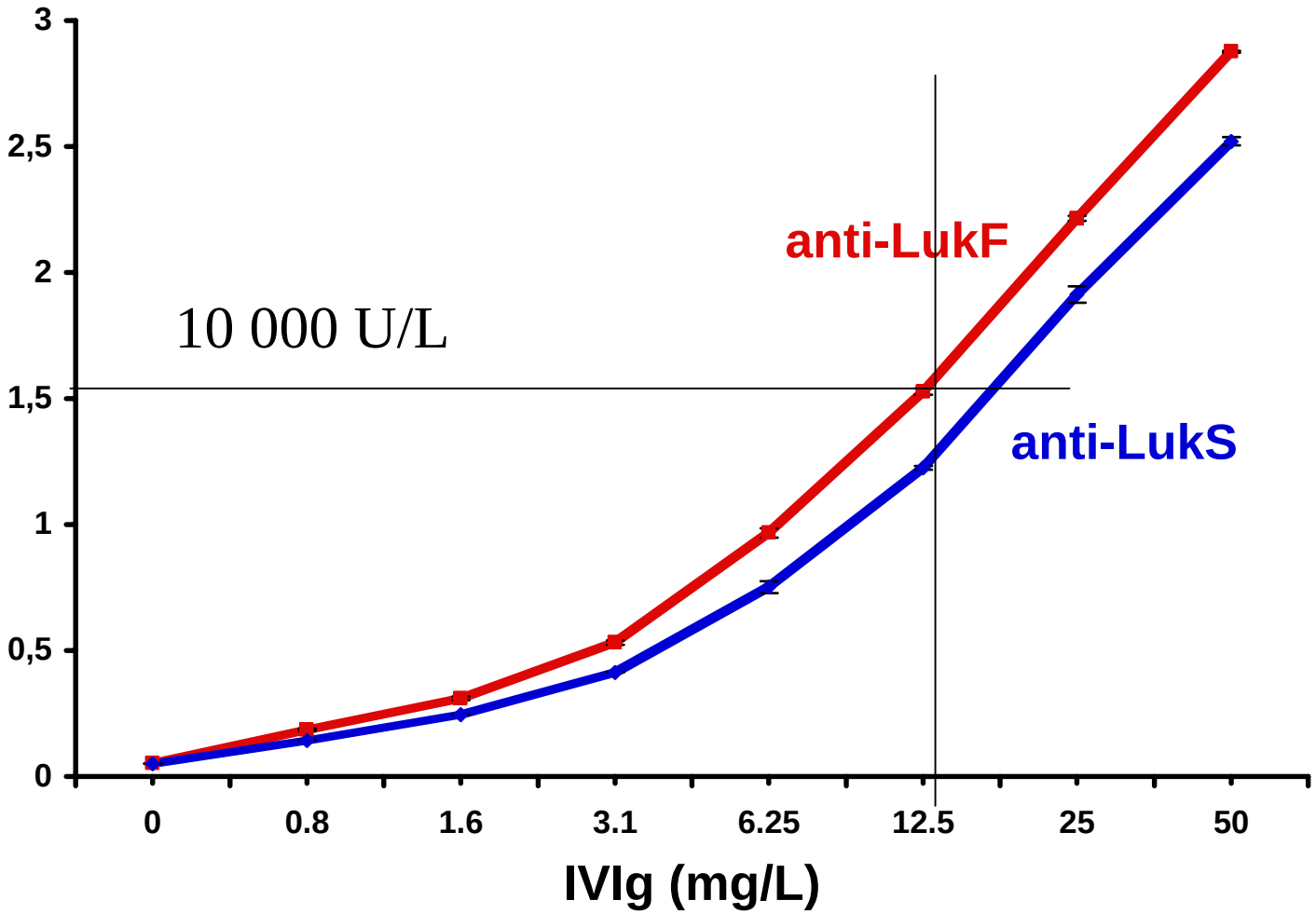
Antibodies against PVL according to the age



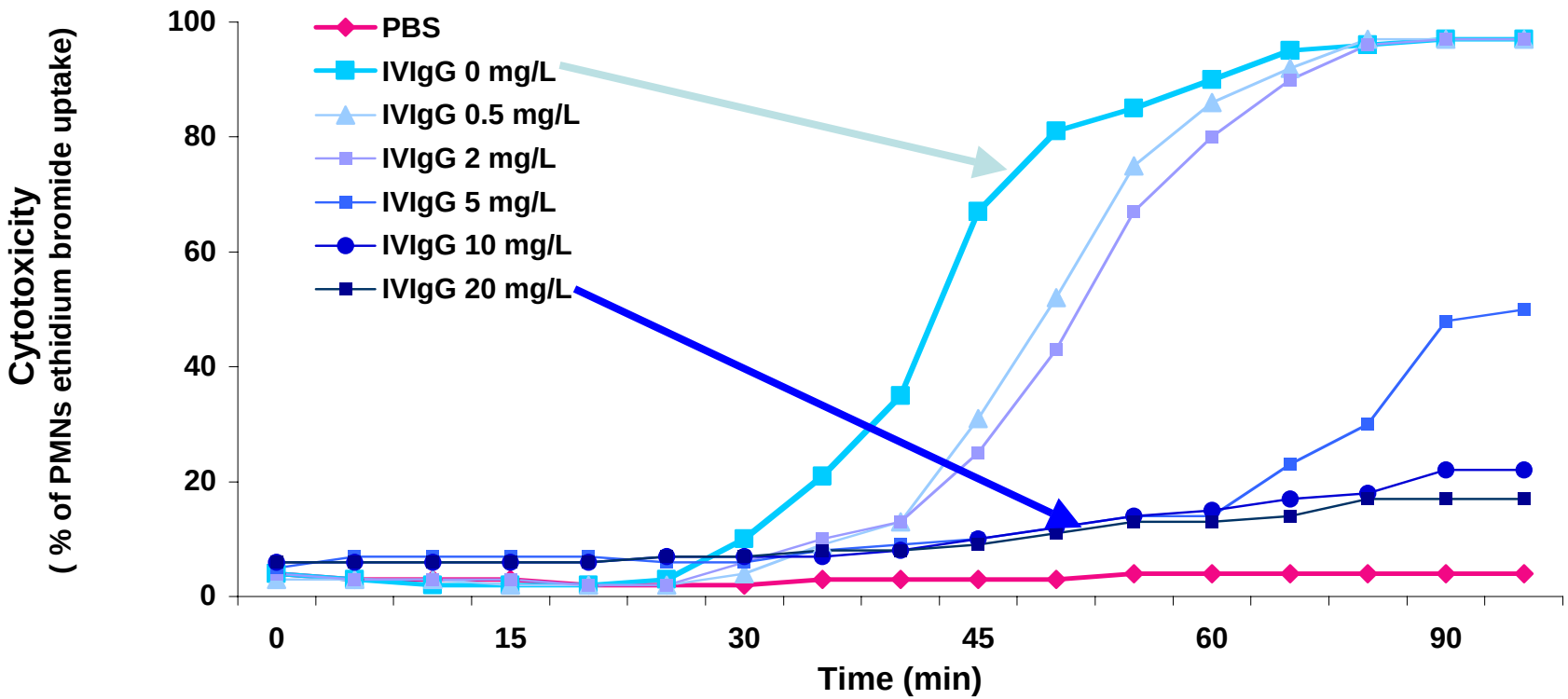
Antibodies against PVL in infected patients



Commercial intravenous immunoglobulins (IVIg) contains PVL-specific antibodies JID, 2004,189:346-53



IVIg inhibition of PVL-induced ethidium bromide uptake by PMNs



--> no clinical trials

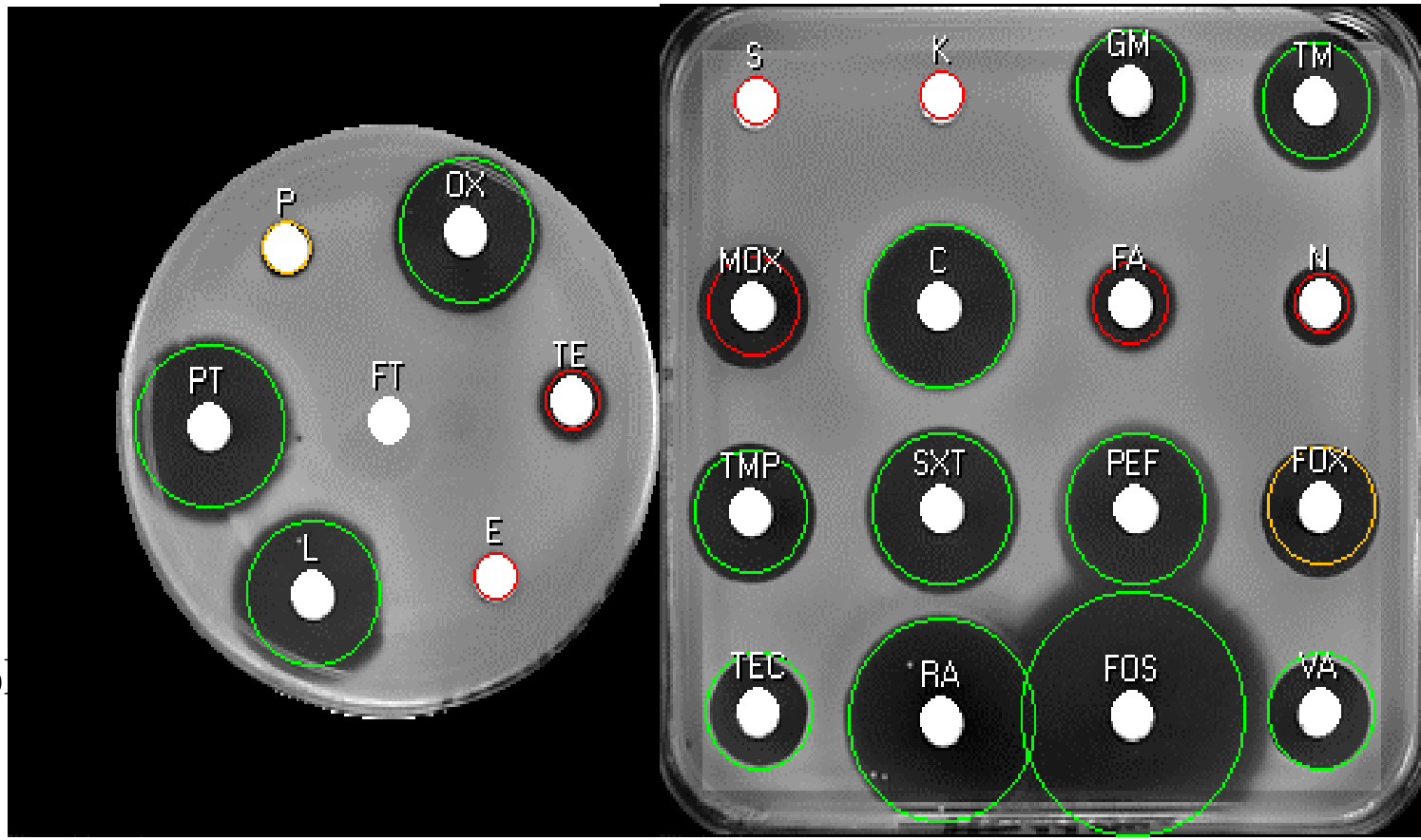
Back to PVL+ C-MRSA

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: chloramphenicol
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

	2001	**2002**	**2003**
MRSA	2 647	2 568	1 333
PVL pattern	21 (0.8%)	17 (0.8%)	9 (0,7%)
Available strains	11	10	6
PVL producing strains	11	10	6
Single PGFE pattern	9	10	6
other PFGE pattern	2 (USA)	0	0

PVL producing MRSA in France 2nd ONERBA Study 2004

Strain	Total		Hospitals		Private labs	
	N	%	N	%	N	%
S. aureus	13840	100	11126	100	2714	100
MRSA	3901	28	3249	29	652	24
PVL pattern	56	**1.4**	55	**1.7**	1	**0.1**
PVL +	48*		47		1	

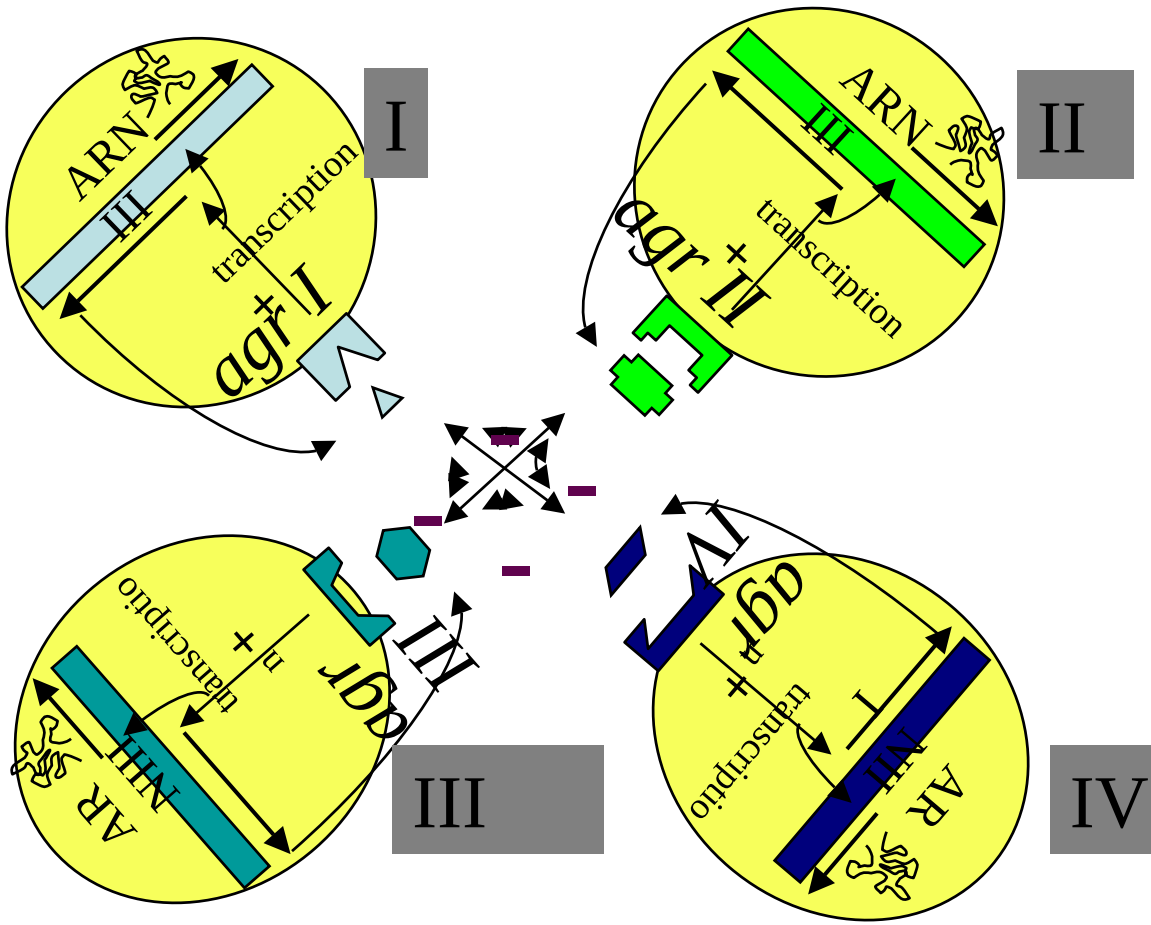
* 6 strains to be tested, 2 strains not available

Patients with PVL+ C-MRSA are mainly detected in hospitals
New emerging clones are detected: with the tst gene

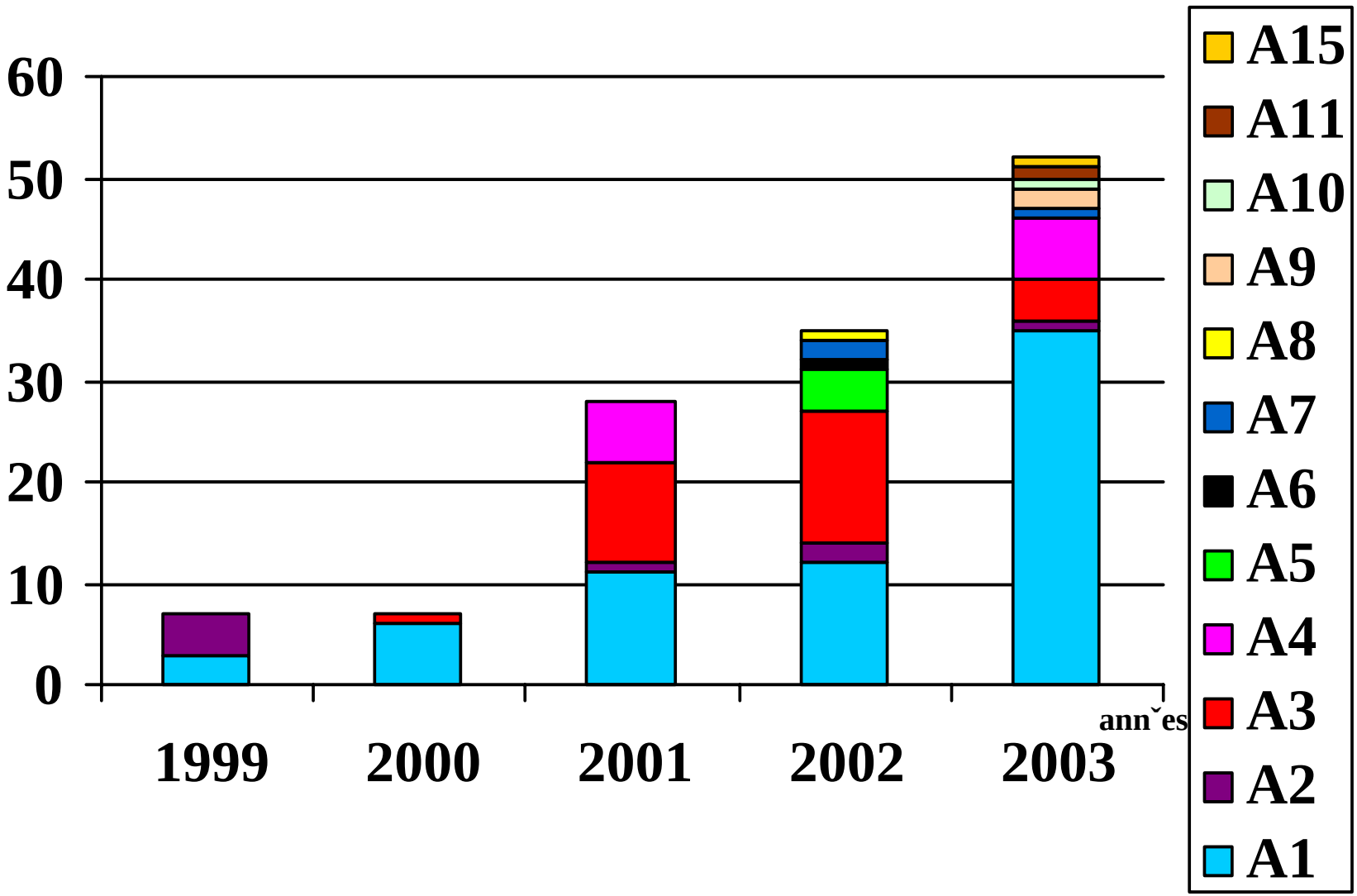
Characterization of the clones of the PVL+ C-MRSA

Identification and diversification of the PVL+ C-MRSA clones

- A clone is recognized by determination of:
  - sequence type
  - *spa* type
  - SCC*mec* type
  - *agr* type

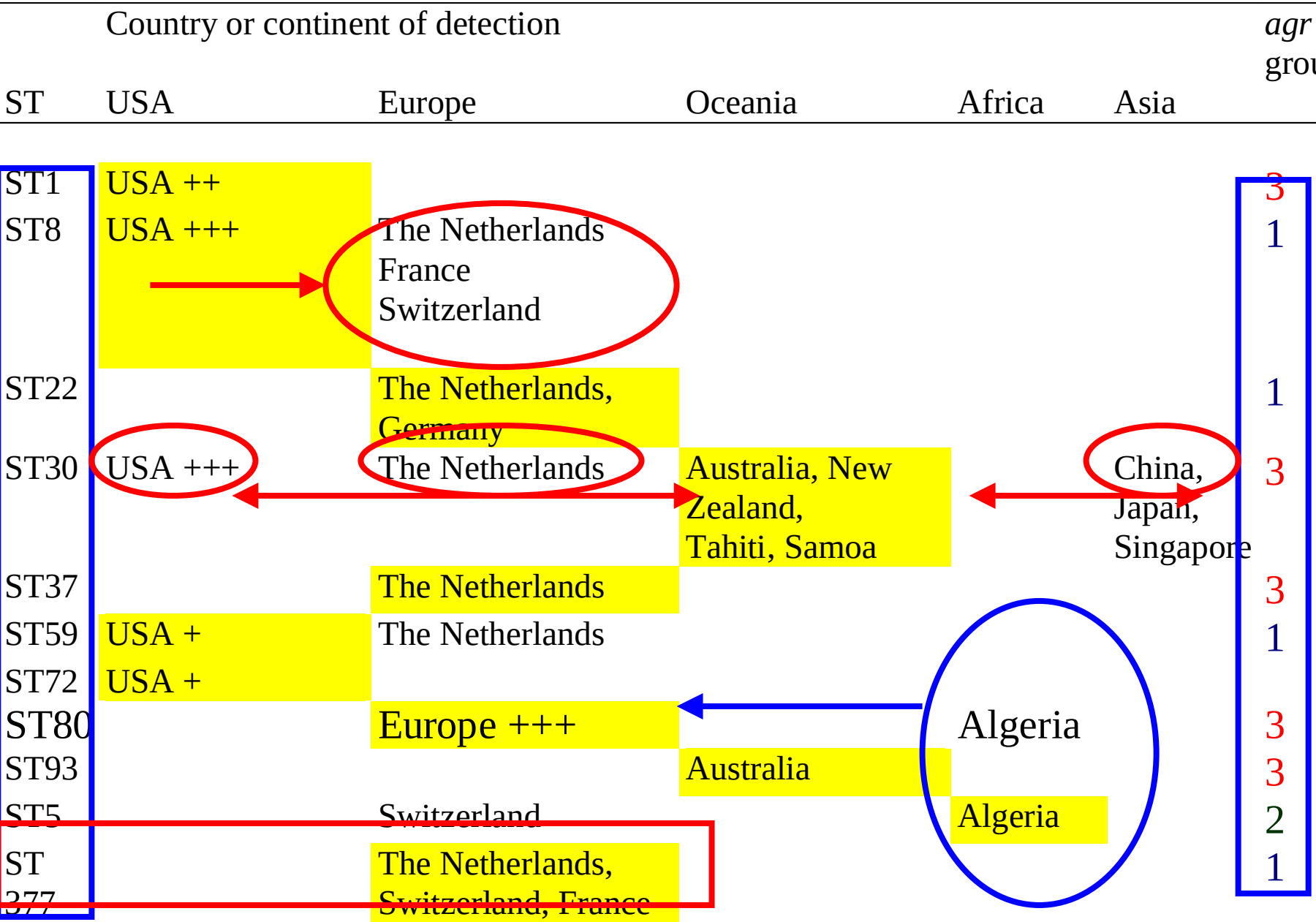


Diversification of PFGE subtypes of PVL+ CA-MRSA of ST80



ST and *agr* types of 228 PVL+ CA-MRSA isolates from the French collection

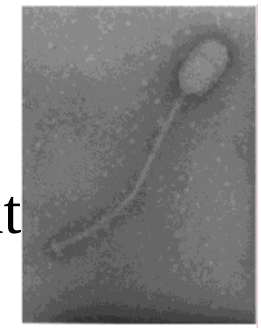
- 203 isolates *agr*3 (89%):
  - 16 isolates ST30: Australia, New Zealand, Samoa, China, Polynesia
  - 154 isolates ST80: Europe, Algeria
  - 29 isolates ST1: USA+++
  - 4 isolates ST93: Australia
  - 1 isolates ST37: Europe
- 22 isolates *agr*1 (9.6%):
  - 14 isolates ST8: USA +++, France (one isolate),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



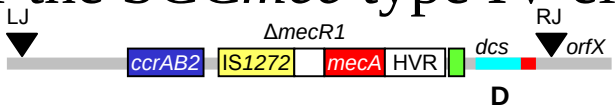
The different clones of PVL+ CA-MRSA (6 STs in 2003, 11 STs in 2004)

eBurst analysis (www.mlst.net)

Transfer of the PVL phage
and/or the SCC*mec* type IV element



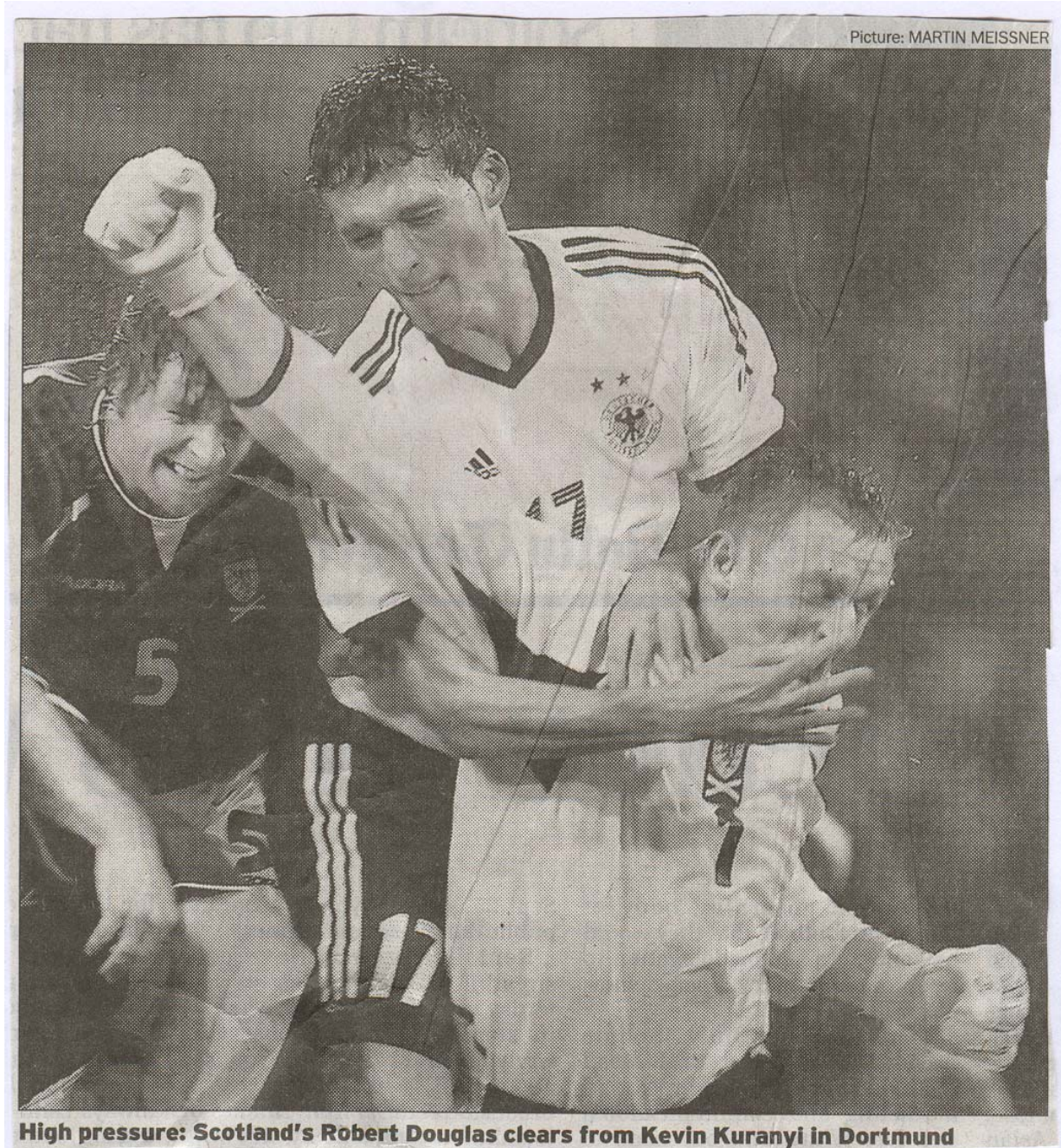
IV



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)

QuickTime™ et un
décompresseur TIFF (LZW)
sont requis pour visionner cette image.

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)

Family	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Total number of persons	5	3	10	4	10	4	12	5	3	6	4
Total number of infected patients	1	3	8	4	7	2	11	4	2	2	2

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)

	*agr*1	*agr2*	*agr*3	Total
*mec*A-	1	5	69	75
*mec*A+	0	25	2	27
Total	1	30	71	102

agr alleles and *mecA* gene in *tst*+ *S. aureus* strains (France 2002-2003)

Similar PFGE types of *tst*+ *S. aureus* isolates

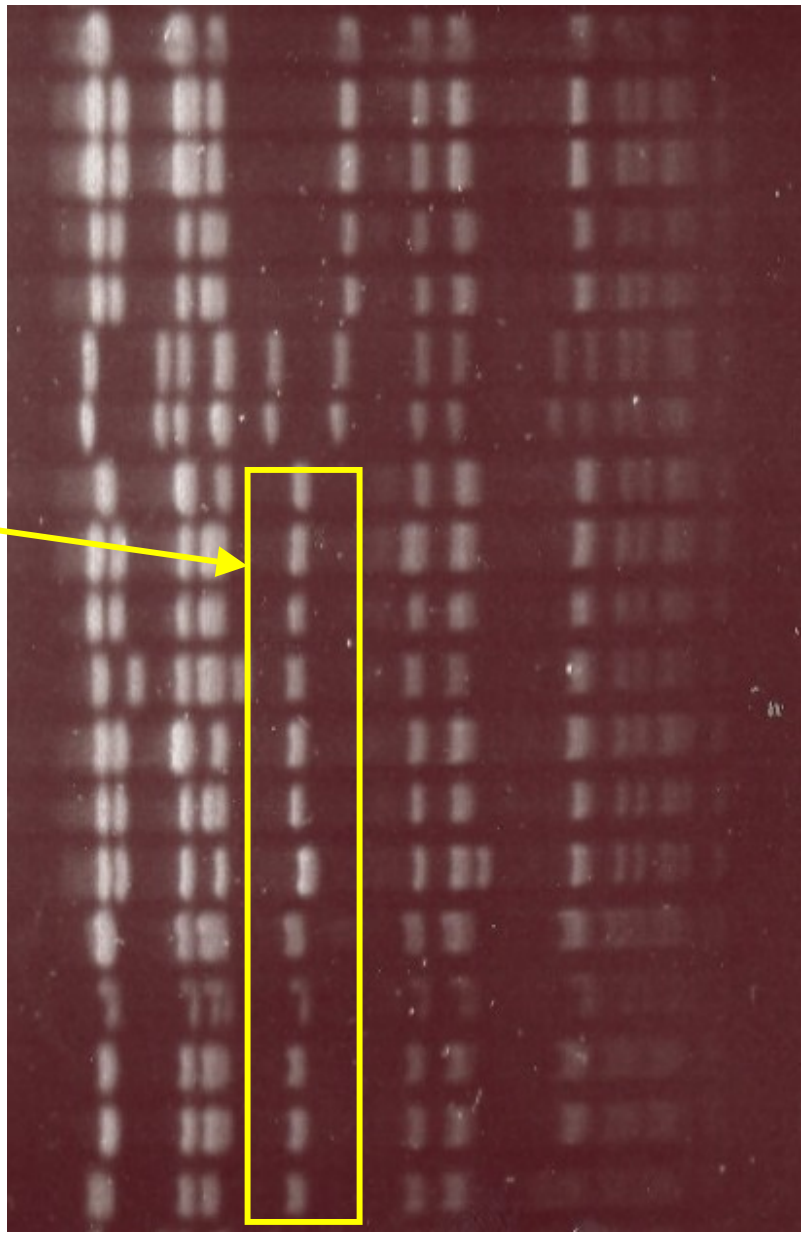
***agr* 2, *mecA*-**

HT 2003 0273
HT 2003 0155
HT 2003 0063
HT 2002 0774
HT 2002 0031
NCTC 8325
NCTC 8325
HT 2003 0849

mecA gene

HT 2003 0769
HT 2003 0768
HT 2003 0749
HT 2003 0695
HT 2002 0780
HT 2002 0277
HT 2002 0256
HT 2002 0255
HT 2002 0212
HT 2002 0188
HT 2002 0132

***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
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