Small Colony Variant et IOA

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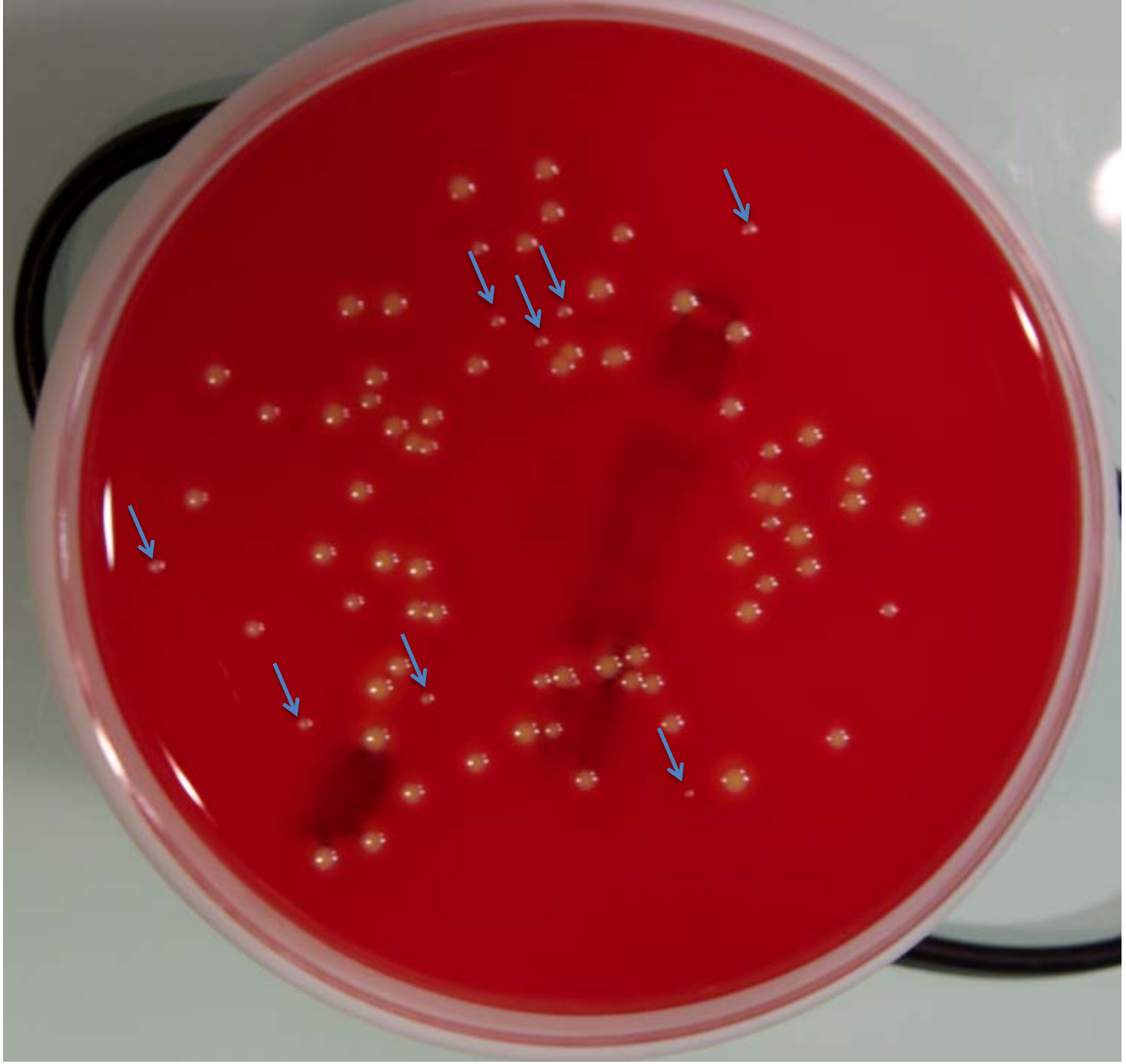
UNIVERSITÉ DE LYON

Hôpitaux de Lyon

STAPHYLOCOQUES
Centre National de Référence

CIRI
Centre International de
Recherche en Infectiologie
Lyon



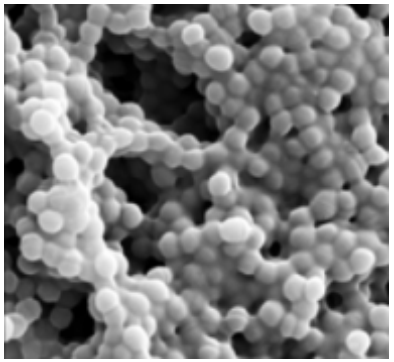
Physiopathologie des IOA

S. aureus = 30% à 75% des IOA

↓

récurrence et chronicité observée chez de nombreux patients

Biofilm



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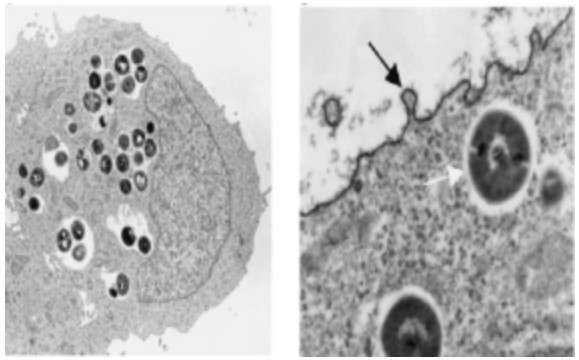
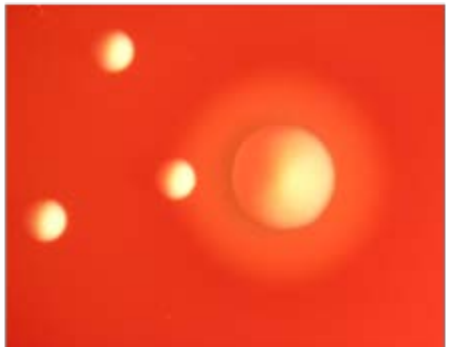


Small Colony Variant



Physiopathologie des IOA

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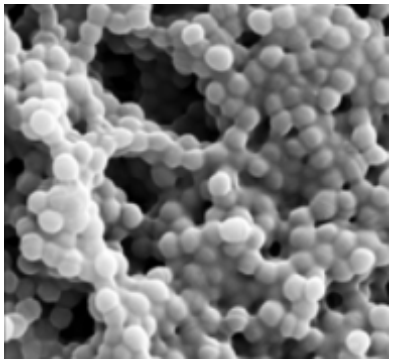

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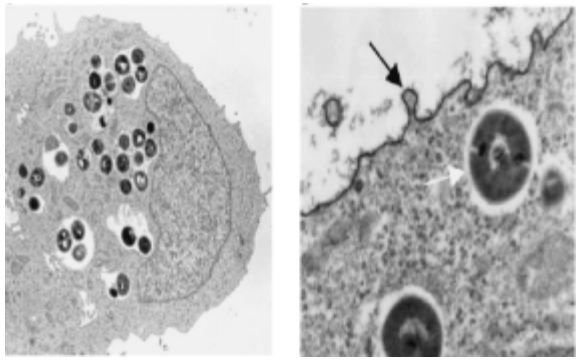
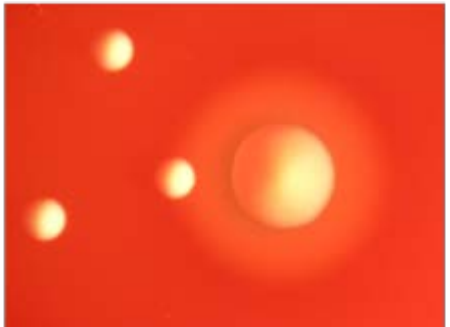
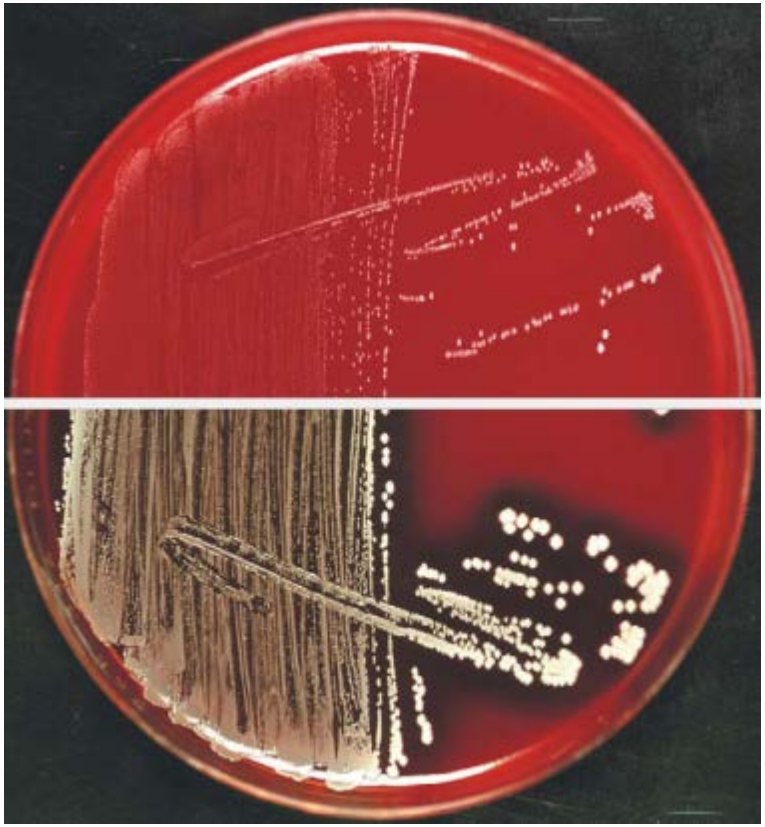


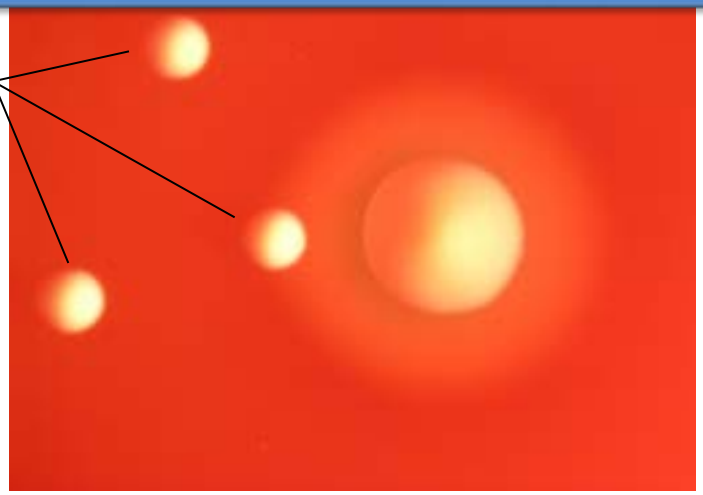
Small Colony Variant



Physiologie des SCV

Même souche (génétiquement)
En bas : *S. aureus* normale
En haut : *S. aureus* SCV



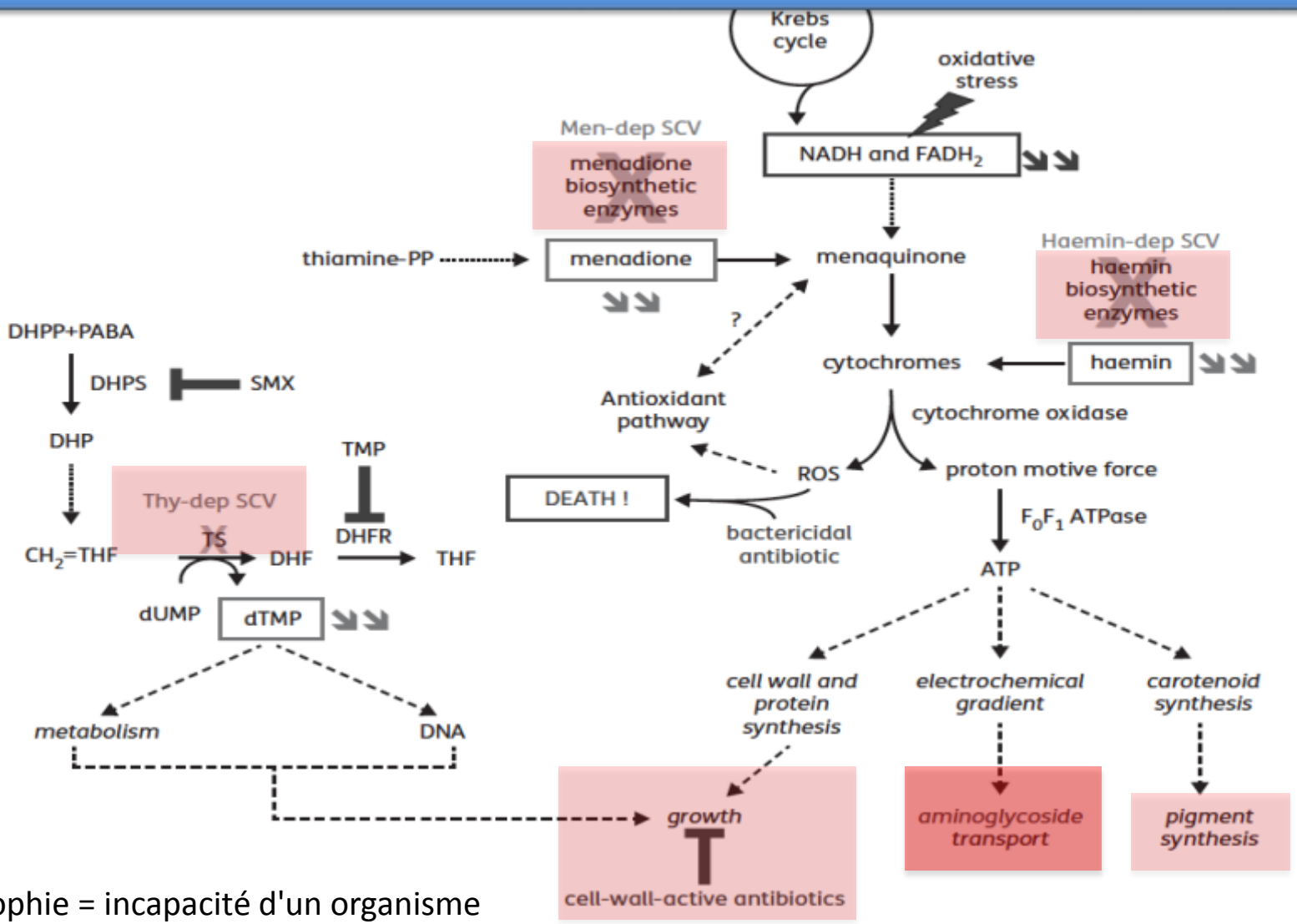


Particularités des micro-colonies *in vitro*:

. adhérence +++
. tps de génération (doublement) x 10
. perte de l 'activité bactéricide de certains ATB
. persistance des bactéries dans des niches cellulaires protectrices (ostéoblastes, cellules endothéliales , …)….
. peu de réaction inflammatoire locale
. à l'origine d'échec thérapeutique

SCV existent chez de nombreuses espèces : ***S. aureus***, *E. coli, P. aeruginosa, P. acnes,* …

Physiologie des SCV



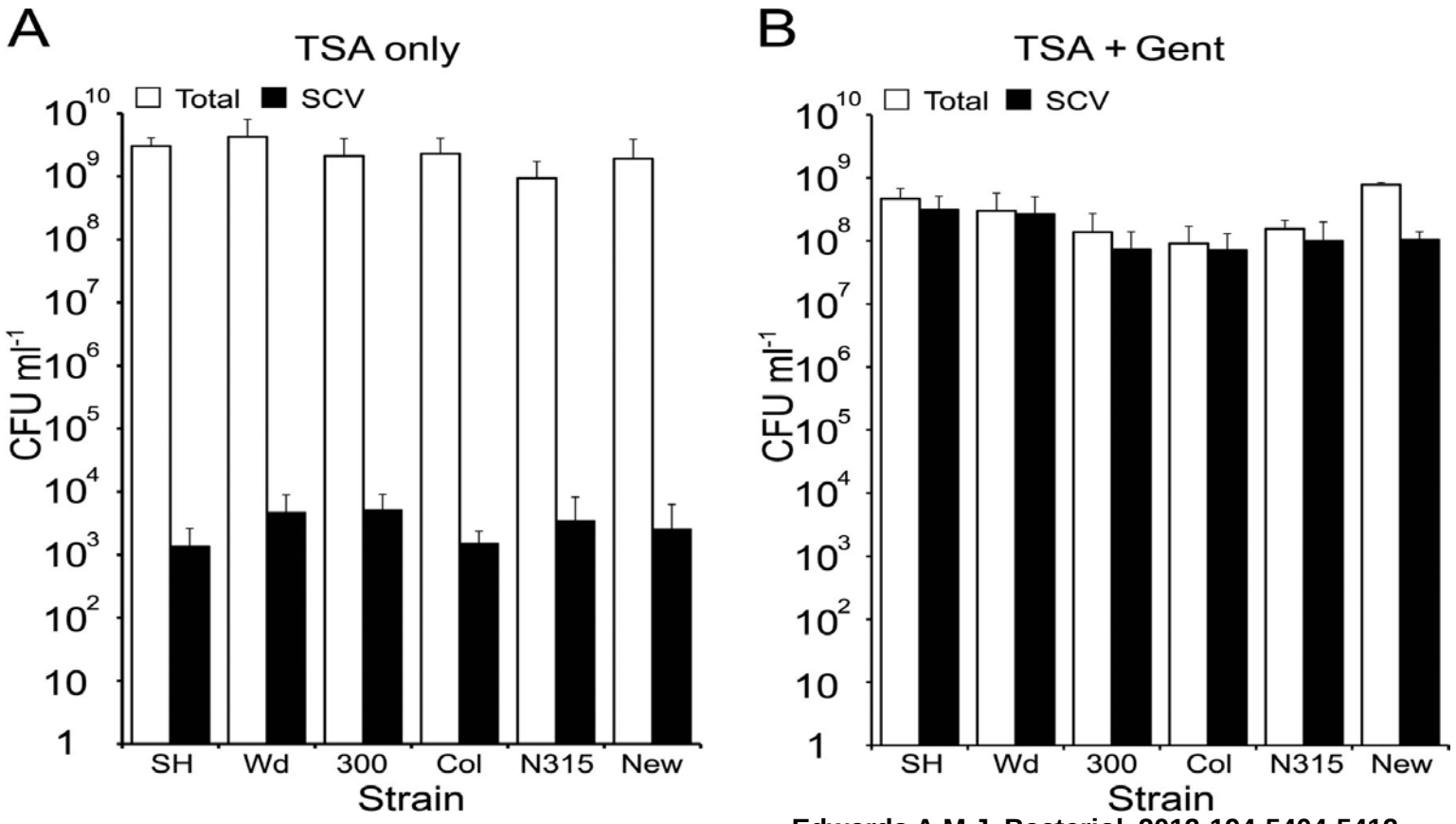
Auxotrophie = incapacité d'un organisme vivant de synthétiser un composé organique nécessaire à son développement.

+ altération large du protéome

Kriegeskorte A, Protéomics 2011

Physiologie des SCV

In vitro



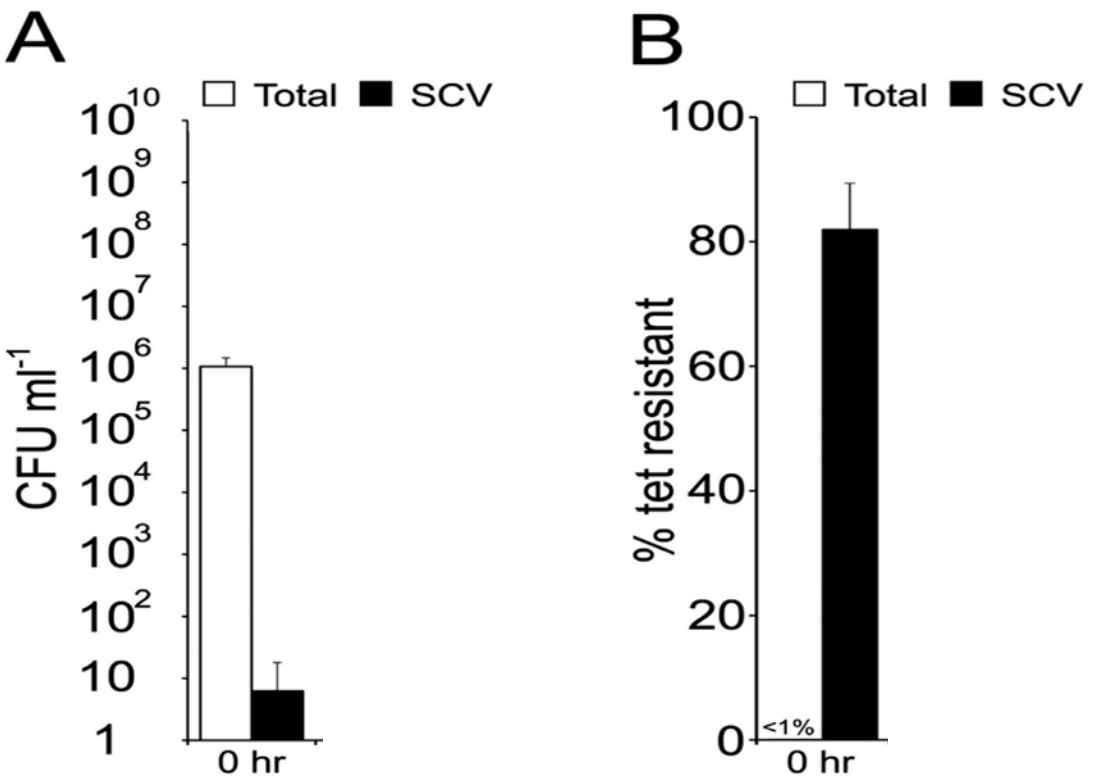
Edwards A M J. Bacteriol. 2012;194:5404-5412

Genetically diverse *S. aureus* cultures contain a small subpopulation of SCVs that expands under selective pressure.

Physiologie des SCV

In vitro

Mélange initial NCP TétraS + SCV TétraR



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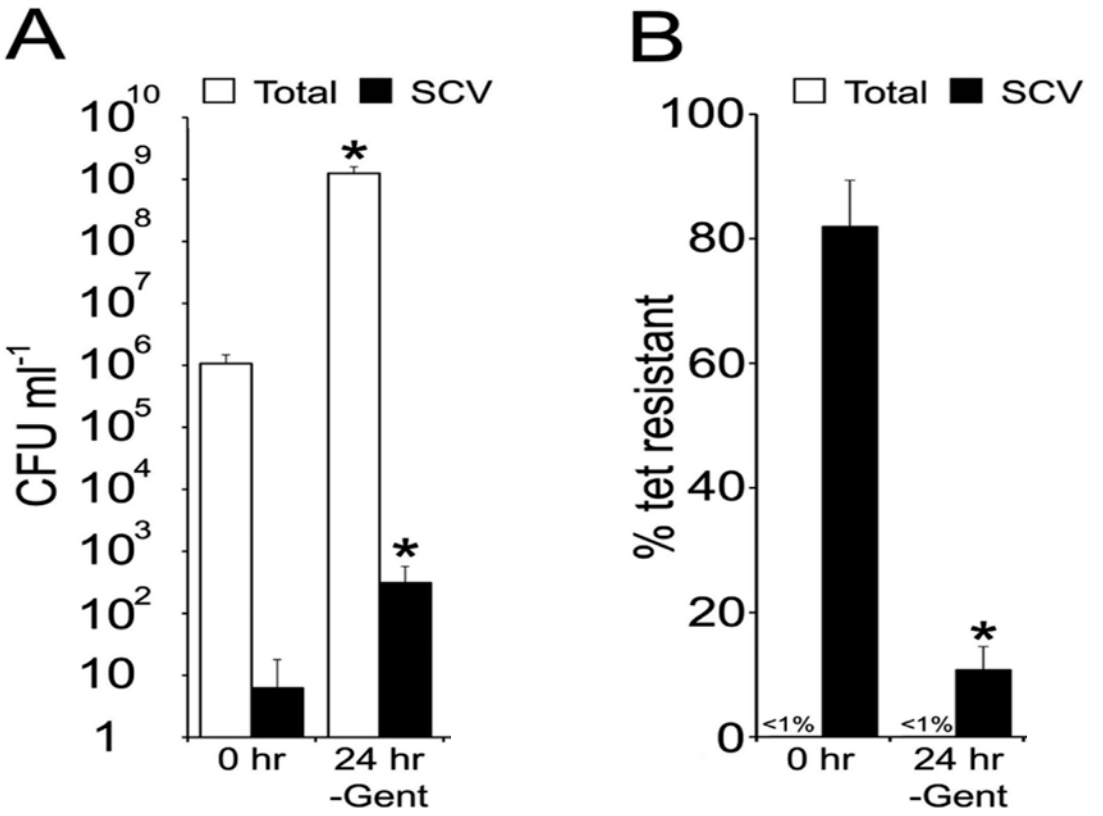
Physiologie des SCV

In vitro

Mélange initial NCP TétraS + SCV TétraR



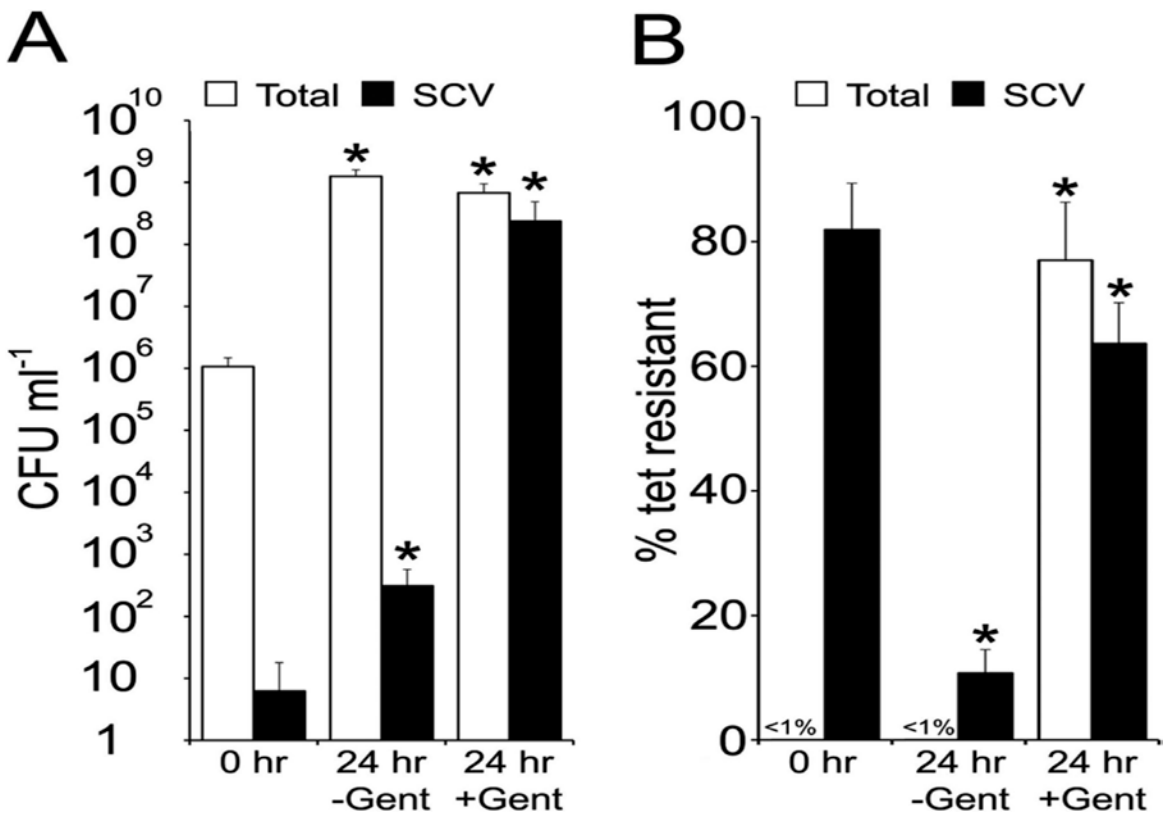
Apparition de SCV TétraS au cours de la croissance sans pression de sélection (ici exposition genta)

Edwards A M J. Bacteriol. 2012;194:5404-5412

Physiologie des SCV

In vitro

Mélange NCP TétraS + SCV TétraR

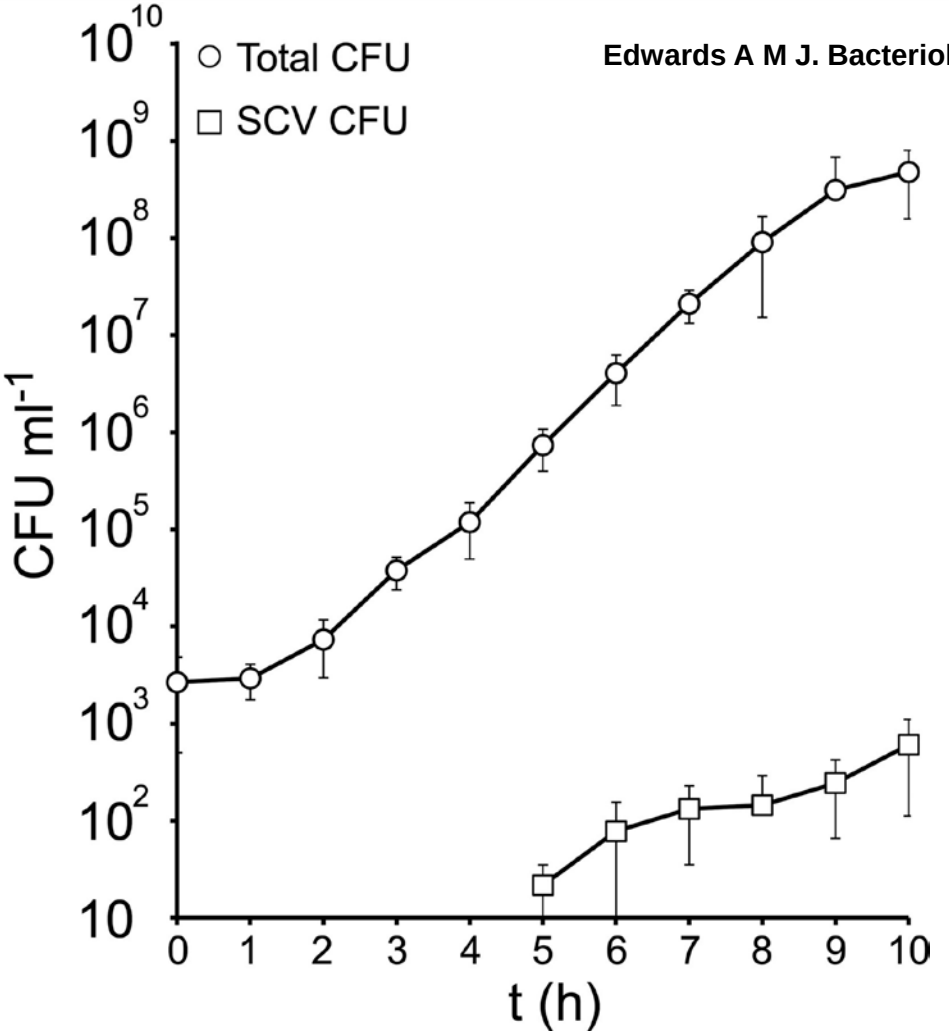


Edwards A M J. Bacteriol. 2012;194:5404-5412

The SCV subpopulation is dynamic in the absence of selective pressure.

Physiologie des SCV

In vitro



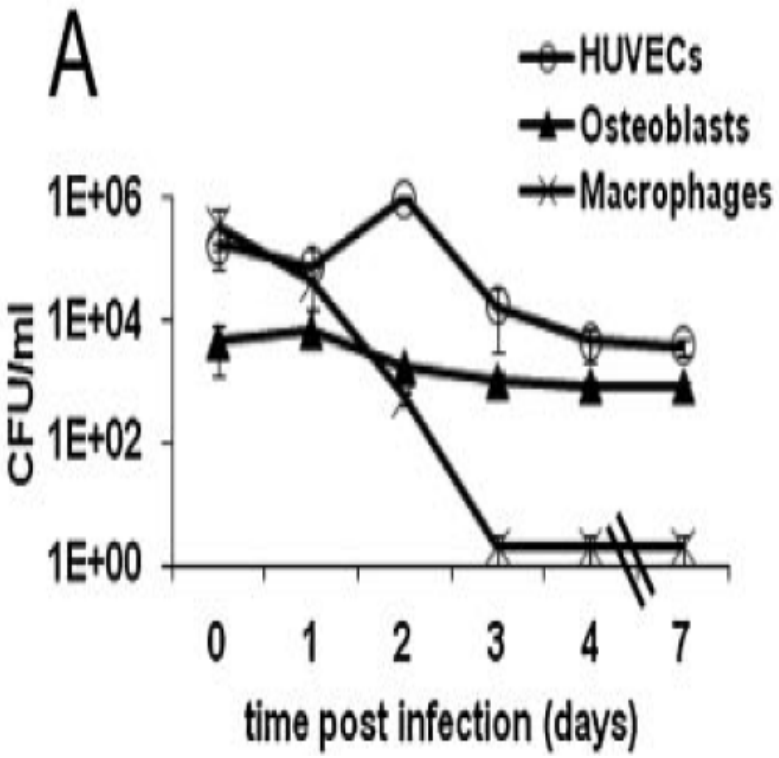
Edwards A M J. Bacteriol. 2012;194:5404-5412

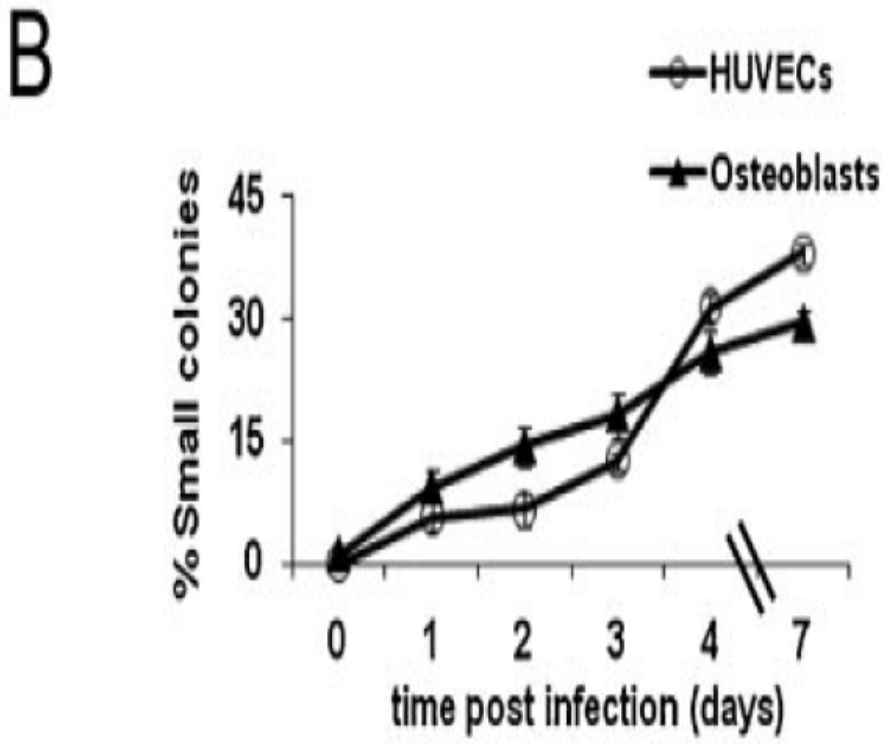
SCVs emerge naturally during exponential-phase of Normal Colony Population growth.

Physiologie des SCV

Modèle cellulaire

Infection culture cellulaire par NCP

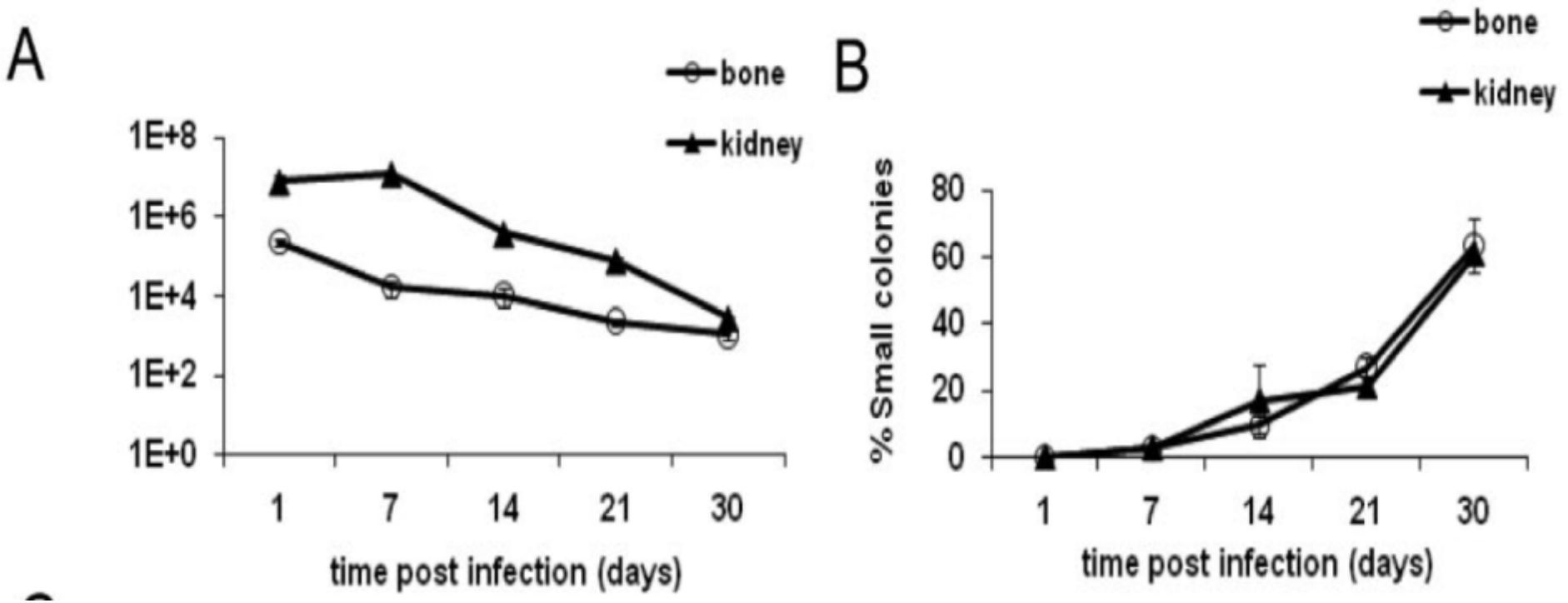




Tuchscherr et al.,2010 EMBO Molecular Medecine

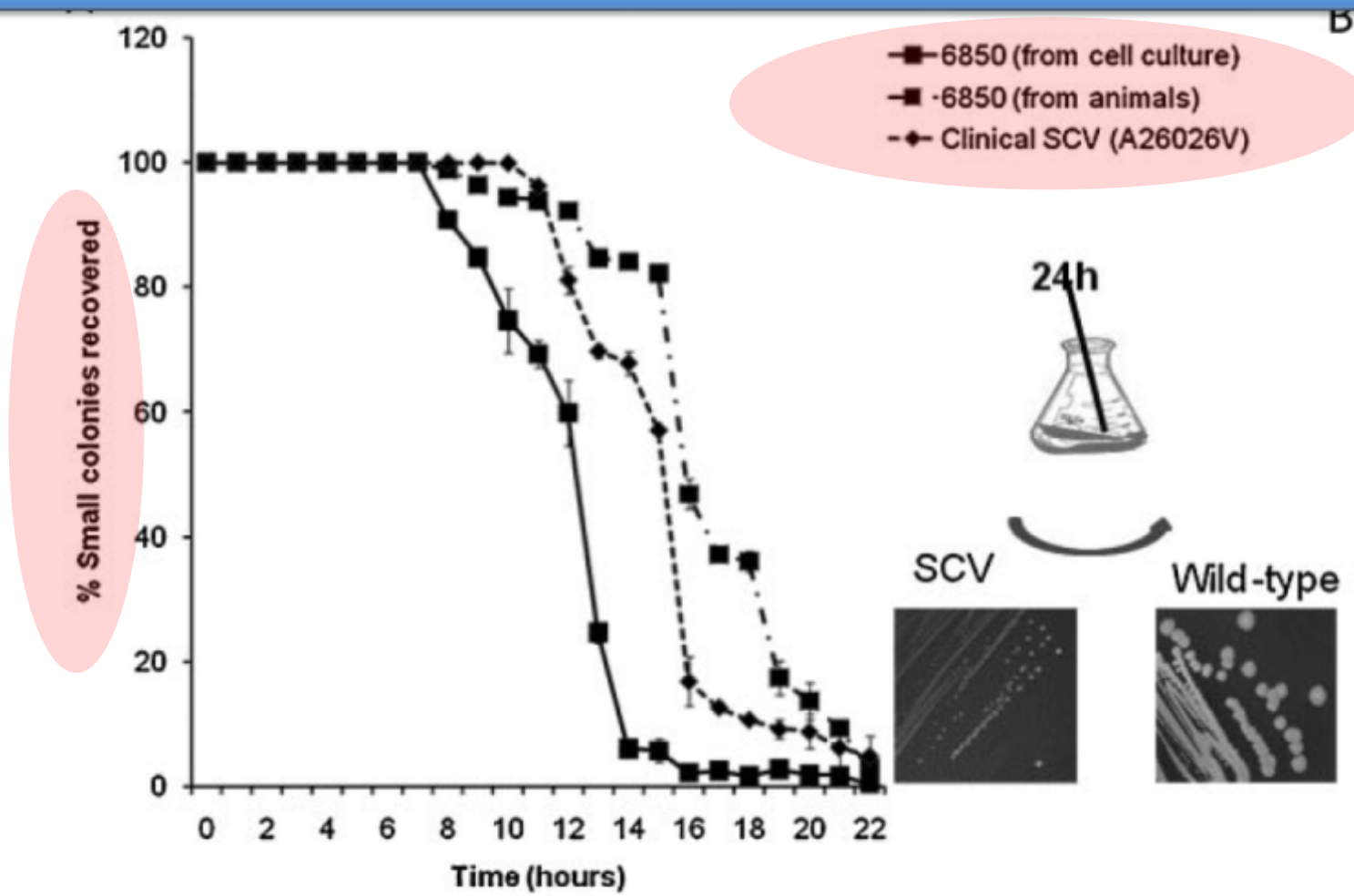
Physiologie des SCV

In vivo



Tuchscherr et al.,2010 EMBO Molecular Medecine

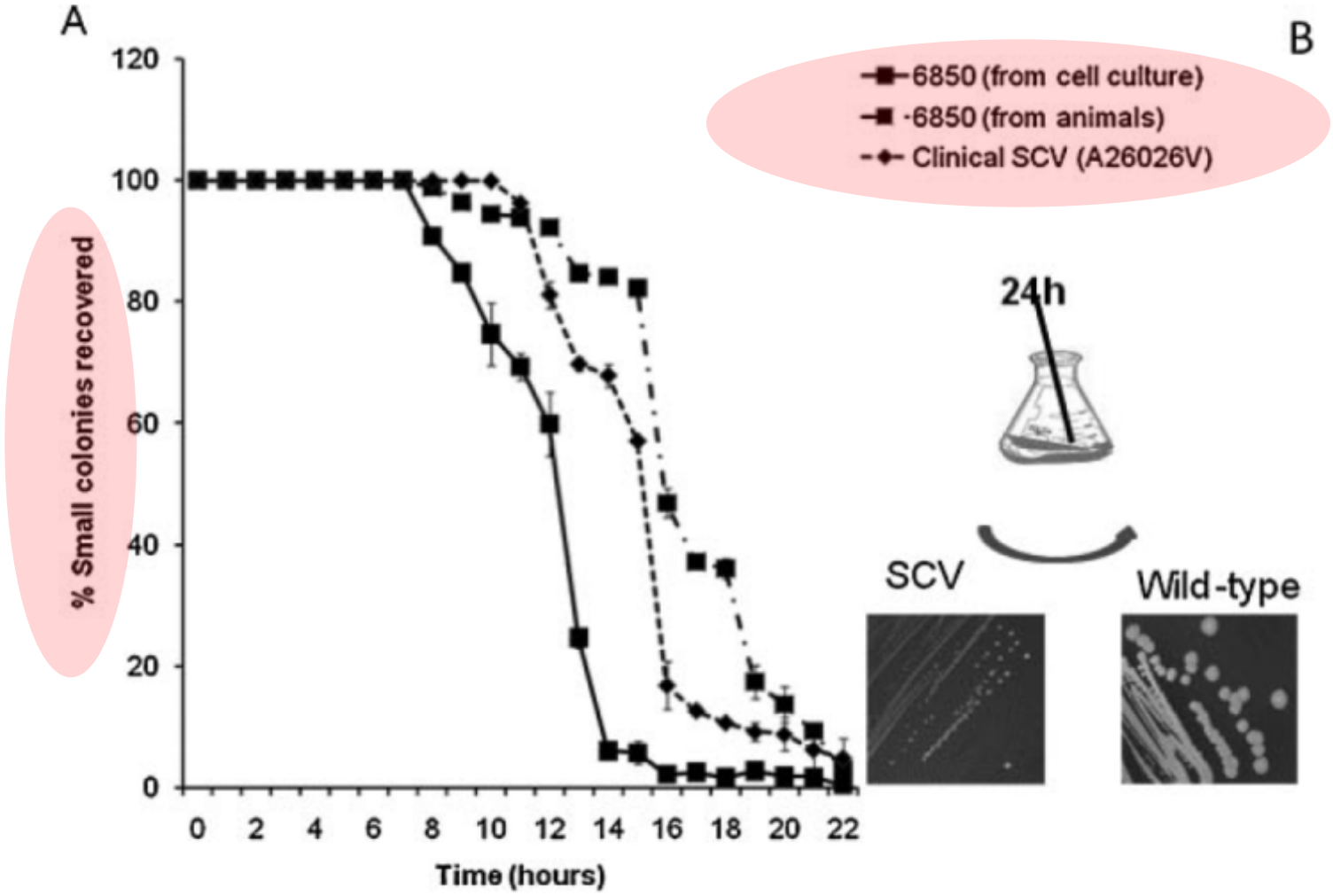
Sélection des SCV



Tuchscherr et al.,2010 EMBO Molecular Medecine

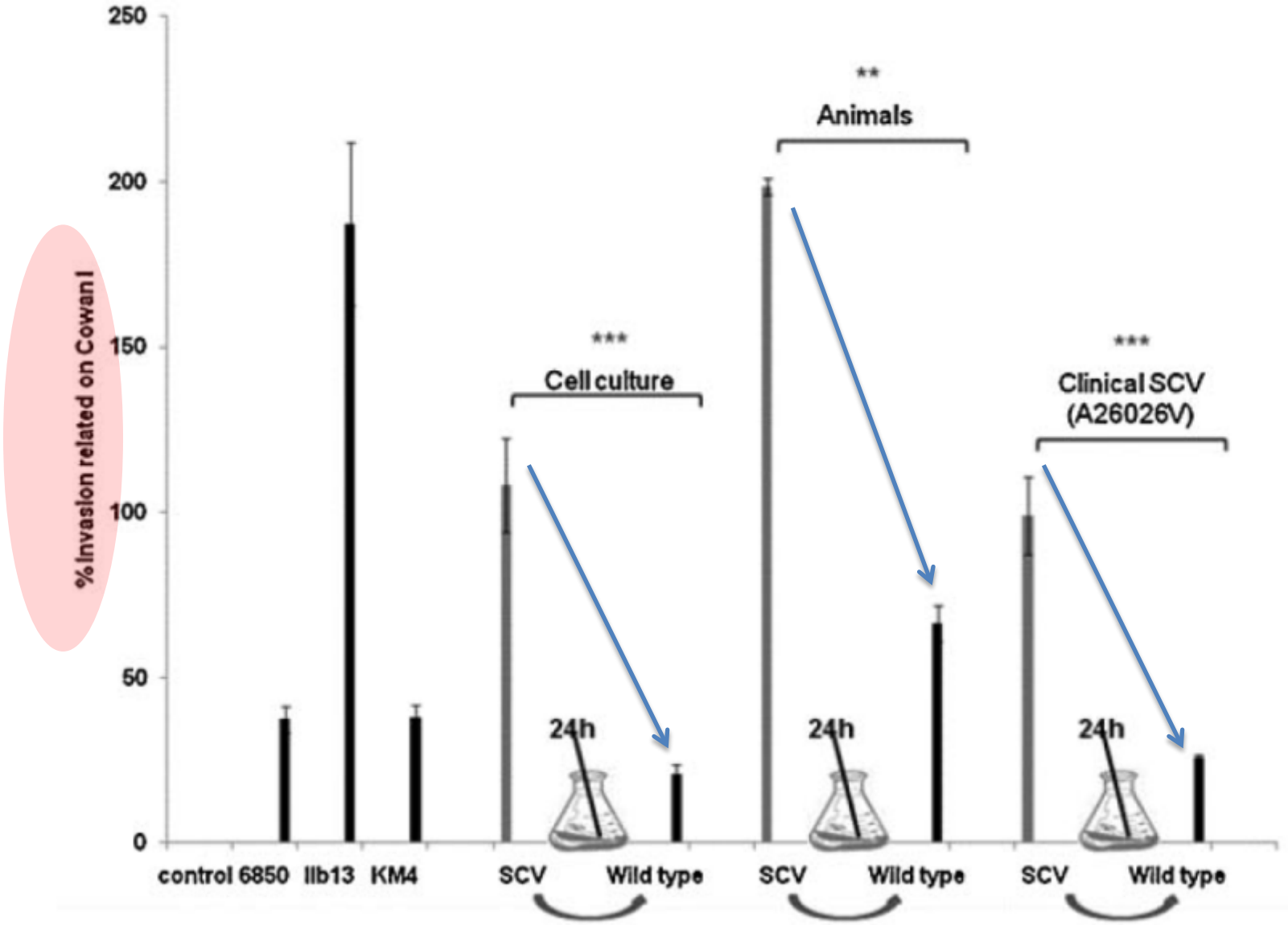
Pb pour le diagnostic : SCV révertants vs. SCV stables

Sélection des SCV



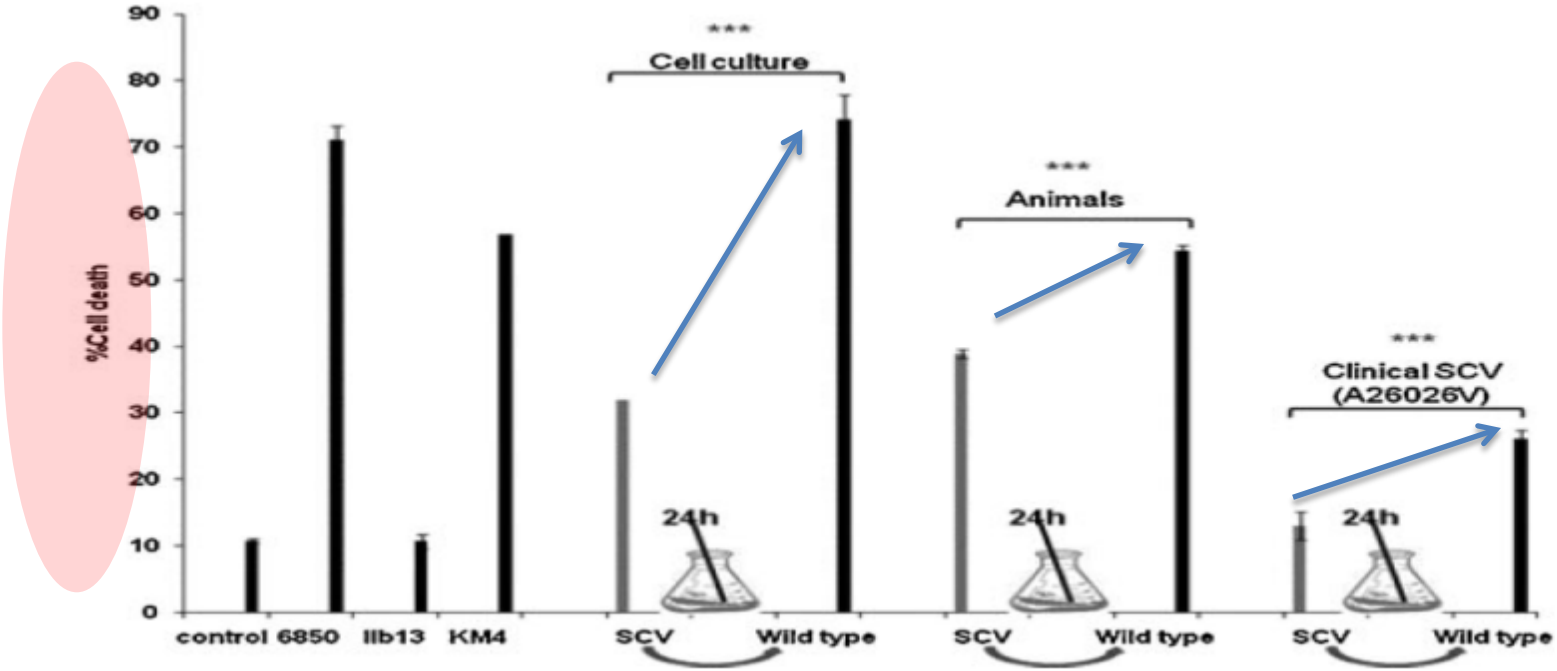
Tuchscherr et al.,2010 EMBO Molecular Medecine

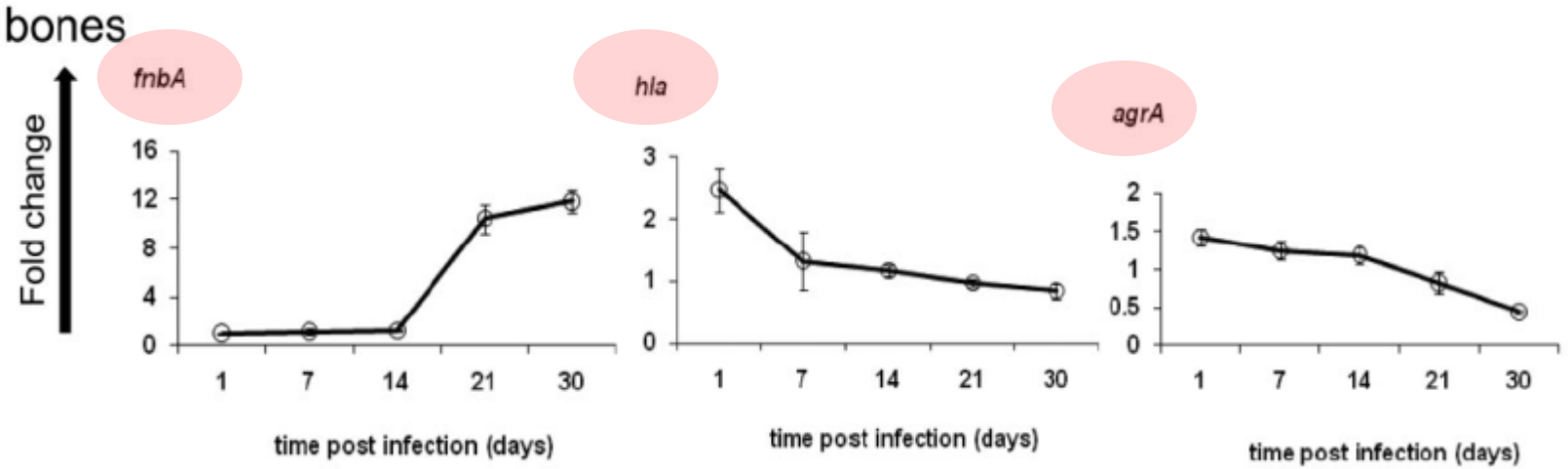
SCV et capacité d'internalisation



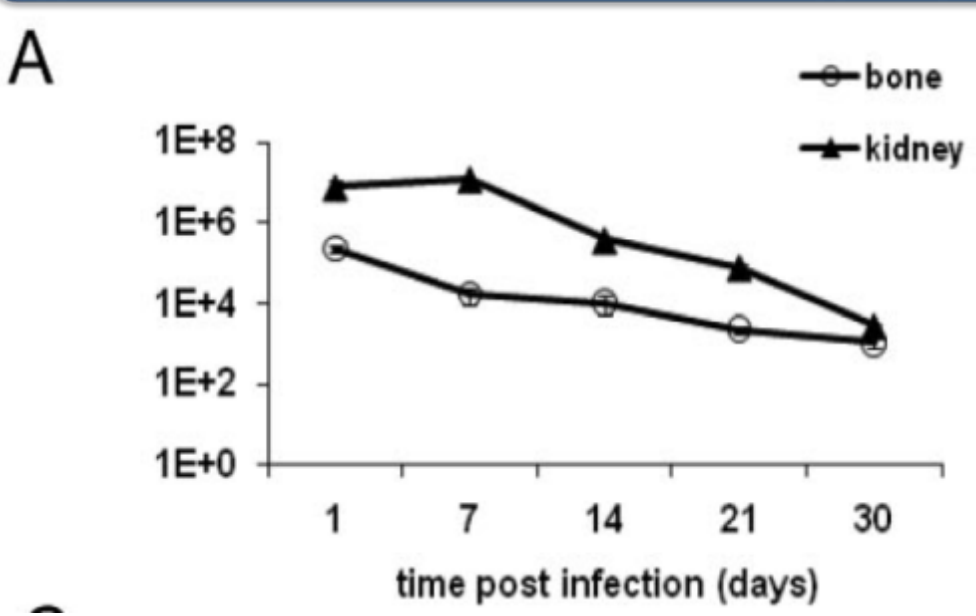
Tuchscherr et al.,2010 EMBO Molecular Medecine

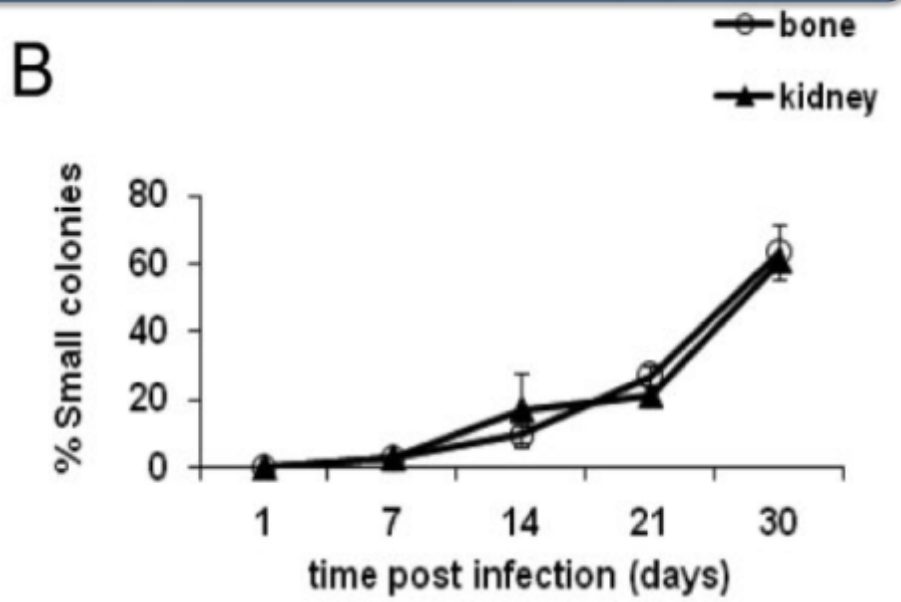
SCV et virulence



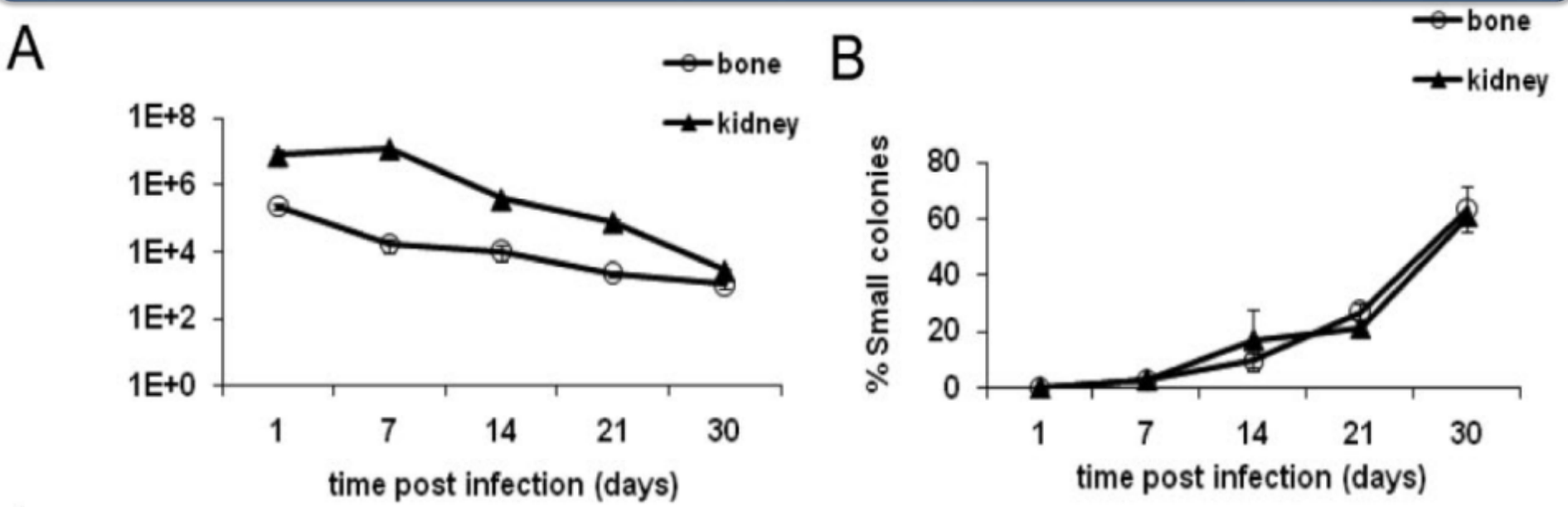


SCV et persistence

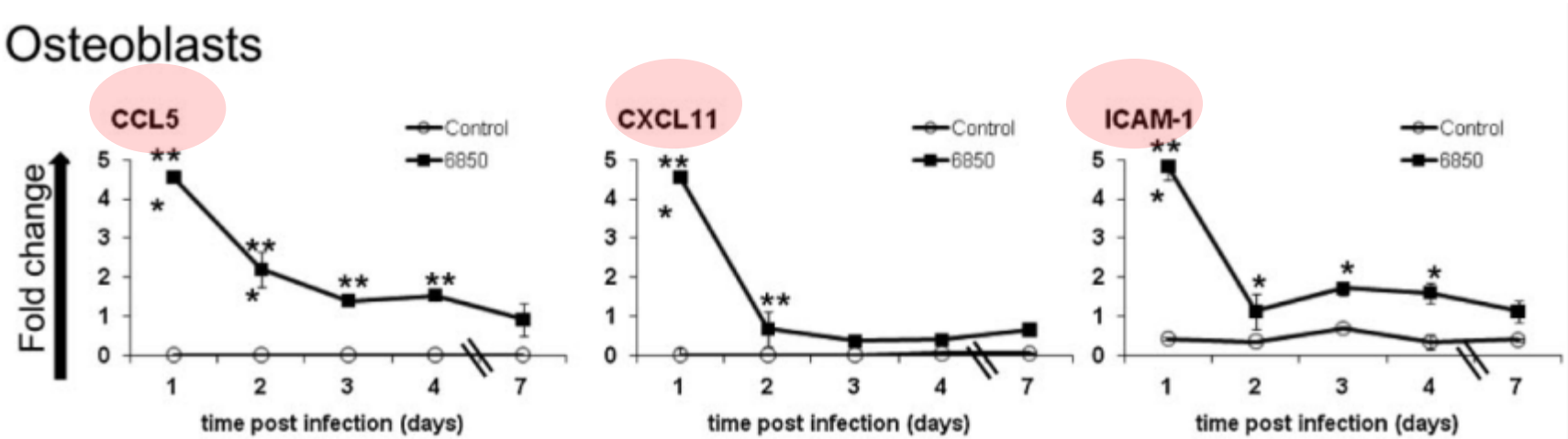




SCV et persistence



Réponse cellulaire

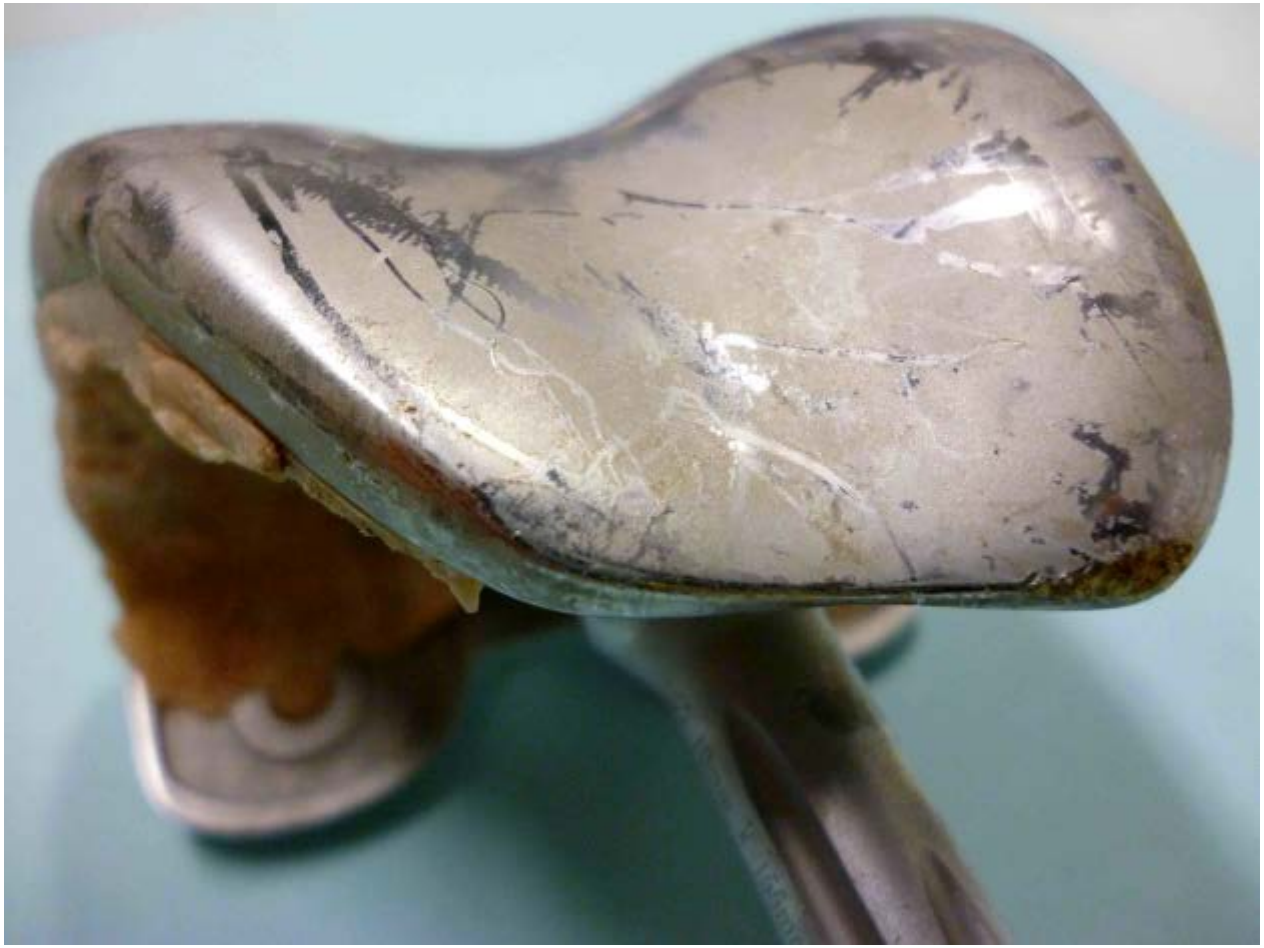


SCV et IOA chronique

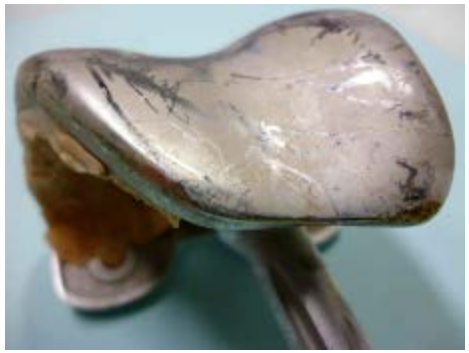
- Correlation SCV et chronicité en clinique:

  oui sur la base de cas cliniques mais pas de large serie publiée ….

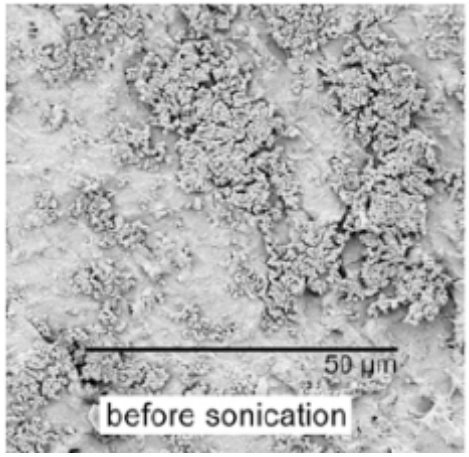
SCV et biofilm

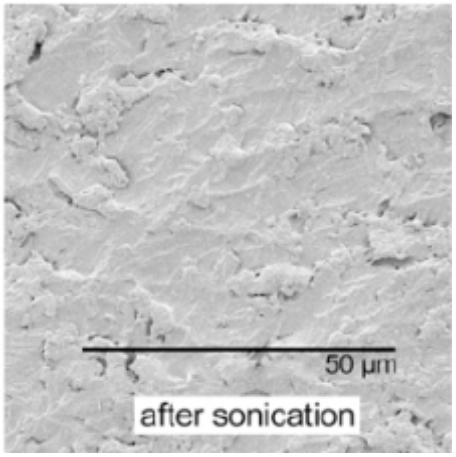


SCV et biofilm



Bactéries collées sur le matériel
Bactéries engluées dans la matrice





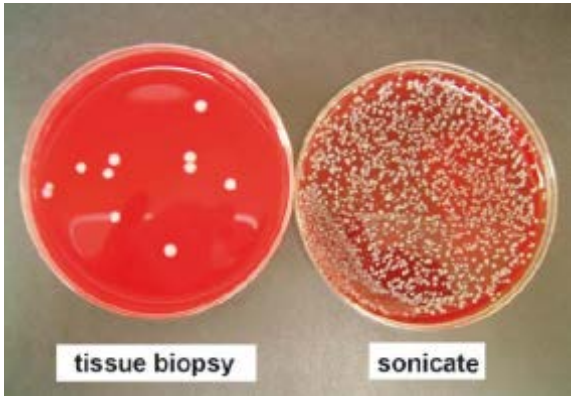
Sonication des prothèses et matériels

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Sonication of Removed Hip and Knee Prostheses for Diagnosis of Infection

Andrej Trampuz, M.D., Kerryl E. Piper, M.S., Melissa J. Jacobson, A.S., Arlen D. Hanssen, M.D., Krishnan K. Unni, M.D., Douglas R. Osmon, M.D., Jayawant N. Mandrekar, Ph.D., Franklin R. Cockerill, M.D., James M. Steckelberg, M.D., James F. Greenleaf, Ph.D., and Robin Patel, M.D.



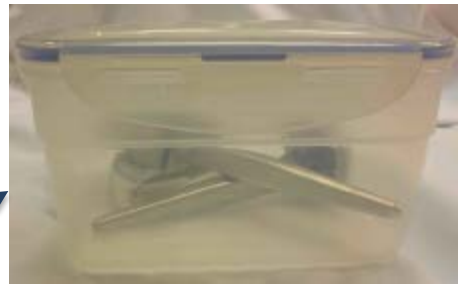
Test	Patients with Prosthetic-Joint Infection (N=79)	Patients with Aseptic Failure (N=252)	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
	*no. of patients with positive specimens**		*% (95% confidence interval)*			
Synovial-fluid culture	18/32	2/108	56.3 (37.7–73.6)	98.1 (93.5–99.8)	90.0 (68.3–98.8)	88.3 (81.2–93.5)
Periprosthetic-tissue culture†						
≥1 positive culture	58	23	73.4 (62.3–82.7)	90.9 (86.6–94.1)	71.6 (60.5–81.1)	91.6 (87.4–94.7)
≥2 positive cultures	48	2	60.8 (49.1–71.6)	99.2 (97.2–99.9)	96.0 (86.3–99.5)	89.0 (84.7–92.4)
Sonicate-fluid culture†‡						
≥1 CFU	64	28	79.0 (68.5–87.3)	88.5 (83.9–92.2)	68.8 (58.4–78.0)	93.9 (88.9–95.8)
≥2 CFU	63	8	79.7 (69.2–88.0)	96.8 (93.8–98.6)	88.7 (79.0–95.0)	93.8 (90.2–96.4)
≥3 CFU	63	5	79.7 (69.2–88.0)	98.0 (95.4–99.4)	92.6 (83.7–97.6)	93.9 (90.3–96.5)
≥4 CFU	62	5	78.5 (67.8–86.9)	98.0 (95.4–99.4)	92.5 (83.4–97.5)	93.6 (89.9–96.2)
≥5 CFU	62	3	78.5 (67.8–86.9)	98.8 (96.6–99.8)	95.4 (87.1–99.0)	93.6 (90.0–96.2)
≥6 CFU	62	3	78.5 (67.8–86.9)	98.8 (96.6–99.8)	95.4 (87.1–99.0)	93.6 (90.0–96.2)
≥7 CFU	60	3	75.9 (65.0–84.9)	98.8 (96.6–99.8)	95.2 (86.7–99.0)	92.6 (89.2–95.7)
≥8 CFU	59	3	74.7 (63.6–83.8)	98.8 (96.6–99.8)	95.2 (86.5–99.0)	92.6 (88.8–95.4)
≥9 CFU	58	3	73.4 (62.3–82.7)	98.8 (96.6–99.8)	95.1 (86.3–99.0)	92.2 (88.4–95.1)
≥10 CFU	57	3	72.2 (60.9–81.7)	98.8 (96.6–99.8)	95.0 (86.1–99.0)	91.9 (88.0–94.8)
≥25 CFU	55	2	69.6 (58.2–79.5)	99.2 (97.2–99.9)	96.5 (87.9–99.6)	91.2 (87.2–94.3)
≥50 CFU	54	1	68.4 (56.9–78.4)	99.6 (97.8–100.0)	98.2 (90.3–100.0)	90.9 (86.9–94.1)
Gram's staining of sonicate fluid	34/76	0/250	44.7 (33.3–56.6)	100.0 (98.5–100.0)	100.0 (89.7–100.0)	85.6 (81.1–89.4)

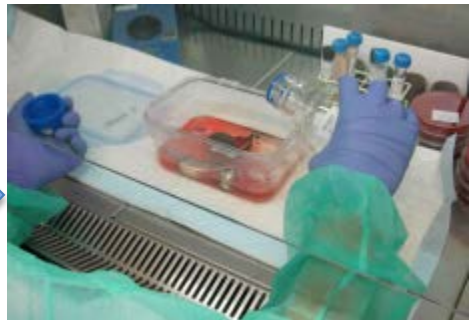
≥ 5 UFC/boîte = infection

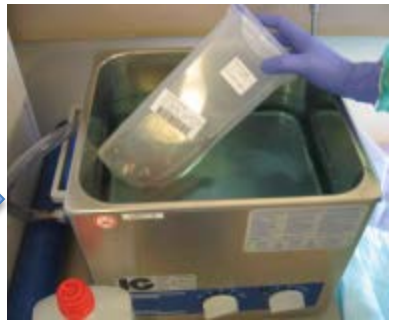
Sensibilité plus importante de la technique de sonication 60,8 % contre 78,5 %

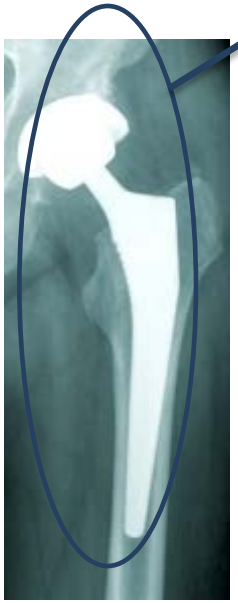
SCV et biofilm

Prothèses explantées

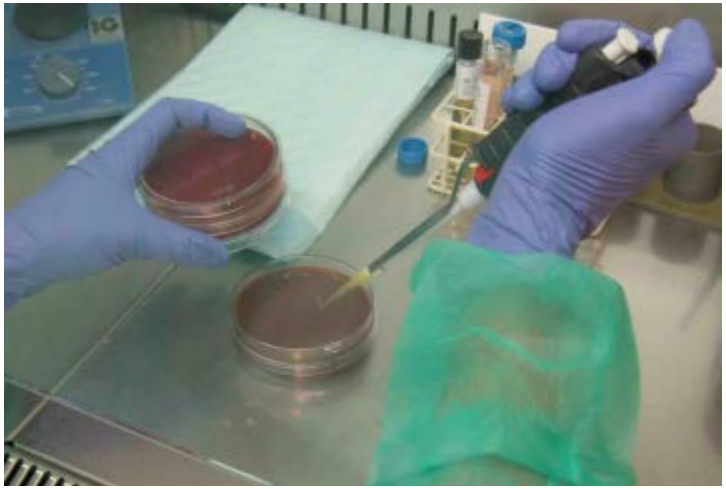






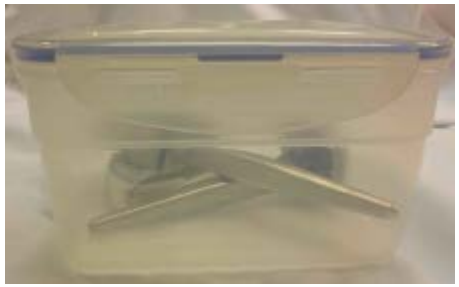


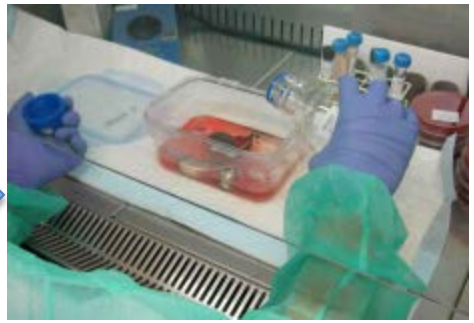
Juin 2011 – Mars 2012

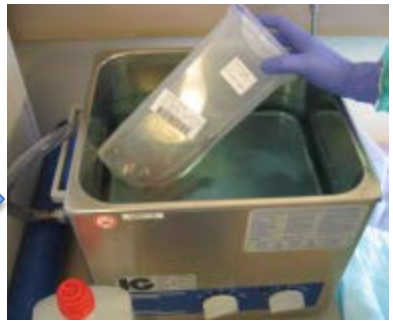


SCV et biofilm

Prothèses explantées

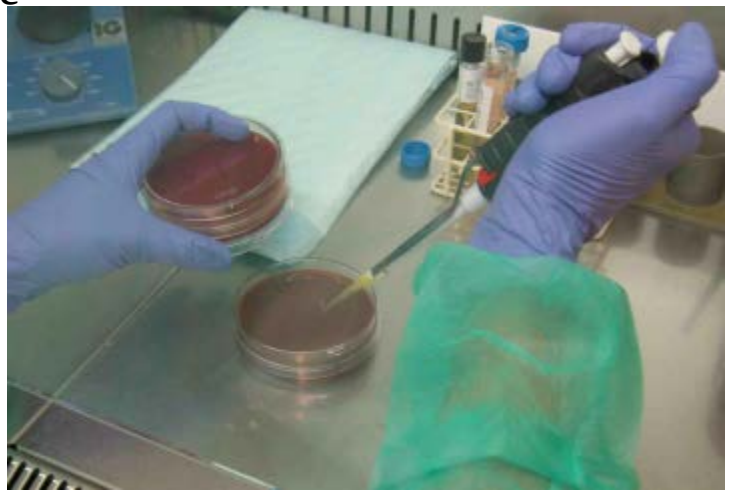




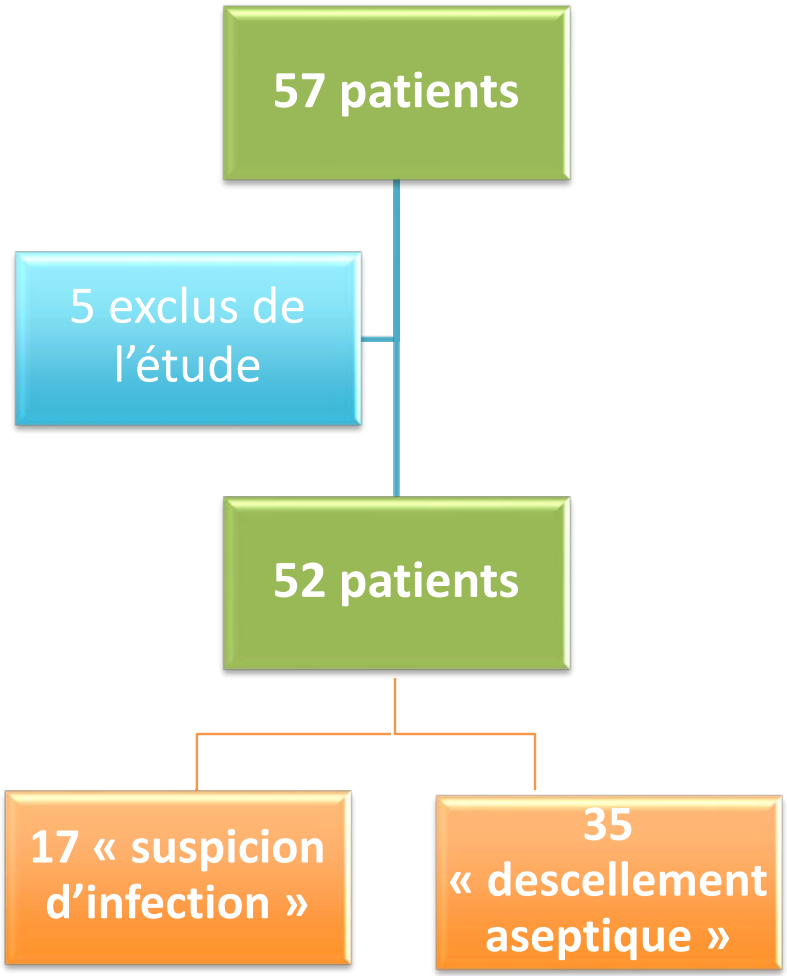


Juin 2011 – Mars 2012

Culture bactérienne classique (1-10 prélèvements per-opératoires)



SCV et biofilm



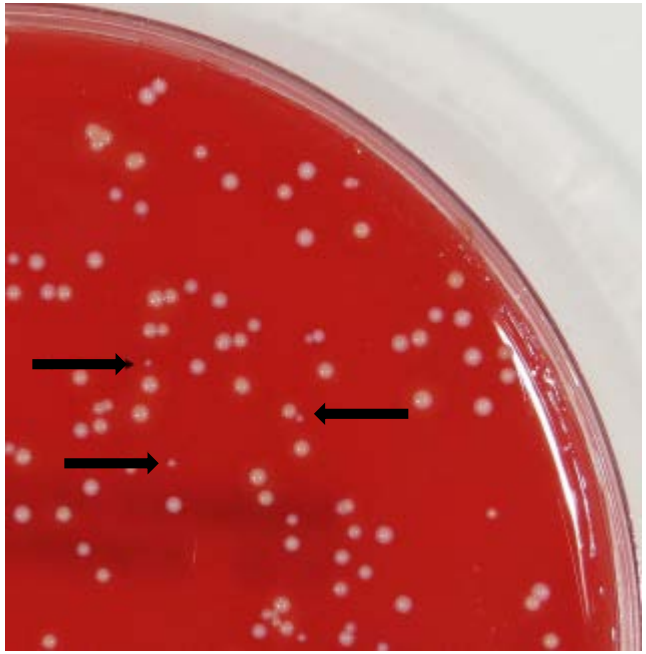
SCV et biofilm

Résultats – Culture classique *VS* sonication (3)

Apport de la sonication dans le diagnostic des SCV

17 ”suspicion d’infection” → 9 cultures + → 6 avec SCV +

S. aureus
S. epidermidis
P. aeruginosa
S. gallolyticus
P. acnes
S. sanguinis



Culture de sonicat

S. aureus « sauvage »

S. aureus SCV

SCV et biofilm

Small colony variant-producing *S aureus* prosthesis joint infection highlighted by sonication and treated with prolonged high doses of daptomycin

Camille Piffaut,[1] Sébastien Lustig,[2,3,4] Frédéric Laurent,[1,2,4,5,6] Christian Chidiac,[2,4,5,7] Tristan Ferry,[2,4,5,6,7] on behalf of the Lyon BJI Study Group

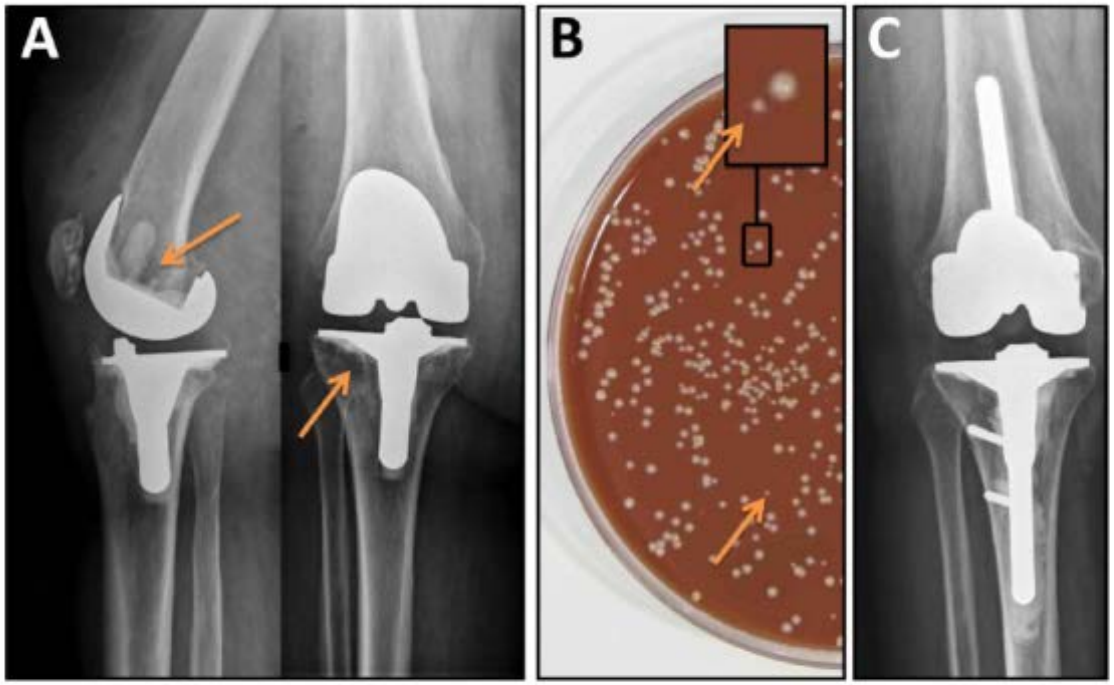
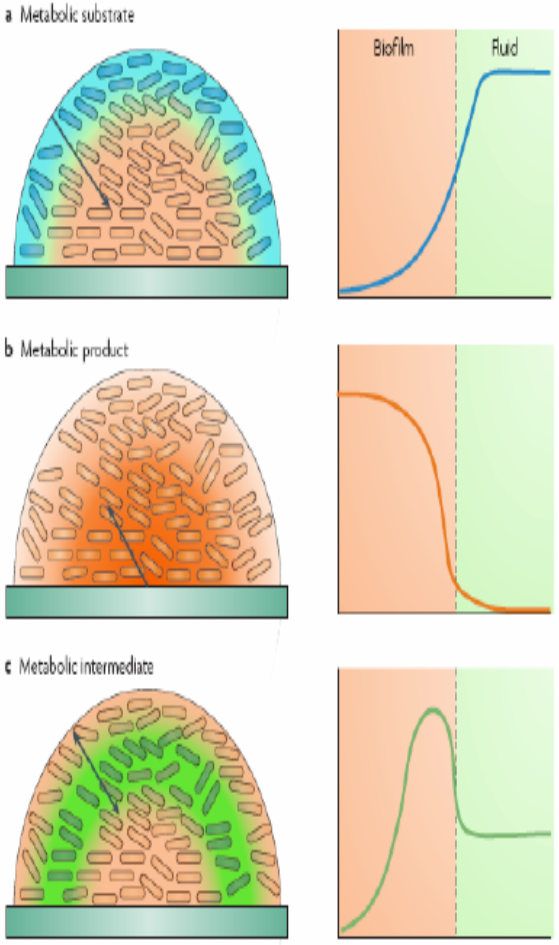


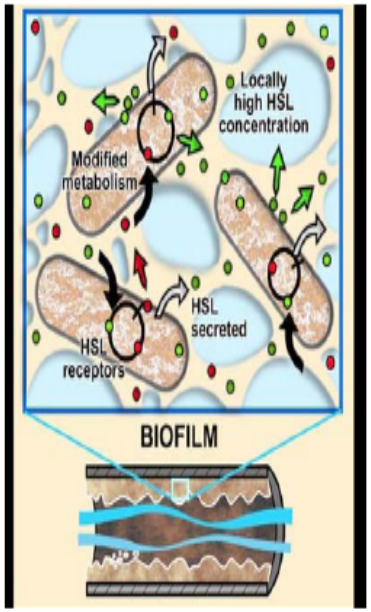
Figure 1 Periprosthetic pathological lucencies (A, arrows), associated with MSSA SCV in cultures (small colonies) obtained with sonication from prosthesis explantation (B, arrows). After reimplantation and a total of 3 months of antimicrobial therapy, no relapse occurred at 1 year (C, x-ray).

SCV et biofilm

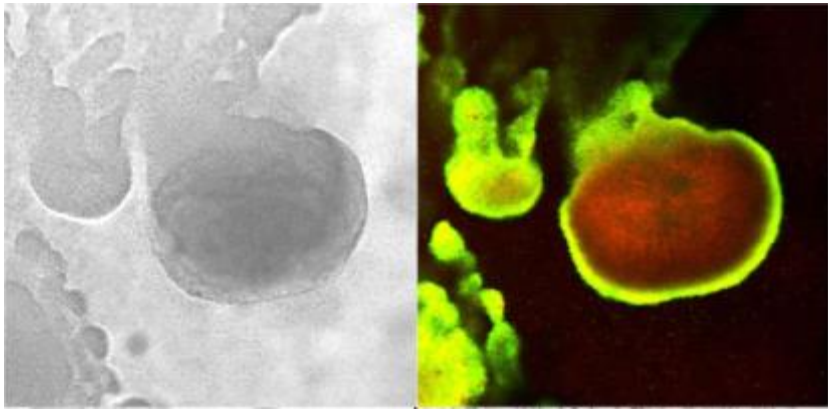
Métabolites primaires



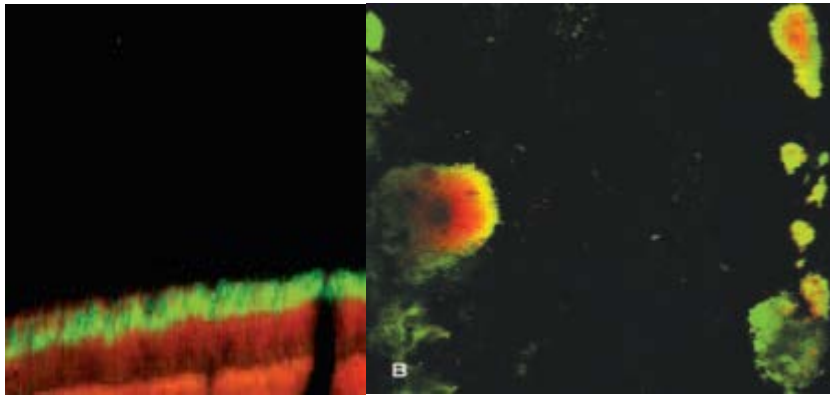
Métabolites secondaires et signaux



Réplication ADN



Synthèse protéique



Stewart & Franklin 2008 Nature Reviews Microbiology 6:199-210

SCV et biofilm

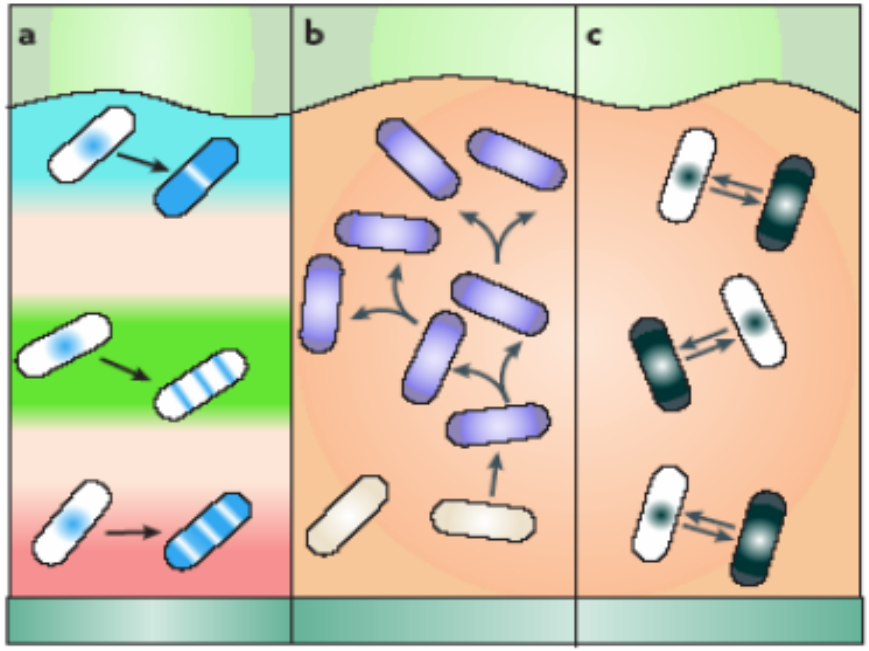
Variation génotypique et phénotypique

1- L'adaptation (a). Perception de conditions environnementales localement différentes auxquelles les cellules répondent.
->réponses adaptatives distinctes en fonction de la localisation dans le biofilm.

2- Variation génotypique et sélection (b). Apparition de variants génétiques (variation de phase) qui se multiplient dans le biofilm en fonction de leur valeur adaptative (fitness).

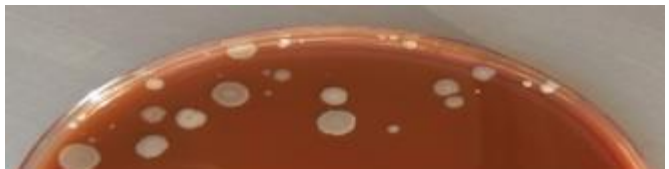
3- Expression stochastique des gènes. Expression génique aléatoire.

Diversification phénotypique : 3 hypothèses



Stewart & Franklin 2008 Nature Reviews Microbiology 6:199-210

→ Sélectionde "phénotype " biofilm



SCV, IOA et diagnostic bactériologique

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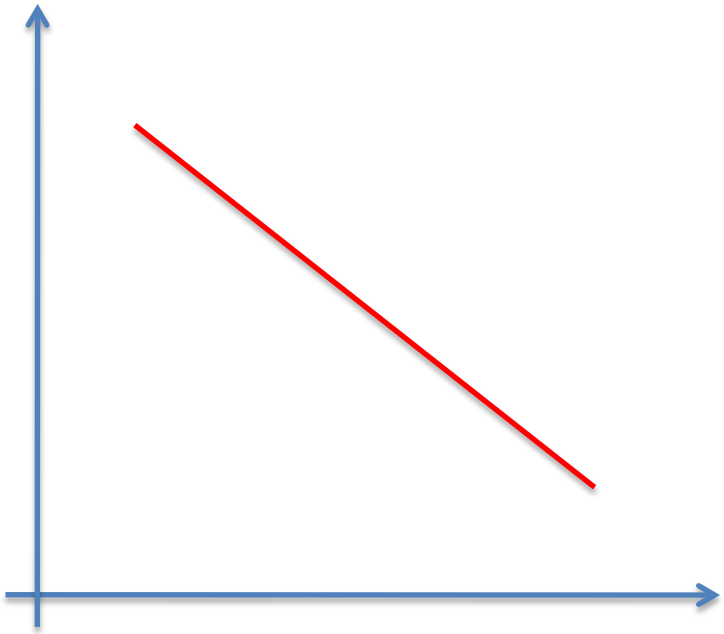
SCV, IOA et diagnostic bactériologique

There is a saying that ”small is beautiful” …. But for SCV small means ”difficult to be seen”

Taux de prélvts +
à SCV



Age et niveau de myopie de la technicienne

SCV, IOA et diagnostic bactériologique

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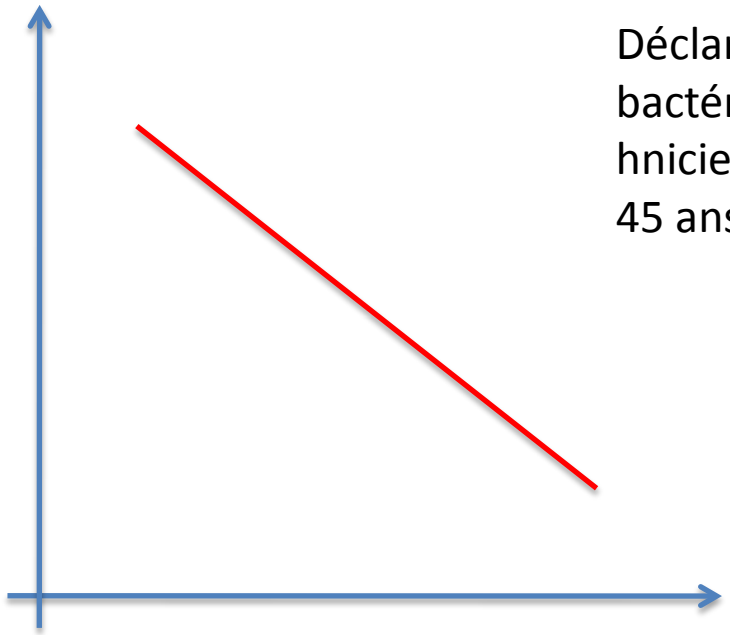

Age et niveau de myopie de la technicienne

Déclarer inapte les bactériologiste/technicien de plus de 45 ans

OU

SCV, IOA et diagnostic bactériologique

- SCV seule ou avec colonies ”classiques”
- Culture lente
- Colonies petites
- Aspect différent (*S. aureus* non pigmenté +++)
- Perte d’hémolyse
- Perte de caractères distinctifs:
  - Ex: *S. aureus* et slidex, coagulase, DNAse, caractères biochimiques

SCV, IOA et diagnostic bactériologique

Table 2. Biochemical characterization of small colony variants of *S. aureus* 6850 obtained after infection of endothelial cells.

Strain (n = 14)	cfu* (n = 660)	Auxotrophy			Coagulase		Hemolysis	
		M	CO_2	H	NS	S	NS	S
E3-2	5	−	−	−	+	NT	+	NT
E3-5	500	+	−	−	−	+	−	−
E3-8	50	−	+	−	−	+	+	+
E3-10	5	−	+	−	−	+	−	−
E4-10	10	−	−	−	+	NT	+	NT
E4-19A	10	+	−	−	−	+	−	+
E4-19B	10	+	−	−	−	+	−	+
E4-19C	10	+	−	−	−	+	−	+
E4-19D	10	+	−	−	−	+	−	+
E4-19E	10	+	−	−	−	+	−	−
E4-19F	10	+	−	−	−	+	−	+
E4-24B	10	−	+	−	−	+	+	+
E4-24D	10	+	−	−	−	+	−	+
E4-24H	10	+	−	−	−	+	−	+

NOTE. M = menadione, H = hemin, NS = nonsupplemented, S = supplemented with menadione or CO_2, NT = not tested because of nonauxotrophism.
* No. of cfu for each strain.

Vesga JID 1996

SCV et diagnostic bactériologique

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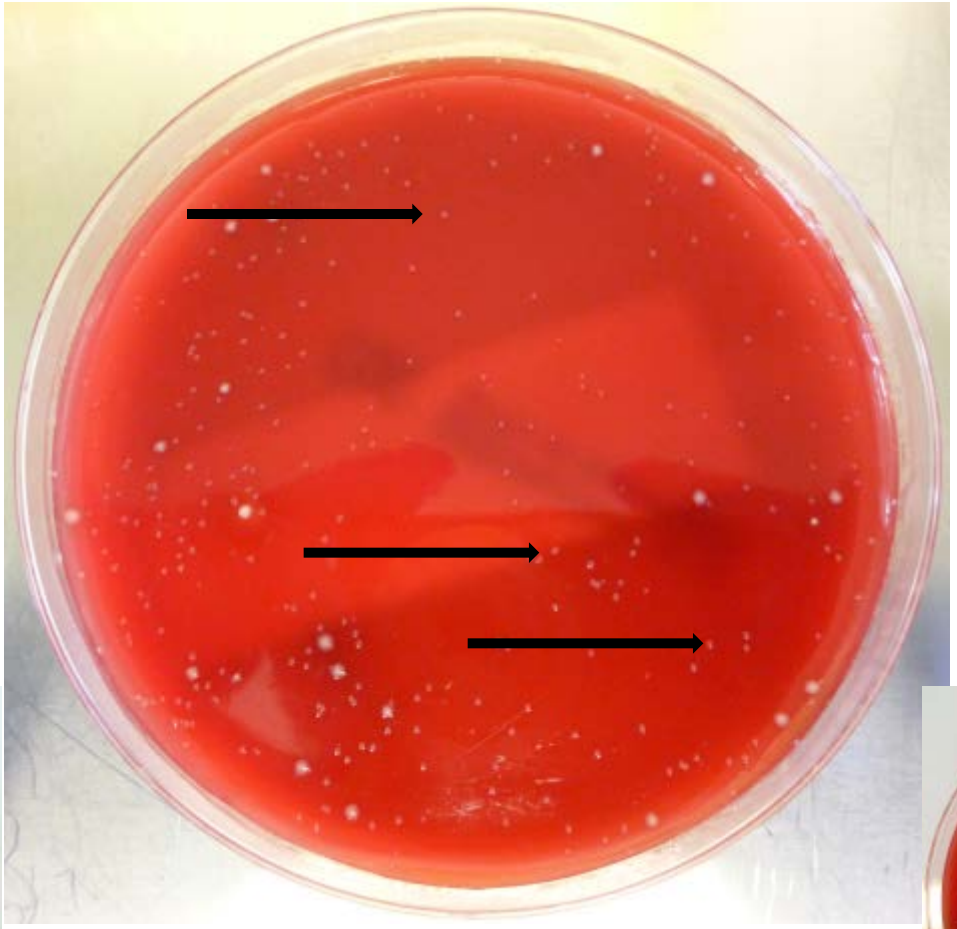
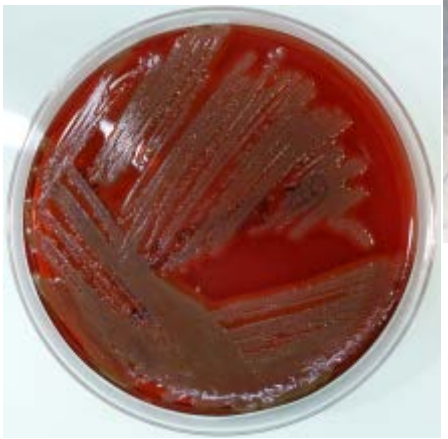
D’où pour IOA

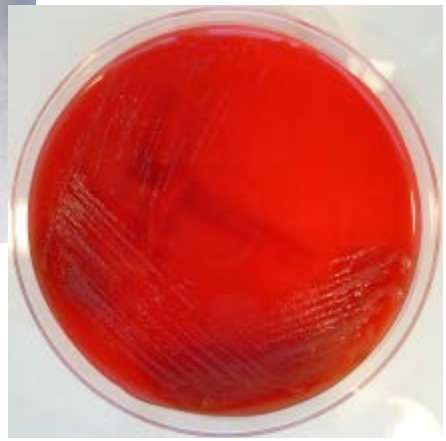
- nécessité examen approfondi
- incubation prolongée
- ATBgramme sur toutes les morphologies
- parfois probablement identification erronée (SCN vs *S. aureus*)

SCV et diagnostic bactériologique





P. aeruginosa



P. aeruginosa SCV

No SCV on standard cultures

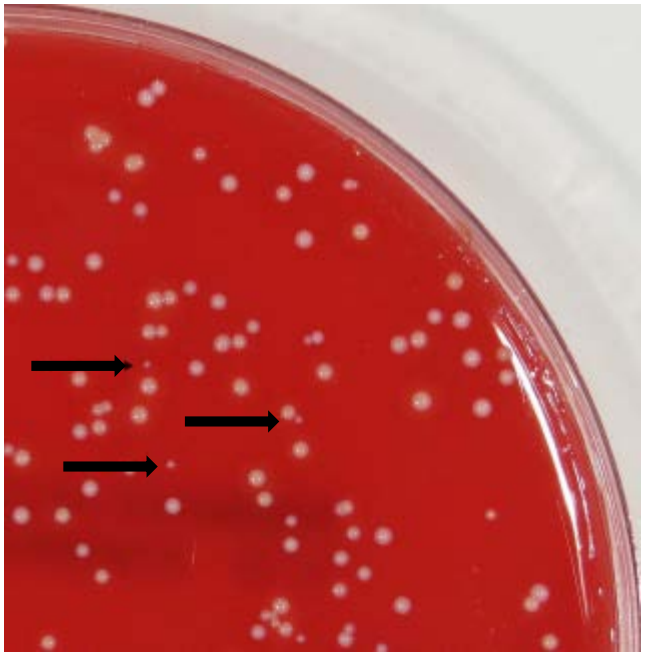
SCV et résistance

Résultats – Culture classique *VS* sonication (3)

Apport de la sonication dans le diagnostic des SCV

17 ”suspicion d’infection” → 9 cultures + → 6 avec SCV +

S. aureus
S. epidermidis
P. aeruginosa
S. gallolyticus
P. acnes
S. sanguinis



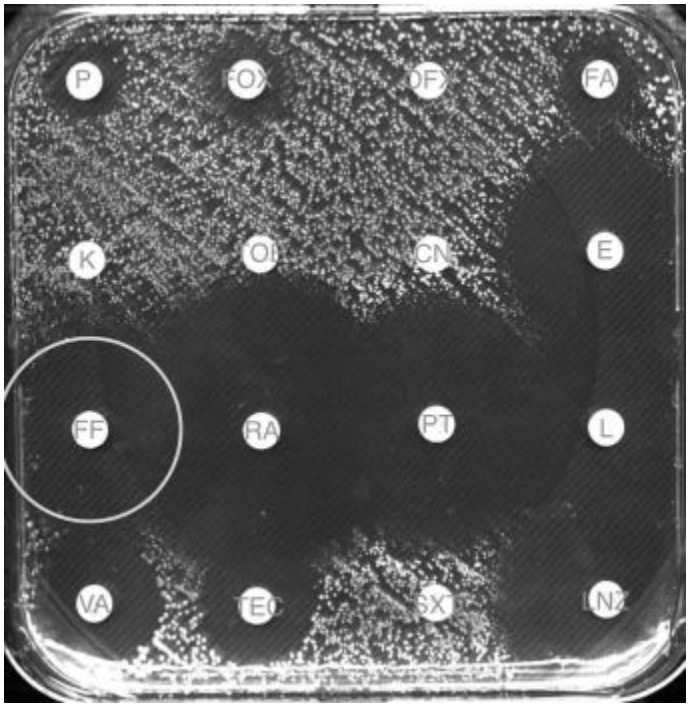
Culture de sonicat

S. aureus « sauvage »

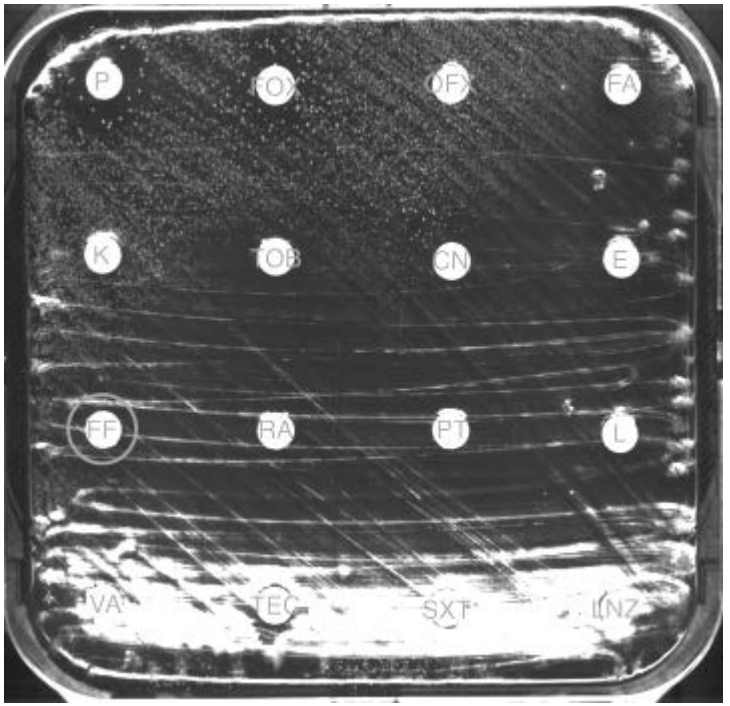
S. aureus SCV

SCV et résistance

- Comparaison des antibiogrammes : SCV plus résistants (n=2)



S. epidermidis



S. epidermidis de phénotype SCV

Choix thérapeutiques orientés et adaptés

SCV et IOA en échec

- Échec de traitement et SCV :

  oui, mais là encore cas cliniques mais pas de grande série documentée

Small colony variant-producing *S aureus* prosthesis joint infection highlighted by sonication and treated with prolonged high doses of daptomycin

Camille Piffaut,[1] Sébastien Lustig,[2,3,4] Frédéric Laurent,[1,2,4,5,6] Christian Chidiac,[2,4,5,7] Tristan Ferry,[2,4,5,6,7] on behalf of the Lyon BJI Study Group

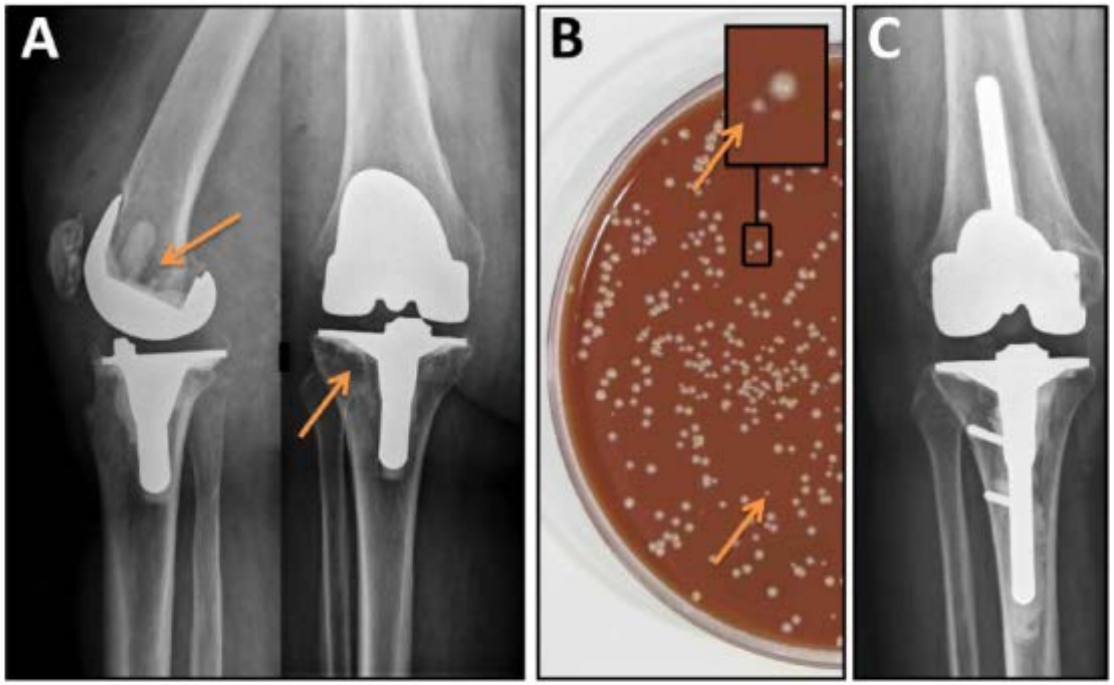


Figure 1 Periprosthetic pathological lucencies (A, arrows), associated with MSSA SCV in cultures (small colonies) obtained with sonication from prosthesis explantation (B, arrows). After reimplantation and a total of 3 months of antimicrobial therapy, no relapse occurred at 1 year (C, x-ray).

SCV et résistance

J Antimicrob Chemother
doi:10.1093/jac/dkt072

Journal of Antimicrobial Chemotherapy

Antibiotic activity against small-colony variants of *Staphylococcus aureus*: review of *in vitro*, animal and clinical data

Laetitia G. Garcia[1], Sandrine Lemaire[1], Barbara C. Kahl[2], Karsten Becker[2], Richard A. Proctor[3], Olivier Denis[4], Paul M. Tulkens[1] and Françoise Van Bambeke[1]*

Conclusions

Although clearly challenging for both the microbiologist and the clinician, SCVs of *S. aureus* remain an ill-explored field, at least with respect to the more appropriate therapeutic options to prevent their emergence on the one hand and to eradicate them when present on the other. Although long-term therapy with gentamicin and antifolate agents is clearly associated with their selection, clinical reports suggest that other drugs may also be incriminated. *In vitro* susceptibility testing should also be performed in conditions that allow SCV susceptibility to be examined (48 h incubation). Clinical investigations specifically targeting SCV-related infections are probably difficult to perform because their diagnosis escapes routine procedures. The present

SCV et résistance

Questions qui reste sans réponse car aucune étude seulement des séries de quelques cas:

- Faut-il traiter différemment les patients avec IOA à SCV + ?
- Faut-il préférer certains ATB ? (activité intracellulaire, intra biofilm, …)
- Faut-il traiter plus longtemps? … mais combien de temps

On aurait envie de répondre oui …

Conclusion

- SCV = physiologique ? Présent tout le temps ?
- Rôle physiopathologique clair in vitro et dans les modèles
- Prise en charge:
  - examiner tous les aspects
  - Identifier tous les aspects (Maldi-tof: colonie blanches qui sont des S. aureus, Strepto non hémolytique qui sont des StreptoA, …)

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Projet possible autour de cette thématique pour le CRIOA / PHRC inter-régional ?

Remerciements

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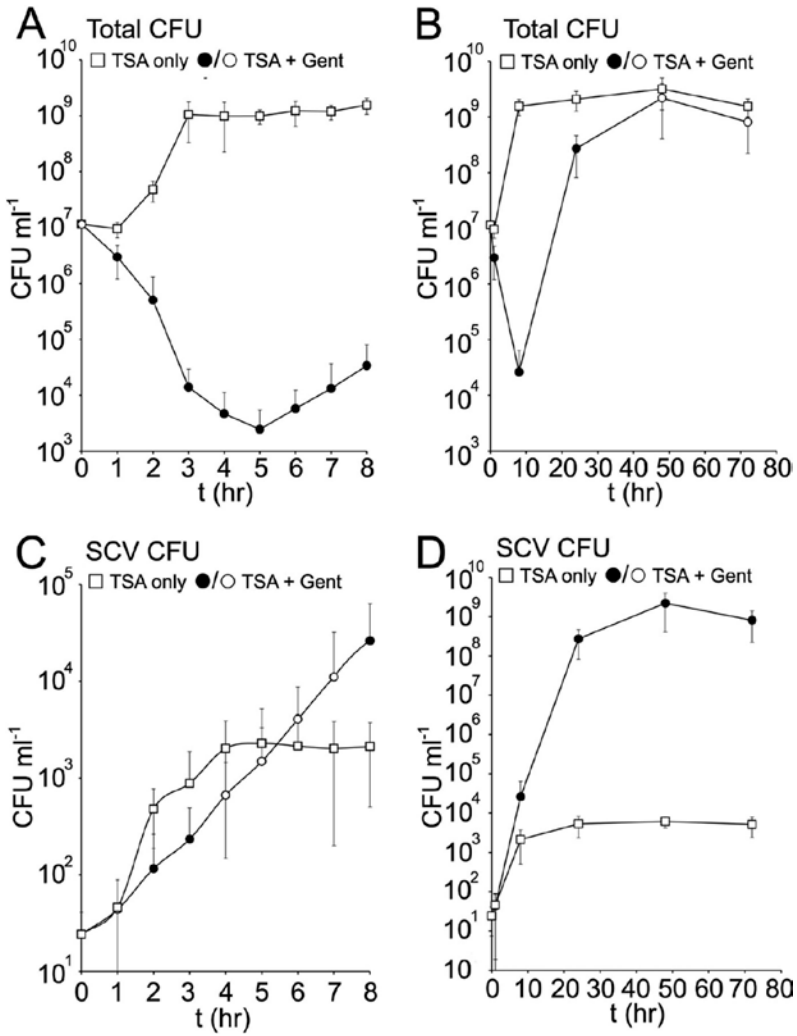
Philippe NEYRET
Sébastien LUSTIG

LYON BJI GROUP ...

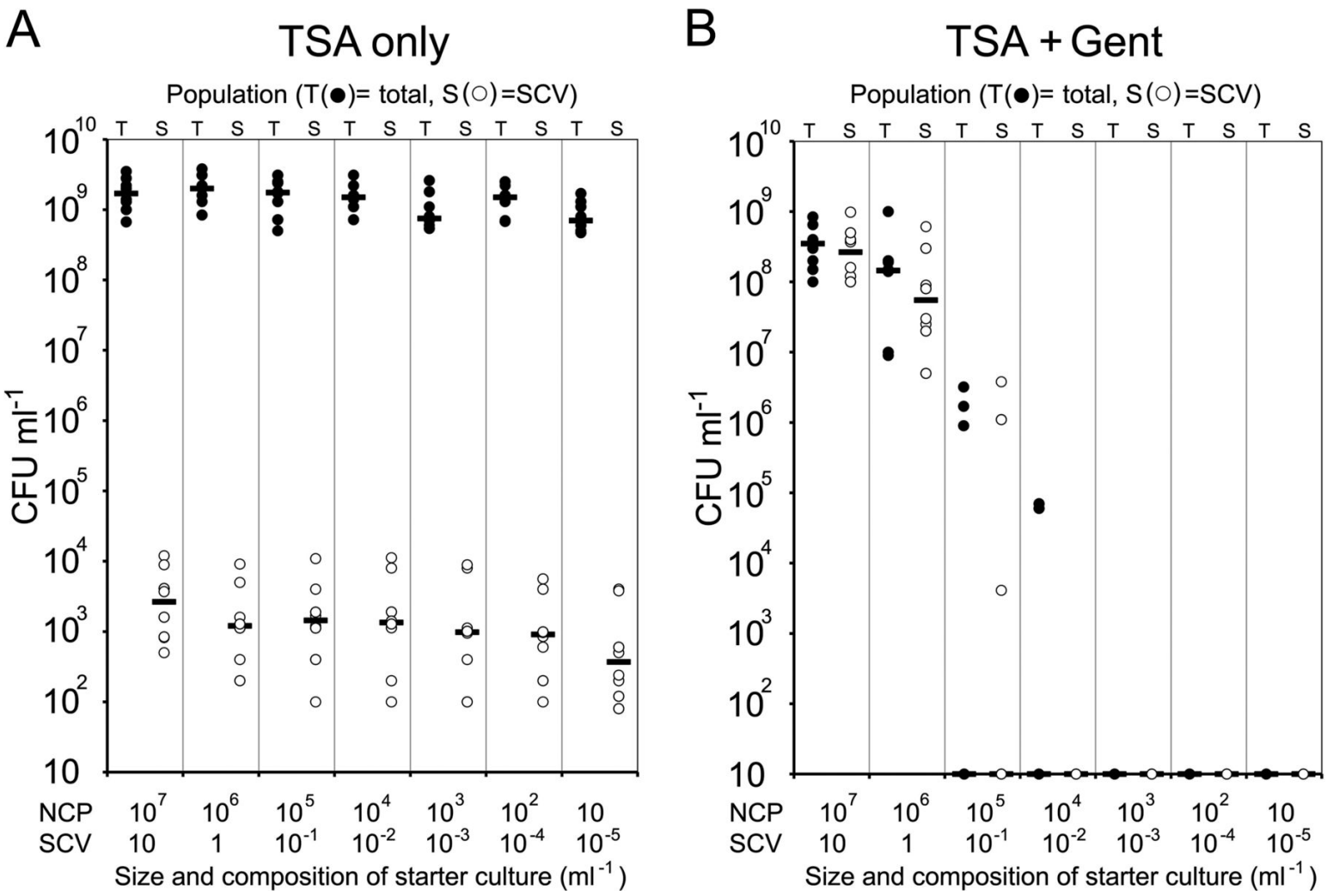
The SCV subpopulation expands more slowly in the presence of gentamicin.



Edwards A M J. Bacteriol. 2012;194:5404-5412

Journal of Bacteriology

SCVs appear in cultures grown from purely NCP inocula.



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