Physiopathologie des infections ostéo-articulaires

Entre virulence et persistance

Florent Valour

Service des maladies infectieuses et tropicales
CRIOAc Lyon
Hospices Civils de Lyon

Frédéric Laurent

Institut des agents infectieux – CNR staphylocoques
CRIOAc Lyon
Hospices Civils de Lyon

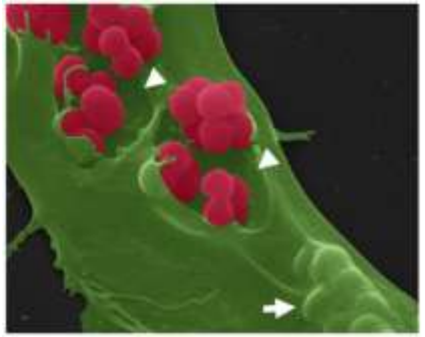
INSERM U1111 – Equipe « Pathogénèse des infections à staphylocoques »
Centre International de Recherche en Infectiologie (CIRI)
Université Claude Bernard Lyon 1

Le programme

MODES DE SURVENUE

BIOFILMS

AGENTS PATHOGENES



RESERVOIR INTRACELLULAIRE

CHRONOLOGIE DES IOA

TIMING IS EVERYTHING

SMALL COLONY VARIANTS
PERSISTERS

Modes de survenue

Modes de survenue (1) : hématogène

Mécanisme le plus fréquent : greffe hématogène au cours d'une bactériémie

- Arthrite sur articulation native +++
- Spondylodiscite +++

→ *S. aureus*

→ Favorisé par anomalie ostéo-articulaire sous-jacente +++

Pathologie articulaire sous-jacente (50%)

- **Polyarthrite rhumatoïde +++ (RR x 3-15)**
  - 30-100/ 100 000 h / an
  - Prédisposition articulaire
  - Immunosuppression locale (+ IS)
  - Rôle du portage de *S. aureus* augmenté ?
- **Autres arthropathies**

  Microcristallines, inflammatoires, dégénératives …

Kaandorp 1995 Arthritis Rheum; Gupta 2001 Rheumatology; Gavet 2005 J Am Geriatr Soc; Margaretten 2007 jama; Mathews 2010 Lancet; Wang 2015 Best Pract Clin Rheumatol; Sapico 1996 Clin Infect Dis

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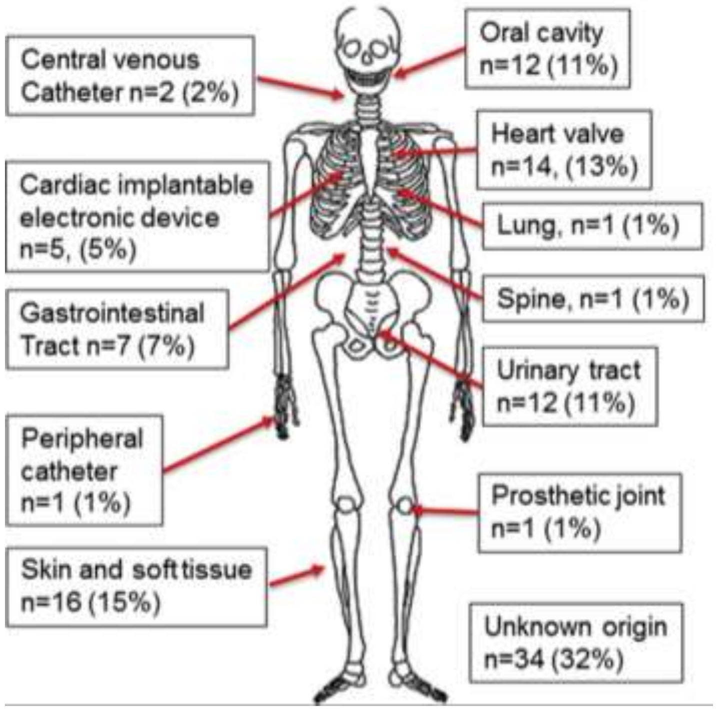
- Infection de prothèse articulaire

Bactériémie chez des patients porteurs de prothèse : 3,8% (Honkanen, 14 378 patients)

Origine (Rako, 106 patients)


Central venous Catheter n=2 (2%)
Cardiac implantable electronic device n=5, (5%)
Gastrointestinal Tract n=7 (7%)
Peripheral catheter n=1 (1%)
Skin and soft tissue n=16 (15%)
Oral cavity n=12 (11%)
Heart valve n=14, (13%)
Lung, n=1 (1%)
Spine, n=1 (1%)
Urinary tract n=12 (11%)
Prosthetic joint n=1 (1%)
Unknown origin n=34 (32%)

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BacT/ALERT
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Risque de greffe bactérienne sur prothèse en cas de bactériémie

S. aureus **= 20-40% vs autres germes = 1-2%**

Sendi	2010	30	40%
Lalani	2008	29	38%
Murdoch	2001	44	34%
Papadimitriou-Olivgeris	2022	135	28%
Honkanen	2021	542	21%
Dufour	2019	143	19%

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Facteurs de risque
- Prothèse de genou
- ATCD de révision
- Bactériémie communautaire
- Âge < 70 ans

Modes de survenue (2) : inoculation directe

Ponctions

- Exceptionnel : 1 / 10 000 à 1 / 100 000

Infiltrations

- Rare : < 0,04%

Chirurgie

- Arthroscopie : 0,04-0,4%
- Si reconstruction : 0,1-2,3%
- *S. aureus* : 35-70%

FR (étude SOFCOT)
- Tabac
- Diabète
- Obésité
- Sportifs

Autres : traumatismes, morsures …

Bauer 2015 OTSR; Van Tongel 2007 Am J Sports Med; Wang 2009 Arthroscopy; Wang 2014 KSSTA; Nallamshetty 2003 Clin Imaging; Geirsson 2008 Ann Rheum Dis

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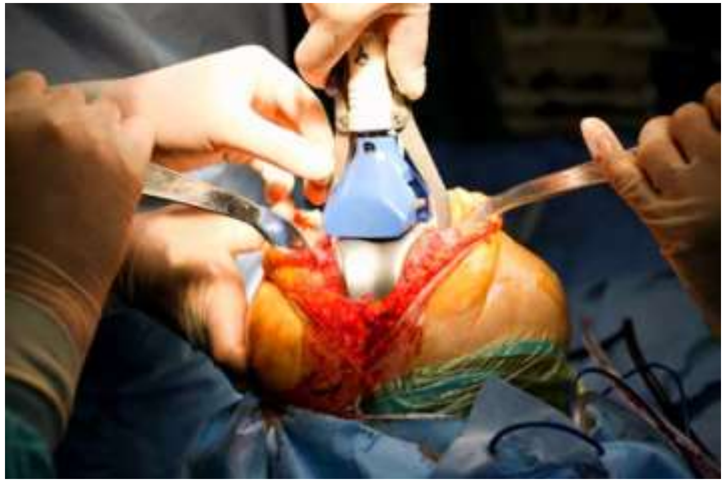
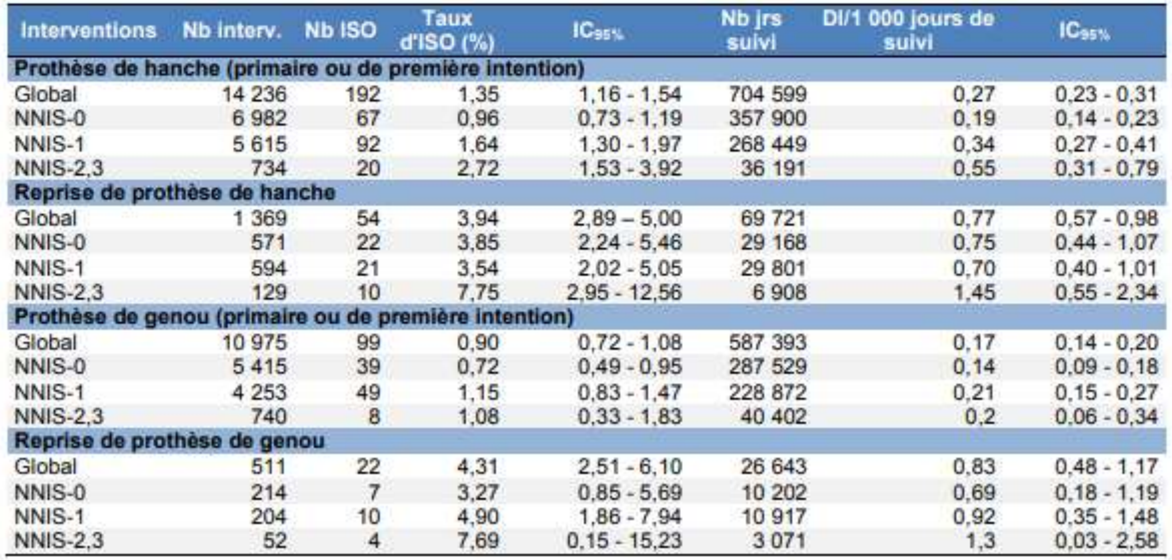
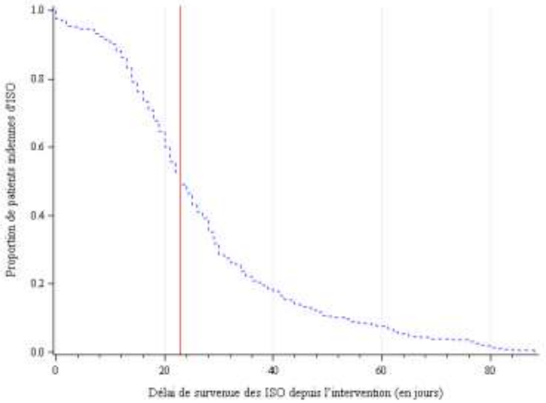




Interventions	Nb interv.	Nb ISO	Taux d'ISO (%)	IC$_{95\%}$	Nb jrs suivi	DI/1 000 jours de suivi	IC$_{95\%}$
Prothèse de hanche (primaire ou de première intention)							
Global	14 236	192	1,35	1,16 - 1,54	704 599	0,27	0,23 - 0,31
NNIS-0	6 982	67	0,96	0,73 - 1,19	357 900	0,19	0,14 - 0,23
NNIS-1	5 615	92	1,64	1,30 - 1,97	268 449	0,34	0,27 - 0,41
NNIS-2,3	734	20	2,72	1,53 - 3,92	36 191	0,55	0,31 - 0,79
Reprise de prothèse de hanche							
Global	1 369	54	3,94	2,89 – 5,00	69 721	0,77	0,57 - 0,98
NNIS-0	571	22	3,85	2,24 - 5,46	29 168	0,75	0,44 - 1,07
NNIS-1	594	21	3,54	2,02 - 5,05	29 801	0,70	0,40 - 1,01
NNIS-2,3	129	10	7,75	2,95 - 12,56	6 908	1,45	0,55 - 2,34
Prothèse de genou (primaire ou de première intention)							
Global	10 975	99	0,90	0,72 - 1,08	587 393	0,17	0,14 - 0,20
NNIS-0	5 415	39	0,72	0,49 - 0,95	287 529	0,14	0,09 - 0,18
NNIS-1	4 253	49	1,15	0,83 - 1,47	228 872	0,21	0,15 - 0,27
NNIS-2,3	740	8	1,08	0,33 - 1,83	40 402	0,2	0,06 - 0,34
Reprise de prothèse de genou							
Global	511	22	4,31	2,51 - 6,10	26 643	0,83	0,48 - 1,17
NNIS-0	214	7	3,27	0,85 - 5,69	10 202	0,69	0,18 - 1,19
NNIS-1	204	10	4,90	1,86 - 7,94	10 917	0,92	0,35 - 1,48
NNIS-2,3	52	4	7,69	0,15 - 15,23	3 071	1,3	0,03 - 2,58



Modes de survenue (2) : inoculation directe

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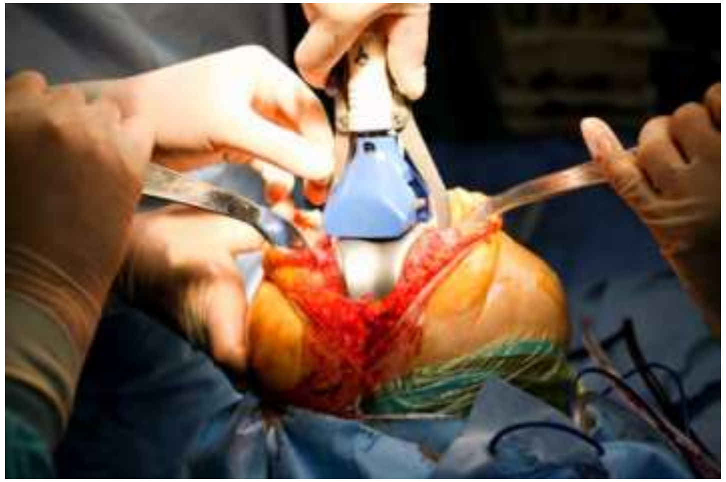
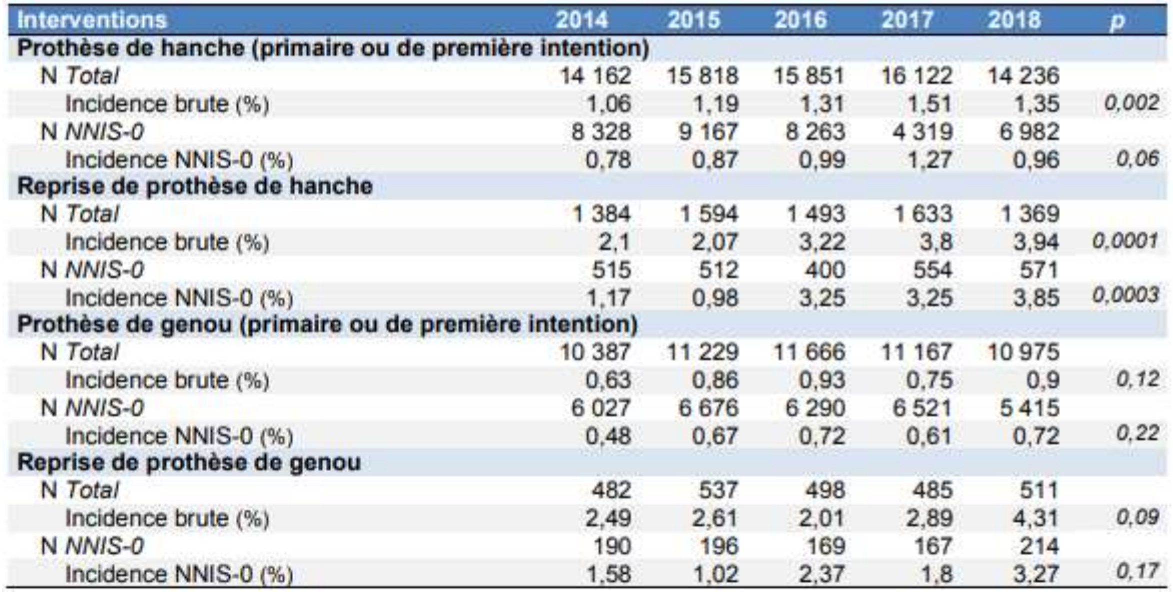
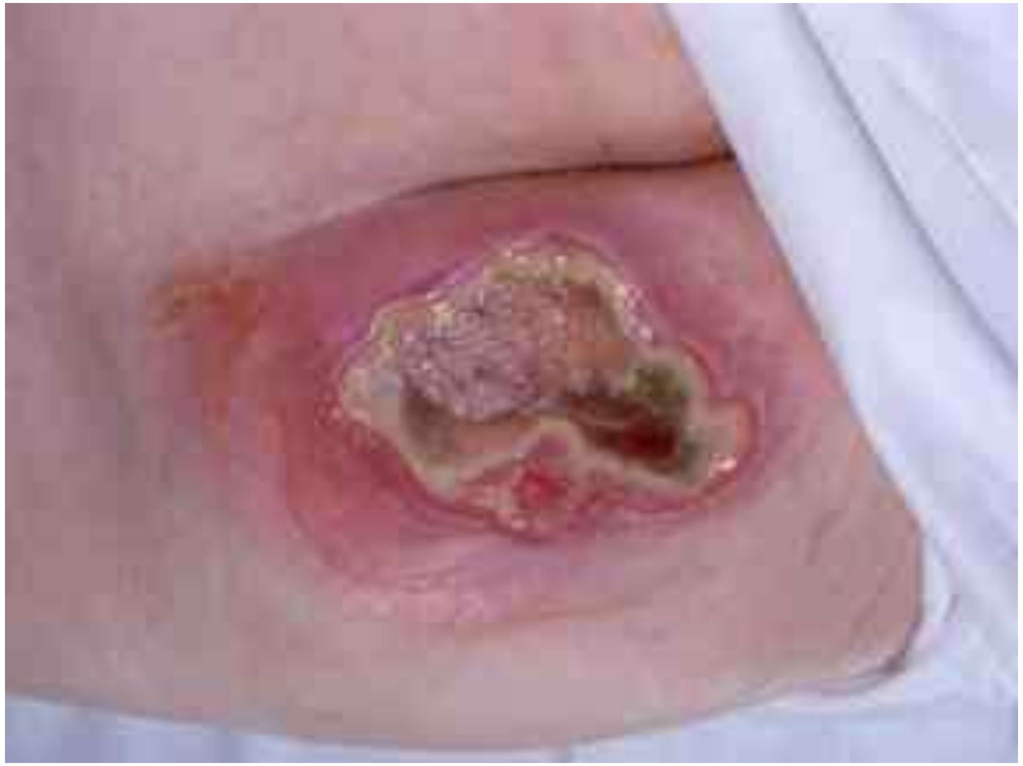
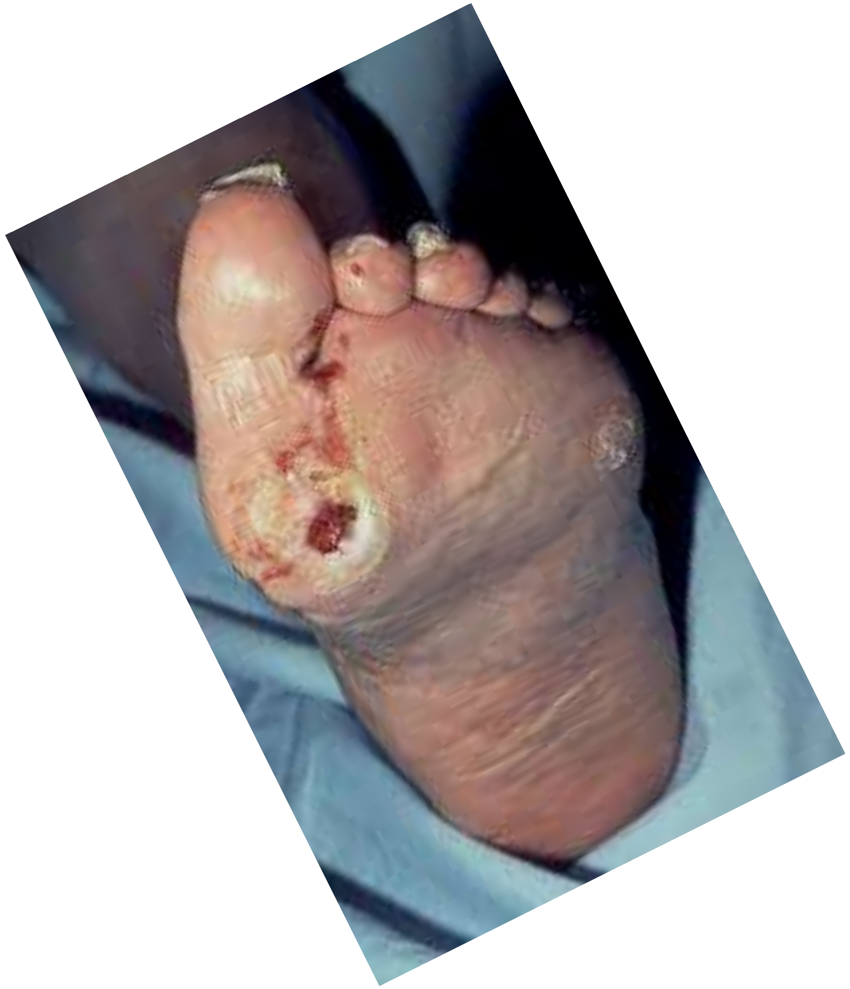




Interventions	2014	2015	2016	2017	2018	*p*
Prothèse de hanche (primaire ou de première intention)						
N *Total*	14 162	15 818	15 851	16 122	14 236	
Incidence brute (%)	1,06	1,19	1,31	1,51	1,35	*0,002*
N *NNIS-0*	8 328	9 167	8 263	4 319	6 982	
Incidence NNIS-0 (%)	0,78	0,87	0,99	1,27	0,96	*0,06*
Reprise de prothèse de hanche						
N *Total*	1 384	1 594	1 493	1 633	1 369	
Incidence brute (%)	2,1	2,07	3,22	3,8	3,94	*0,0001*
N *NNIS-0*	515	512	400	554	571	
Incidence NNIS-0 (%)	1,17	0,98	3,25	3,25	3,85	*0,0003*
Prothèse de genou (primaire ou de première intention)						
N *Total*	10 387	11 229	11 666	11 167	10 975	
Incidence brute (%)	0,63	0,86	0,93	0,75	0,9	*0,12*
N *NNIS-0*	6 027	6 676	6 290	6 521	5 415	
Incidence NNIS-0 (%)	0,48	0,67	0,72	0,61	0,72	*0,22*
Reprise de prothèse de genou						
N *Total*	482	537	498	485	511	
Incidence brute (%)	2,49	2,61	2,01	2,89	4,31	*0,09*
N *NNIS-0*	190	196	169	167	214	
Incidence NNIS-0 (%)	1,58	1,02	2,37	1,8	3,27	*0,17*

Modes de survenue (3) : contiguïté





Agents étiologiques

Agents étiologiques

Bibliographie à interpréter avec précautions

Impact +++

- du recrutement (ex: CRIOAc vs non-CRIOAc)

- des outils diagnostiques (ex: Enfant culture gélose vs milieu Liq vs PCR)

- de l'épidémiologie locale, régionale, nationale
  (ex: Europe vs US (clone com. USA 300 +++))

Agents étiologiques – France PMSI

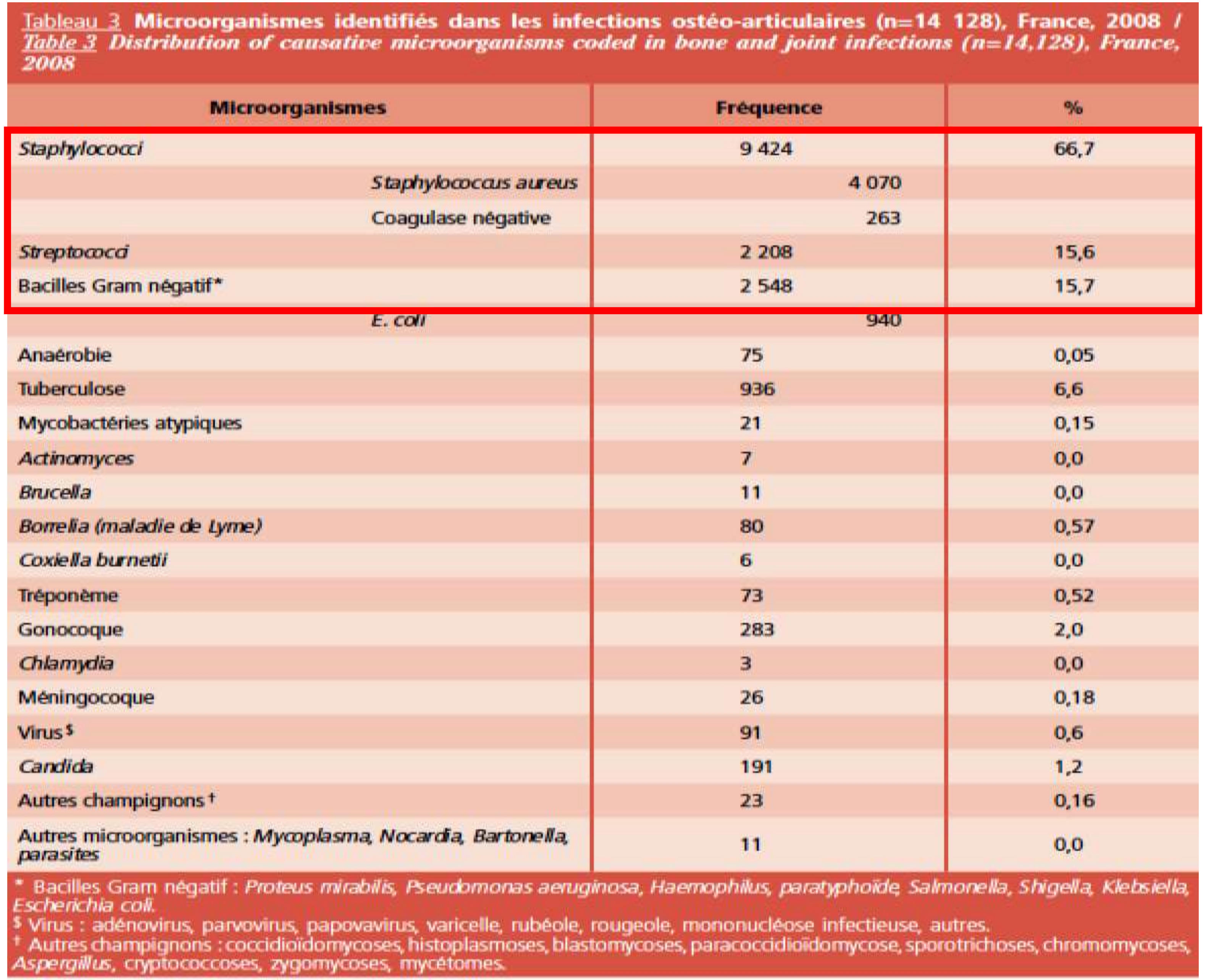


Tableau 3 **Microorganismes identifiés dans les infections ostéo-articulaires (n=14 128), France, 2008** / *Table 3* ***Distribution of causative microorganisms coded in bone and joint infections (n=14,128), France, 2008***

Microorganismes	Fréquence	%
Staphylococci	9 424	66,7
Staphylococcus aureus	4 070	
Coagulase négative	263	
Streptococci	2 208	15,6
Bacilles Gram négatif*	2 548	15,7
E. coli	940	
Anaérobie	75	0,05
Tuberculose	936	6,6
Mycobactéries atypiques	21	0,15
Actinomyces	7	0,0
Brucella	11	0,0
Borrelia (maladie de Lyme)	80	0,57
Coxiella burnetii	6	0,0
Tréponème	73	0,52
Gonocoque	283	2,0
Chlamydia	3	0,0
Méningocoque	26	0,18
Virus $	91	0,6
Candida	191	1,2
Autres champignons †	23	0,16
Autres microorganismes : *Mycoplasma, Nocardia, Bartonella, parasites*	11	0,0

* Bacilles Gram négatif : *Proteus mirabilis, Pseudomonas aeruginosa, Haemophilus, paratyphoïde, Salmonella, Shigella, Klebsiella, Escherichia coli.*

$ Virus : adénovirus, parvovirus, papovavirus, varicelle, rubéole, rougeole, mononucléose infectieuse, autres.

† Autres champignons : coccidioïdomycoses, histoplasmoses, blastomycoses, paracoccidioïdomycose, sporotrichoses, chromomycoses, *Aspergillus*, cryptococcoses, zygomycoses, mycétomes.

Agents étiologiques – Adultes / Articulation native

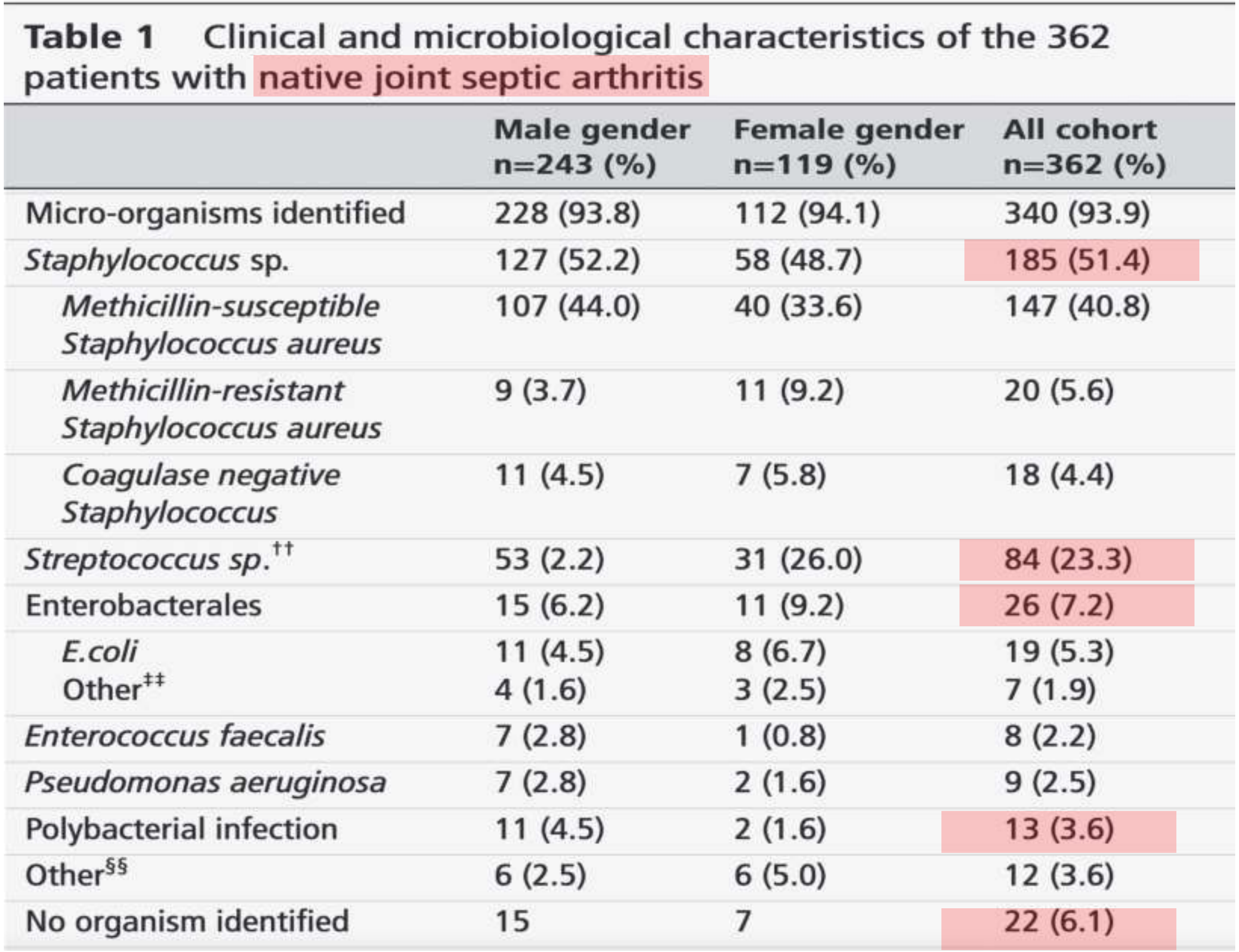


Table 1 Clinical and microbiological characteristics of the 362 patients with native joint septic arthritis

	Male gender n=243 (%)	**Female gender n=119 (%)**	**All cohort n=362 (%)**
Micro-organisms identified	228 (93.8)	112 (94.1)	340 (93.9)
Staphylococcus sp.	127 (52.2)	58 (48.7)	185 (51.4)
Methicillin-susceptible Staphylococcus aureus	107 (44.0)	40 (33.6)	147 (40.8)
Methicillin-resistant Staphylococcus aureus	9 (3.7)	11 (9.2)	20 (5.6)
Coagulase negative Staphylococcus	11 (4.5)	7 (5.8)	18 (4.4)
Streptococcus sp.††	53 (2.2)	31 (26.0)	84 (23.3)
Enterobacterales	15 (6.2)	11 (9.2)	26 (7.2)
E.coli	11 (4.5)	8 (6.7)	19 (5.3)
Other‡‡	4 (1.6)	3 (2.5)	7 (1.9)
Enterococcus faecalis	7 (2.8)	1 (0.8)	8 (2.2)
Pseudomonas aeruginosa	7 (2.8)	2 (1.6)	9 (2.5)
Polybacterial infection	11 (4.5)	2 (1.6)	13 (3.6)
Other§§	6 (2.5)	6 (5.0)	12 (3.6)
No organism identified	15	7	22 (6.1)

Richebé P, et al., Ann Rheum Dis. 2022

Management and outcome of native joint septic arthritis: a nationwide survey in French rheumatology departments, 2016-2017.

Agents étiologiques - Adultes / Articulation native vs prothètique

Linke S, J Orthop Surg Res. 2022 Jan 6;17(1):9.
January 2018 to January 2020 / Germany / 60 NJI = Native joint infections

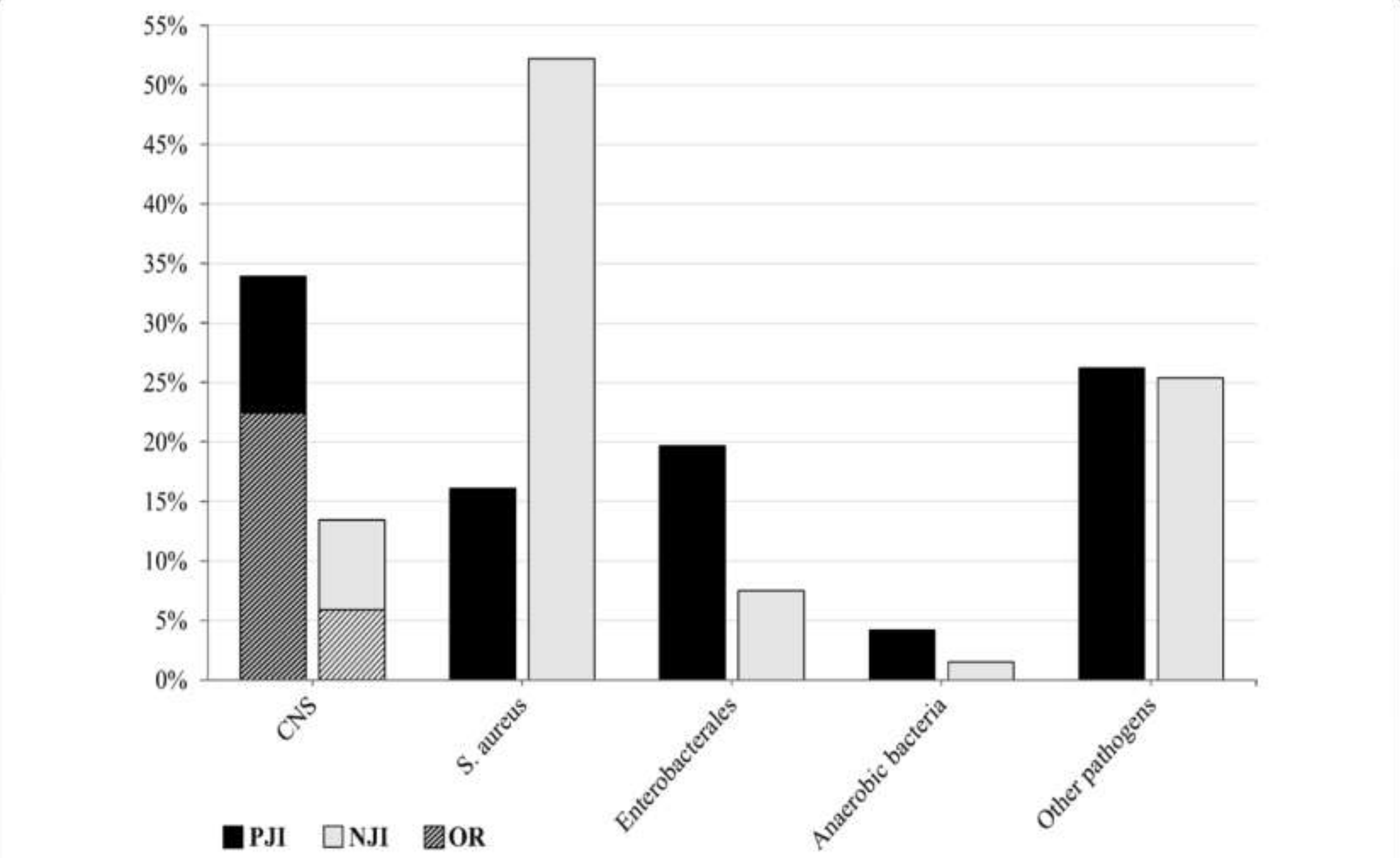
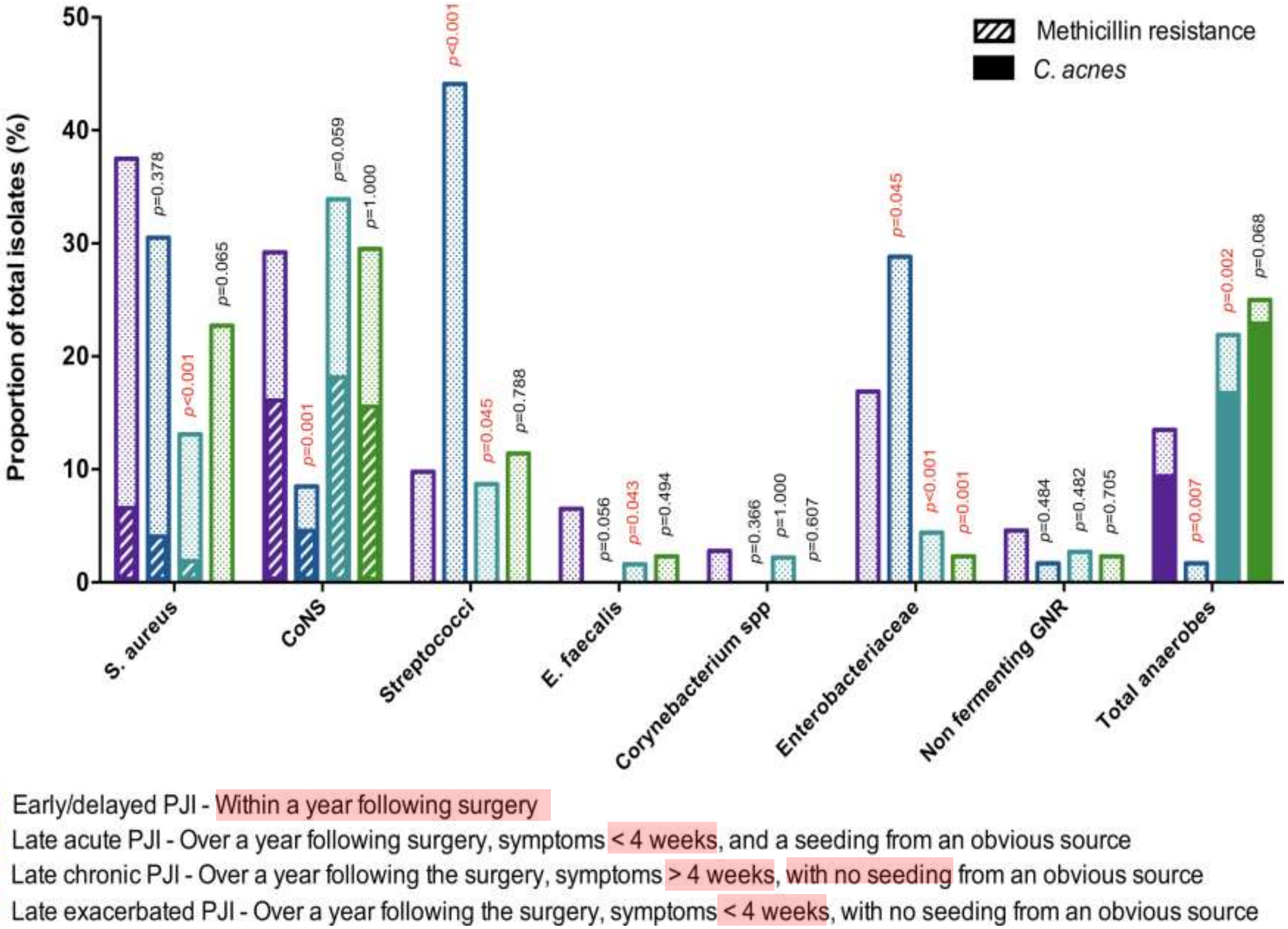


Fig. 1 Detection rates of pathogen classes in PJI and NJI. PJI: Periprosthetic joint infection. NJI: Native joint infection. OR: Oxacillin resistance. X-axis: pathogen classes. Y-axis: detection rates

NJI = Native joint infections	n/%
Other pathogens	17 [25.4]
Enterococcus faecalis	4 [6]
Pseudomonas aeruginosa	1 [1.5]
Streptococcus dysgalactiae	4 [6]
Propionibacterium acnes	1 [1.5]
Streptococcus agalactiae	0 [0]
Enterococcus faecium	0 [0]
Streptococcus pneumoniae	3 [4.5]
Streptococcus gallolyticus	0 [0]
Streptococcus oralis	1 [1.5]
Streptococcus pyogenes	1 [1.5]
Acinetobacter baumannii	1 [1.5]
Candida albicans	0 [0]
Listeria monocytogenes	0 [0]
Morganella morganii	1 [1.5]

Agents étiologiques – Adultes PJI



▨ Early/delayed PJI - Within a year following surgery

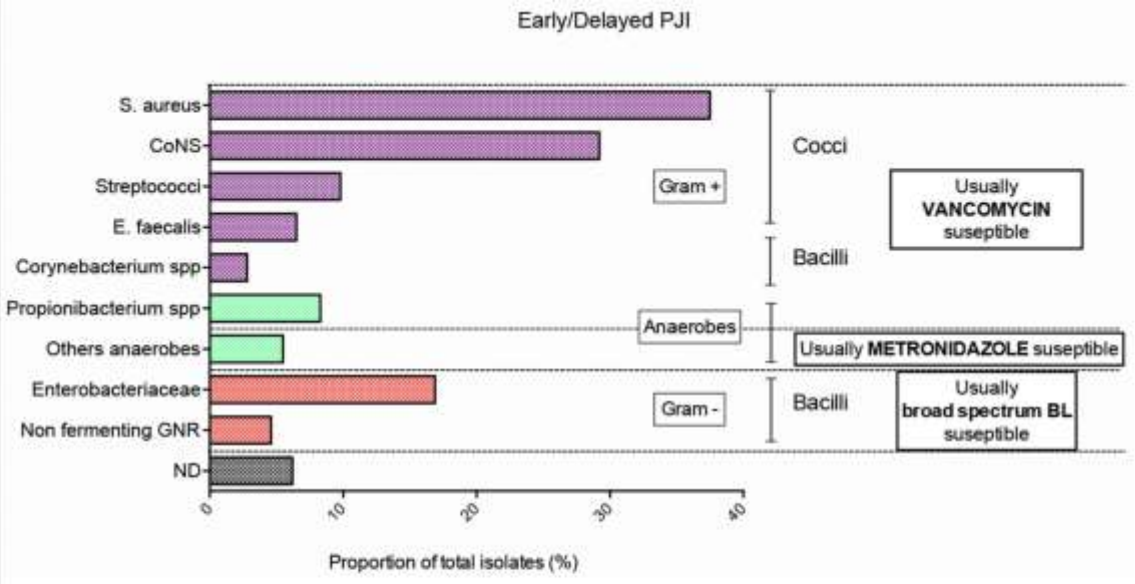
▨ Late acute PJI - Over a year following surgery, symptoms < 4 weeks, and a seeding from an obvious source

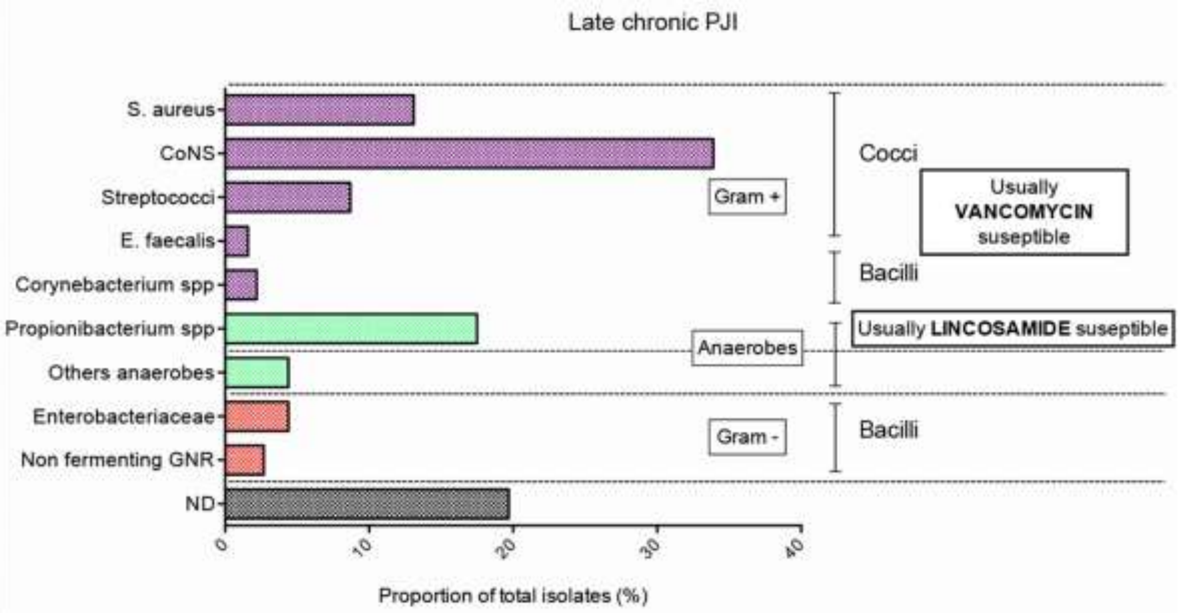
▨ Late chronic PJI - Over a year following the surgery, symptoms > 4 weeks, with no seeding from an obvious source

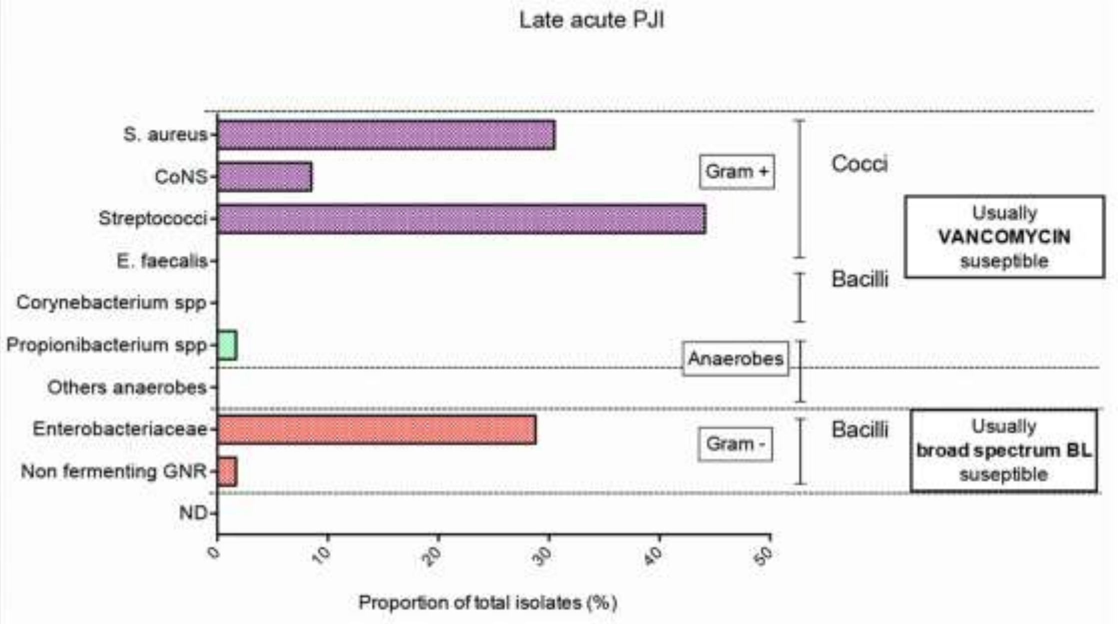
▨ Late exacerbated PJI - Over a year following the surgery, symptoms < 4 weeks, with no seeding from an obvious source

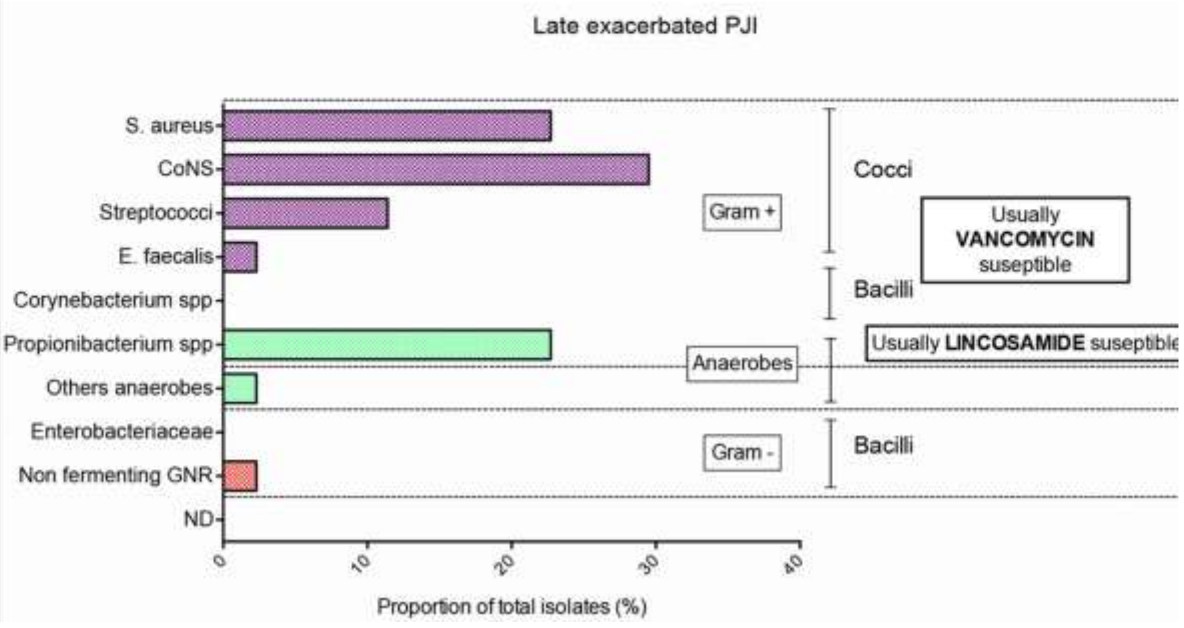
Prospective cohort study
Complex bone and joint infection
Between 2011 and 2016
First episode of PJI in CRIOAc Lyon
Not osteosynthesis
Not external device–related

Agents étiologiques – Adultes PJI


Early/Delayed PJI


Late chronic PJI


Late acute PJI

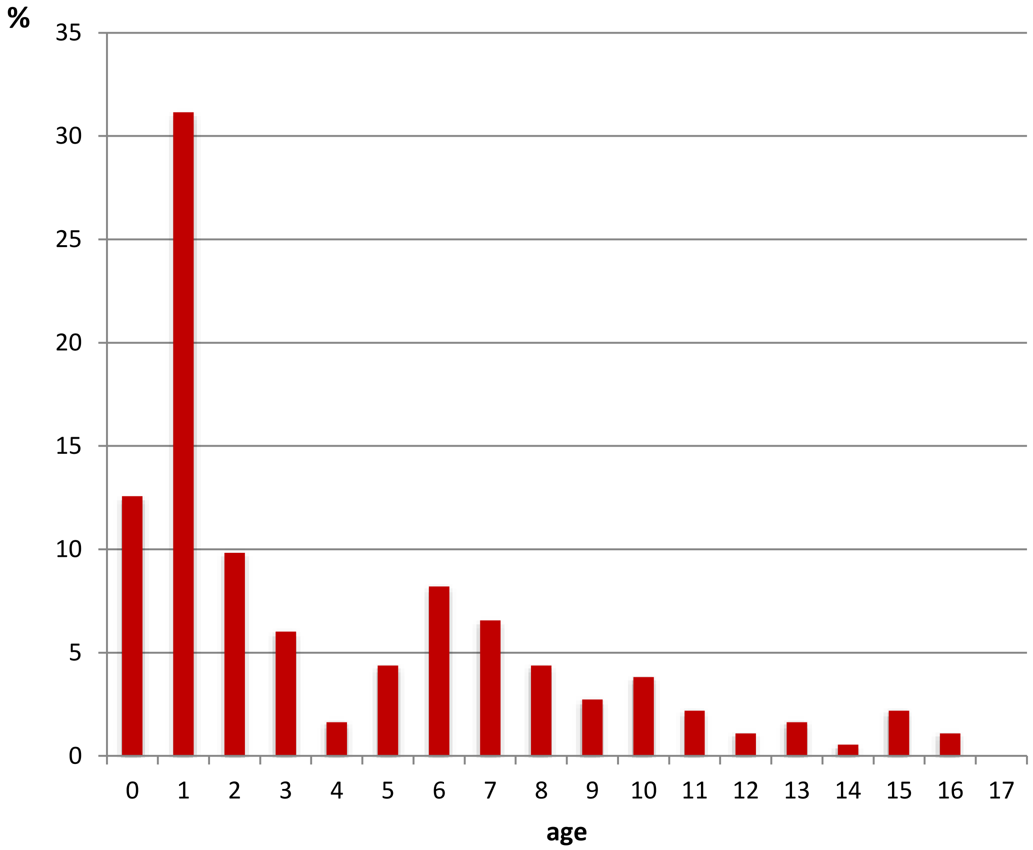

Late exacerbated PJI

Agents étiologiques – Enfants

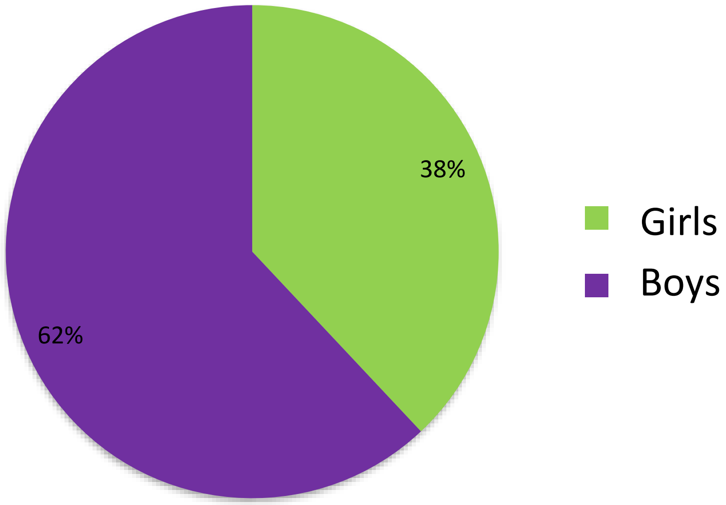
Among the 191 included patients, **183 were confirmed as BJI**

Distribution according to age and gender

Medium age: 4,6 years and median age:**2,1 years**

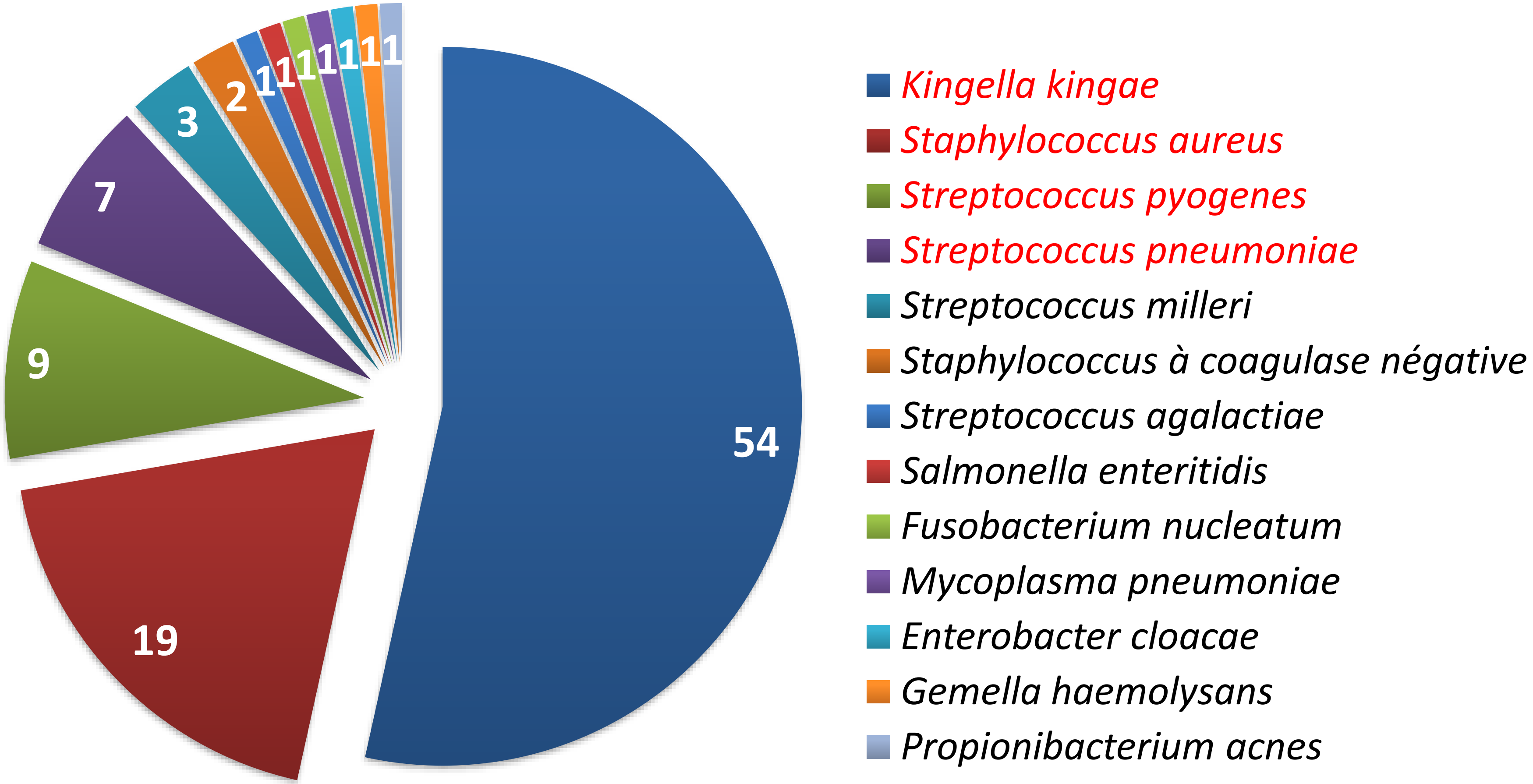


Sexe ratio: **1,6**
(114 M / 69 F)



Agents étiologiques – Enfants

Among 183 proven BJI , **101 were bacteriologically documented** (all methods gathered)



72% of the cases

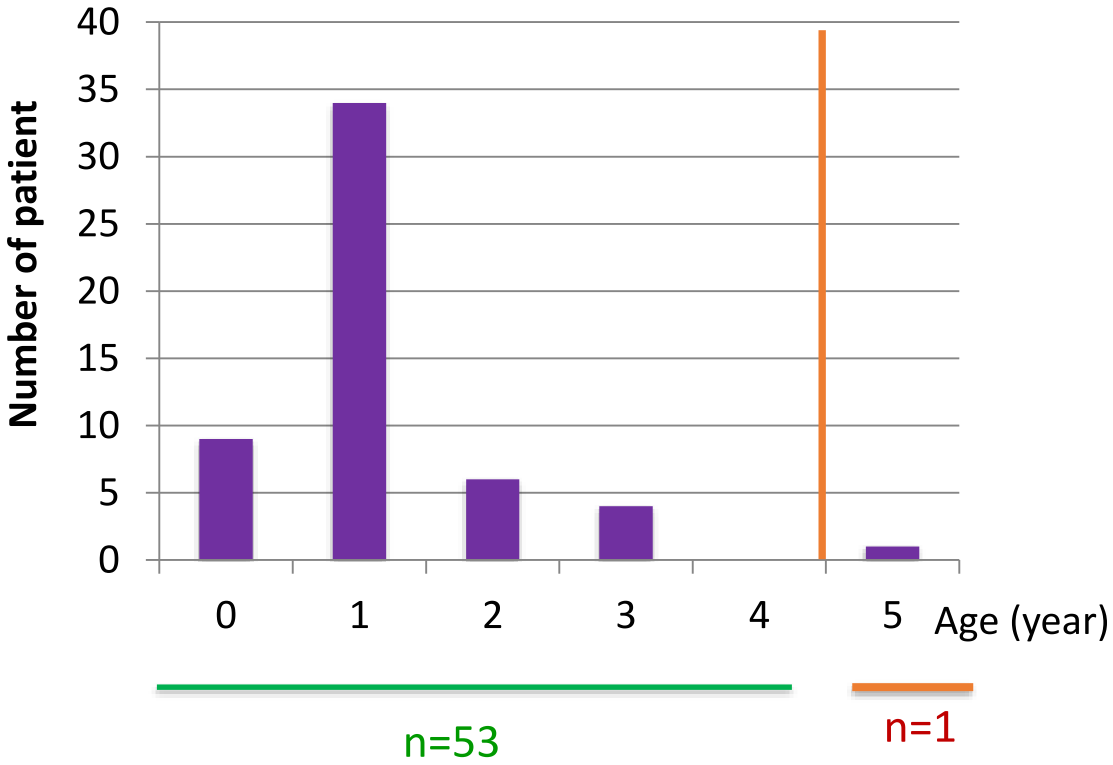
=

K. kingae* + *S. aureus

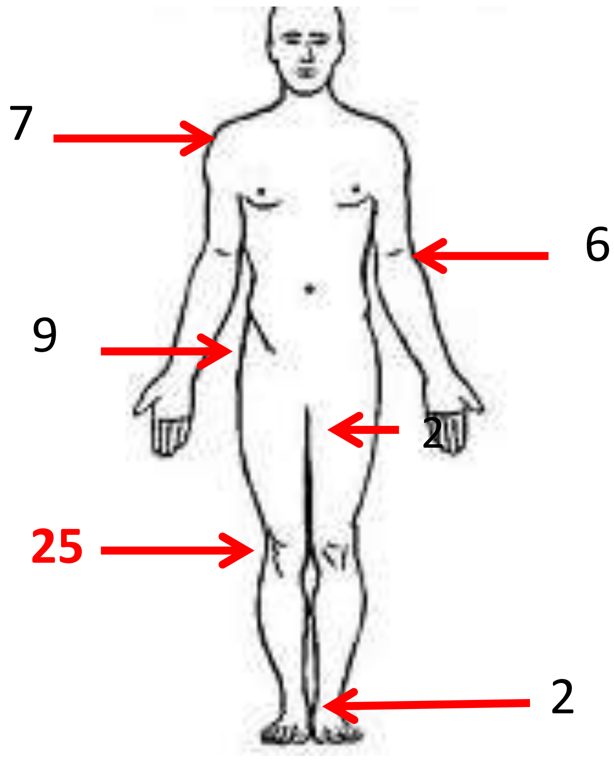
Agents étiologiques – Enfants

Kingella kingae

- Epidemiology
  - Recovered from 54 patients
  - Sexe ratio: 31 M and 23 F (1,3)
  - Median age: 17 months; (0-5 years)



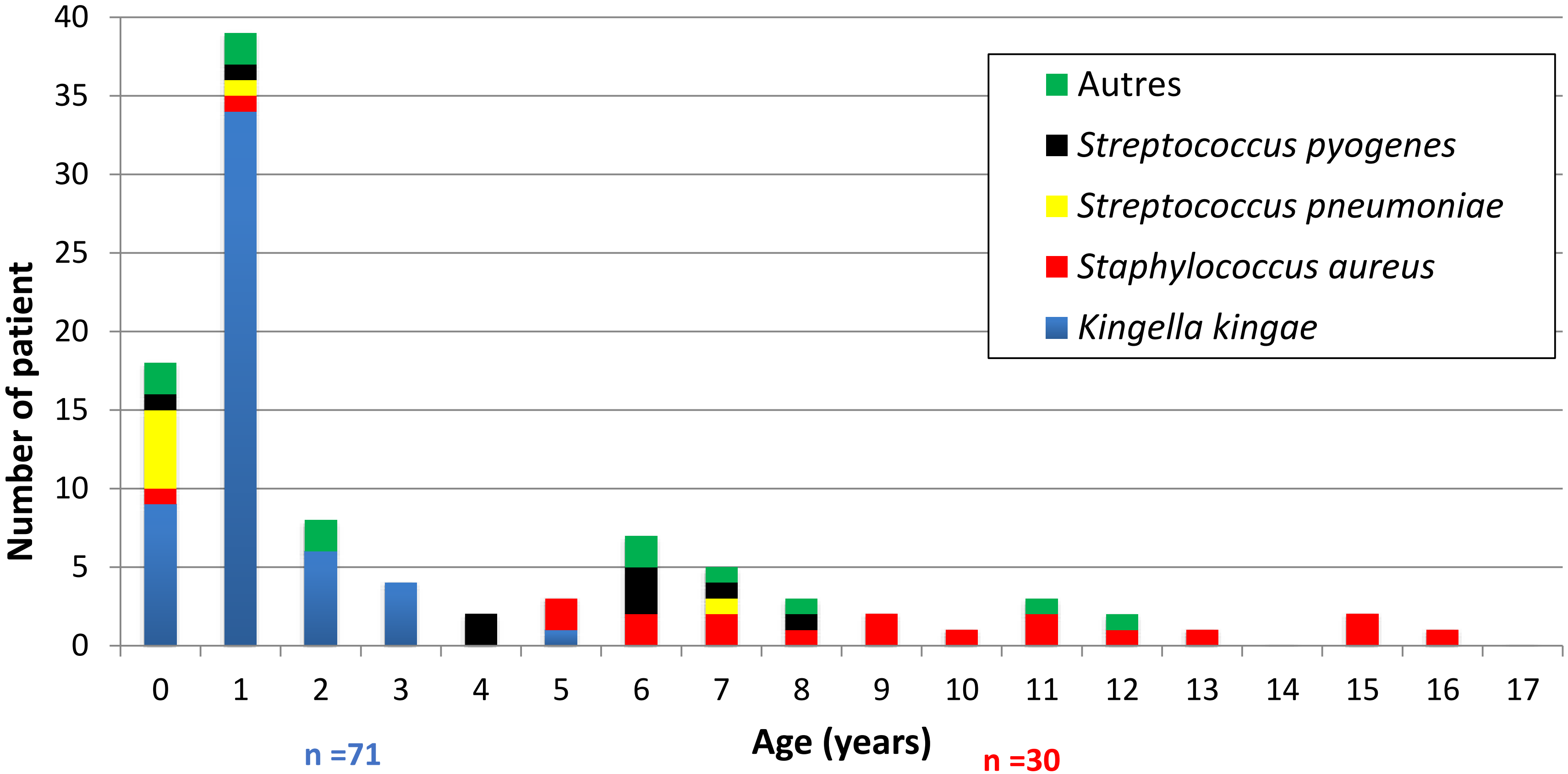
- Location



Vertebral: 1

Agents étiologiques – Enfants

Distribution of the 101 pathogensaccording to the patient **age**



n =71

K. kingae
74% of the cases

S. pneumoniae: 8 %
S. pyogenes: 2.8 %
S. aureus: 1.4%

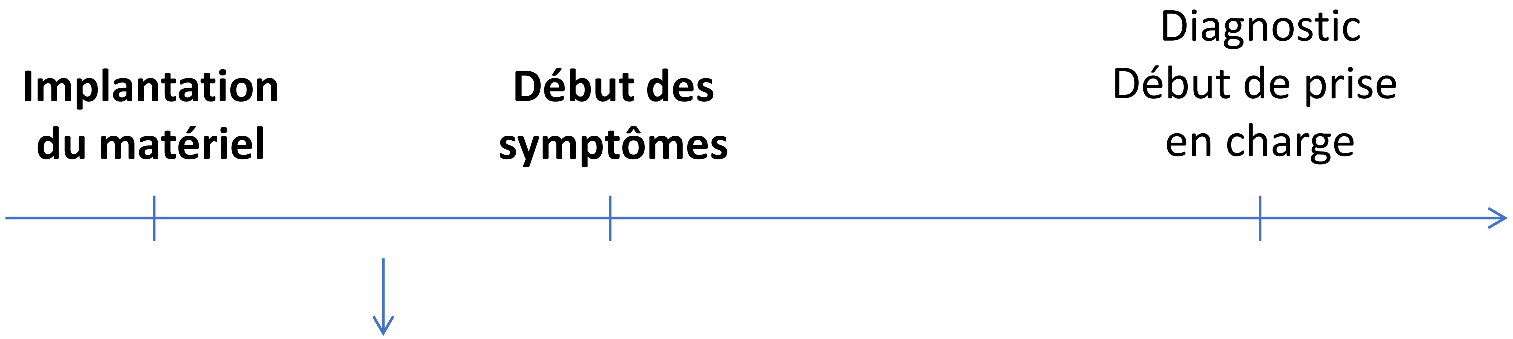
n =30

S. aureus
57% of the cases

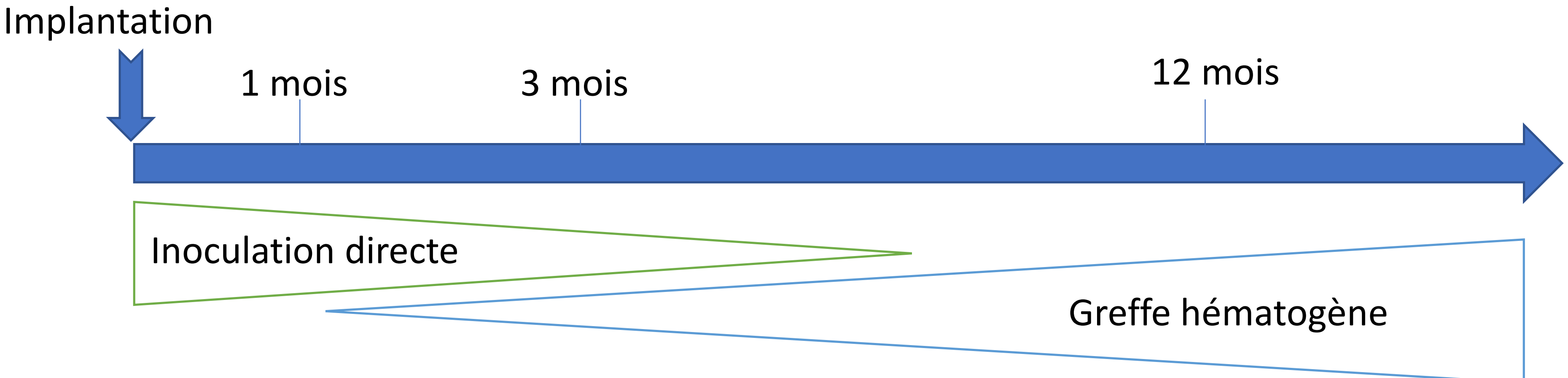
S. pyogenes: 20 %
K. kingae: 3 %
S. pneumoniae: 3 %

Chronologie des IOA

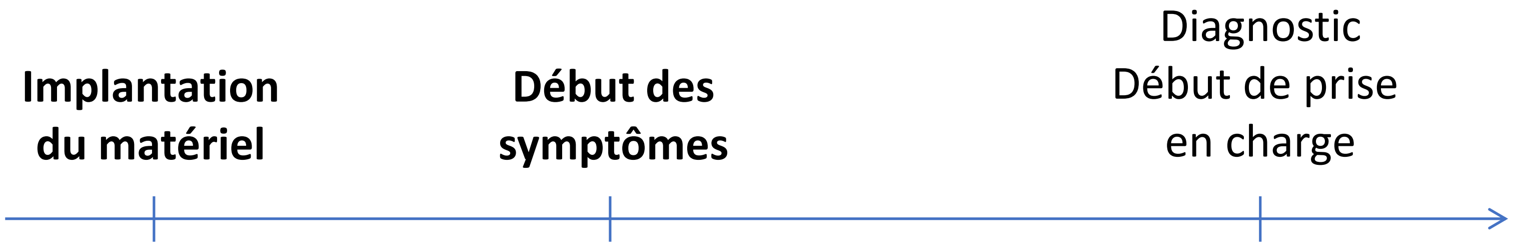
Chronologie (1) : délai de survenue


Implantation du matériel
Début des symptômes
Diagnostic
Début de prise en charge

PRECOCE (< 1-3 mois)	Inoculation chirurgicale +++ Germes à croissance rapide : *S. aureus*, BGN, streptocoques
RETARDEE (1-3 à 12-24 ms)	Inoculation chirurgicale + Germes indolents : SCN, corynebactéries, *Propionibacterium*
TARDIVES (> 12-24 mois)	Hématogènes ++


Implantation
1 mois
3 mois
12 mois
Inoculation directe
Greffe hématogène

Chronologie (1) : délai de survenue



Implantation du matériel

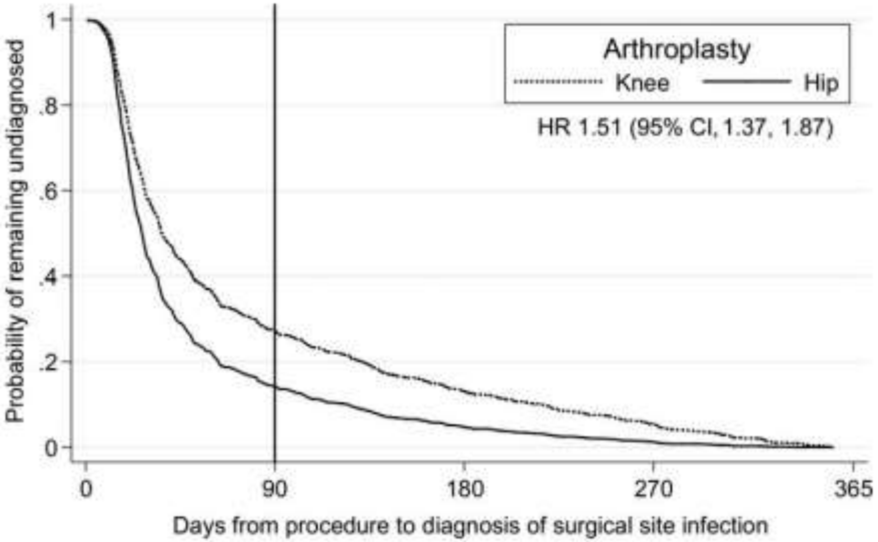
Début des symptômes

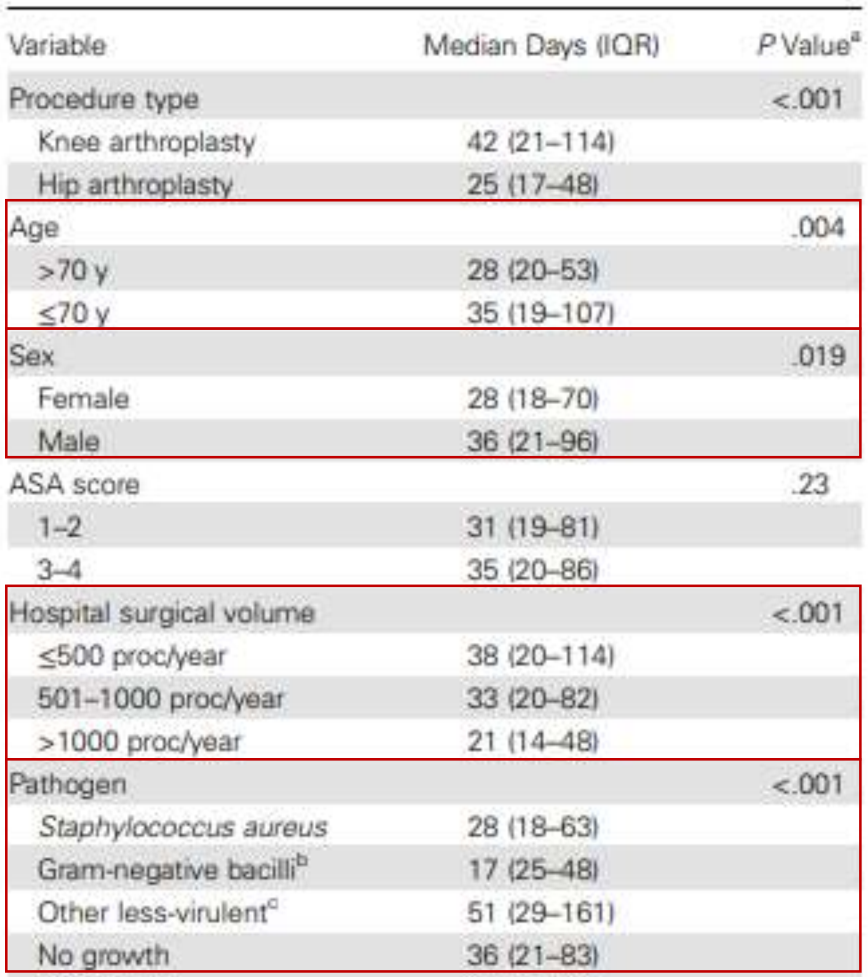
Diagnostic
Début de prise en charge

Delay in Diagnosis of Invasive Surgical Site Infections Following Knee Arthroplasty Versus Hip Arthroplasty

Sarah S. Lewis,[1,2] Kristen V. Dicks,[1,2] Luke F. Chen,[1,2] Michael P. Bolognesi,[3] Deverick J. Anderson,[1,2] Daniel J. Sexton,[1,2] and Rebekah W. Moehring[1,2,4]

661 PJI dans l'année post-implantation





Variable	Median Days (IQR)	*P* Value[a]
Procedure type		<.001
Knee arthroplasty	42 (21–114)	
Hip arthroplasty	25 (17–48)	
Age		.004
>70 y	28 (20–53)	
≤70 y	35 (19–107)	
Sex		.019
Female	28 (18–70)	
Male	36 (21–96)	
ASA score		.23
1–2	31 (19–81)	
3–4	35 (20–86)	
Hospital surgical volume		<.001
≤500 proc/year	38 (20–114)	
501–1000 proc/year	33 (20–82)	
>1000 proc/year	21 (14–48)	
Pathogen		<.001
Staphylococcus aureus	28 (18–63)	
Gram-negative bacilli[b]	17 (25–48)	
Other less-virulent[c]	51 (29–161)	
No growth	36 (21–83)	

Chronologie (2) : durée d'évolution

1993 Mohamed, 14 ans, pas d'antécédent

Tableau brutal : fièvre à 39°C avec frissons, douleurs et impotence fonctionnelle du MI gauche
Arthrite du genou clinique + douleur intense à la palpation de l'épiphyse fémorale inférieure
Hémocultures positives à S. aureus méti-S (par ailleurs multi-sensible)
Radiographies simples normale
Echo : épanchement articulaire, pas de collection. Ponction positive à MSSA

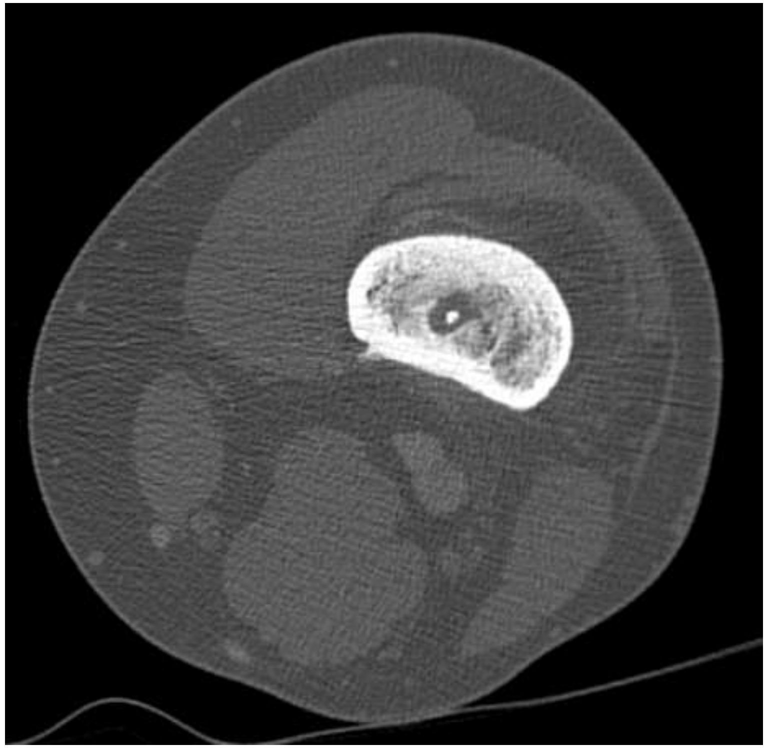
Diagnostic d'**OSTÉOMYÉLITE AIGUË HÉMATOGÈNE**
OXACILLINE IV + acide fusidique
Rash à J8 : relais à J8 par CLINDAMYCINE per os pour 6 semaines au total

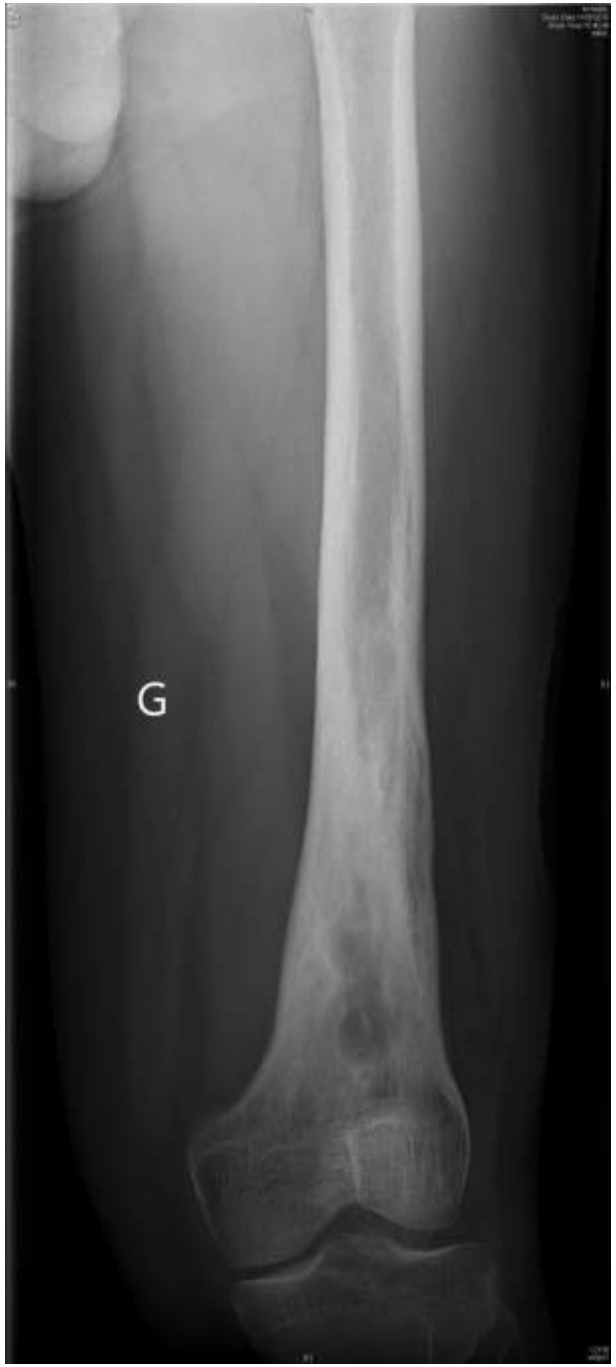
Bonne évolution immédiate
Douleurs récidivantes (quelques jours, tous les hivers)

Chronologie (2) : durée d’évolution

2015-16 Mohamed, 36 ans, devenu préparateur de commandes

Persistance de l’épisode douloureux hivernal …
Douleurs devenant continues, insomniantes avec boiterie





Chronologie (2) : durée d’évolution

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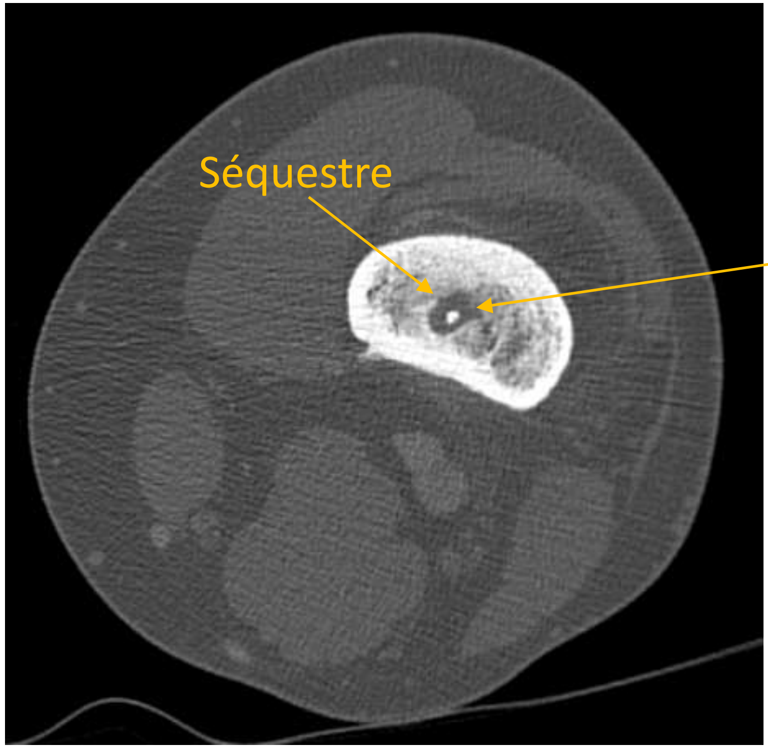
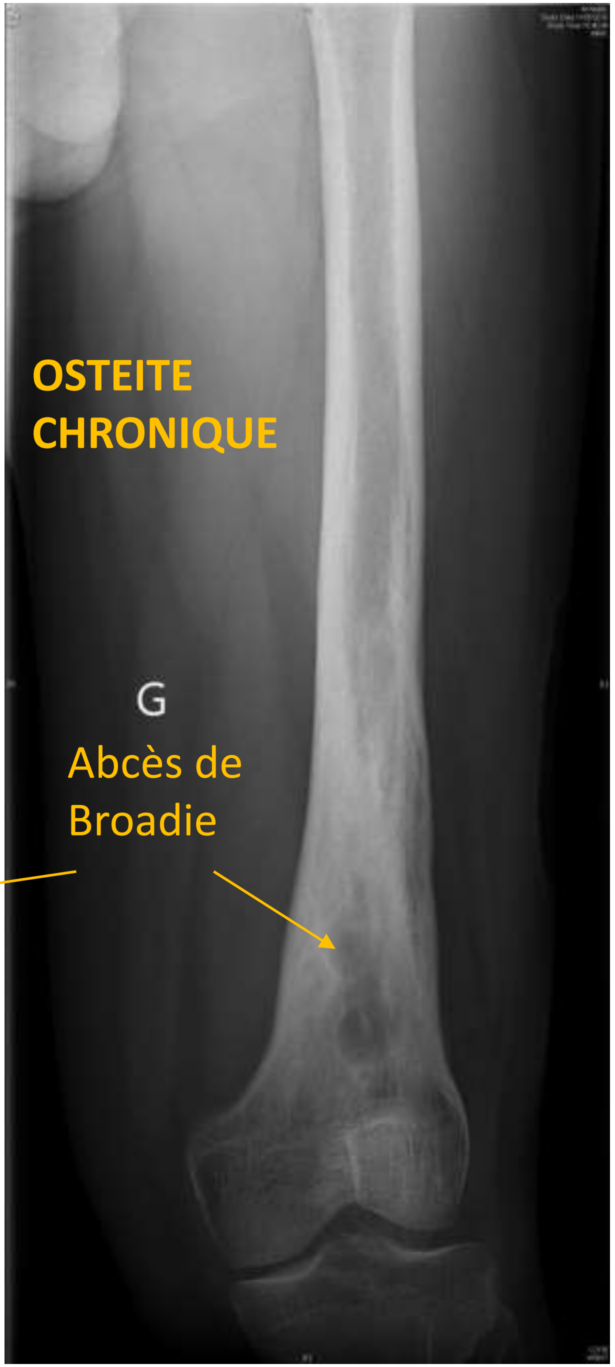



OSTEITE CHRONIQUE
G
Abcès de Broadie

Chronologie (2) : durée d'évolution

2015-16 Mohamed, 36 ans, devenu préparateur de commandes

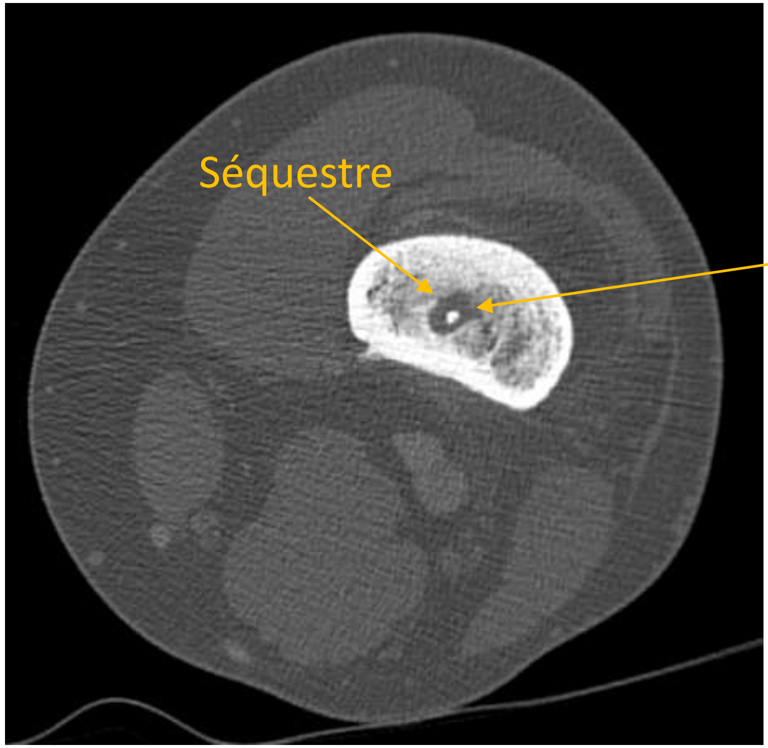
Persistance de l'épisode douloureux hivernal …
Douleurs devenant continues, insomniantes avec boiterie

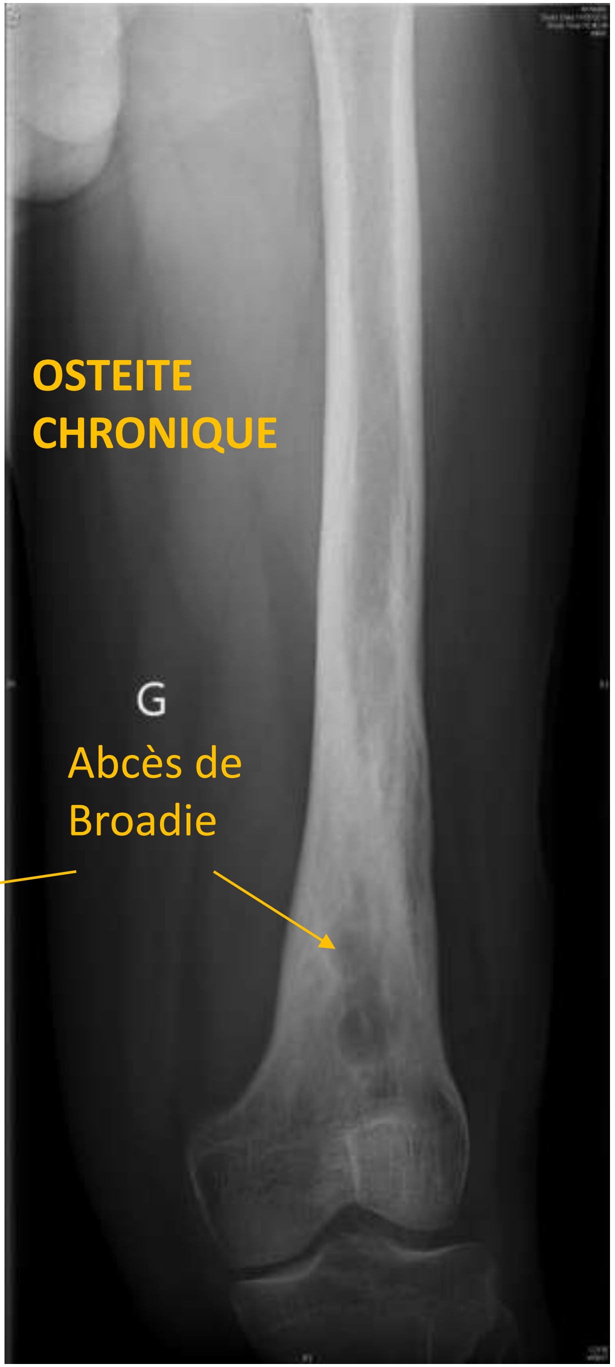
OSTEITE (très) CHRONIQUE

Fémorotomie, curetage
Prlvmts : stériles, PCR 16+ MSSA – Redon

CEFAZOLINE – RIFAM (2 sem)
RIFAM – OFLOXACINE (total : 3 ms)

Bonne évolution




OSTEITE CHRONIQUE
G
Abcès de Broadie

Staphylococcus aureus : entre virulence et persistance

Facteurs associés à la paroi

Adhesines (MSCRAMMs)
Fibronectin-BPs
Collagen-BP
Bone sialoprotein-BP

Protéine A
binding to Ig and vWF

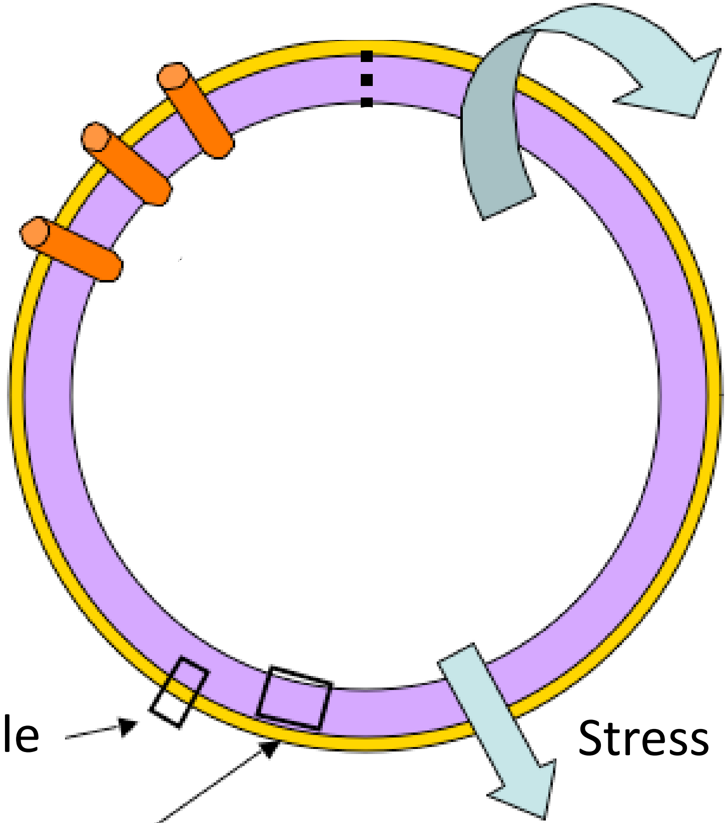
Echappement du système immunitaire

Facteurs de virulence sécrétés

Exotoxines

- Toxines superantigéniques : *TSST-1, SEA, …*
- Hémolysines : *α-toxin, β-toxin …*
- Pore-forming toxins : *PVL …*
- Enzymes : *Staphylokinase, proteases, lipases, …*

Adhesines (SERAMs)
Coagulase, Efb, Emp …



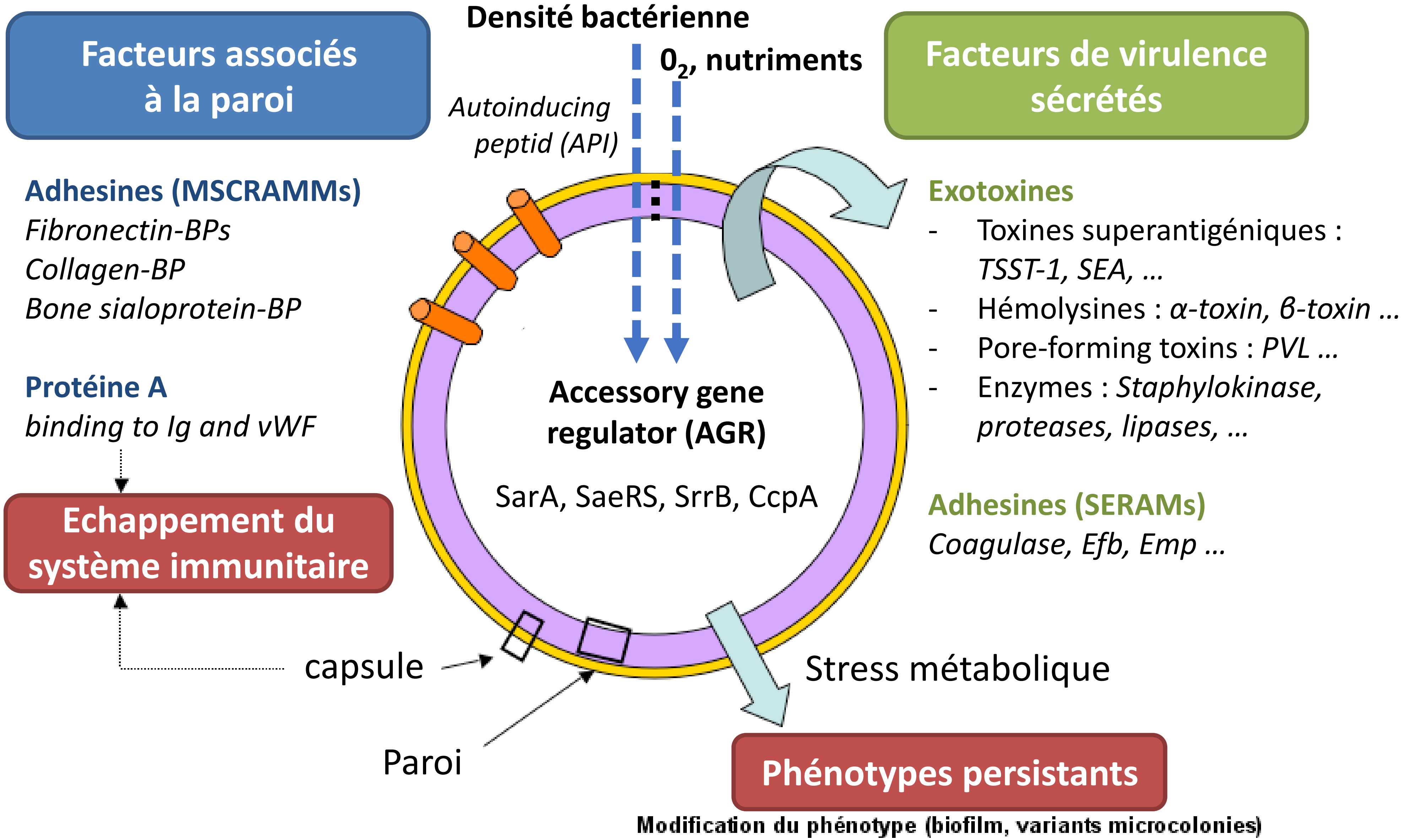
Stress métabolique

Phénotypes persistants

Modification du phénotype (biofilm, variants microcolonies)

D'après Ferry et al. Curr Infec Dis Rep 2005

Staphylococcus aureus : entre virulence et persistance



Facteurs associés à la paroi

Adhesines (MSCRAMMs)
Fibronectin-BPs
Collagen-BP
Bone sialoprotein-BP

Protéine A
binding to Ig and vWF

Echappement du système immunitaire

capsule

Paroi

Densité bactérienne

0_2, nutriments

Autoinducing peptid (API)

Accessory gene regulator (AGR)

SarA, SaeRS, SrrB, CcpA

Facteurs de virulence sécrétés

Exotoxines

- Toxines superantigéniques : *TSST-1, SEA, …*
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Coagulase, Efb, Emp …

Stress métabolique

Phénotypes persistants

Modification du phénotype (biofilm, variants microcolonies)

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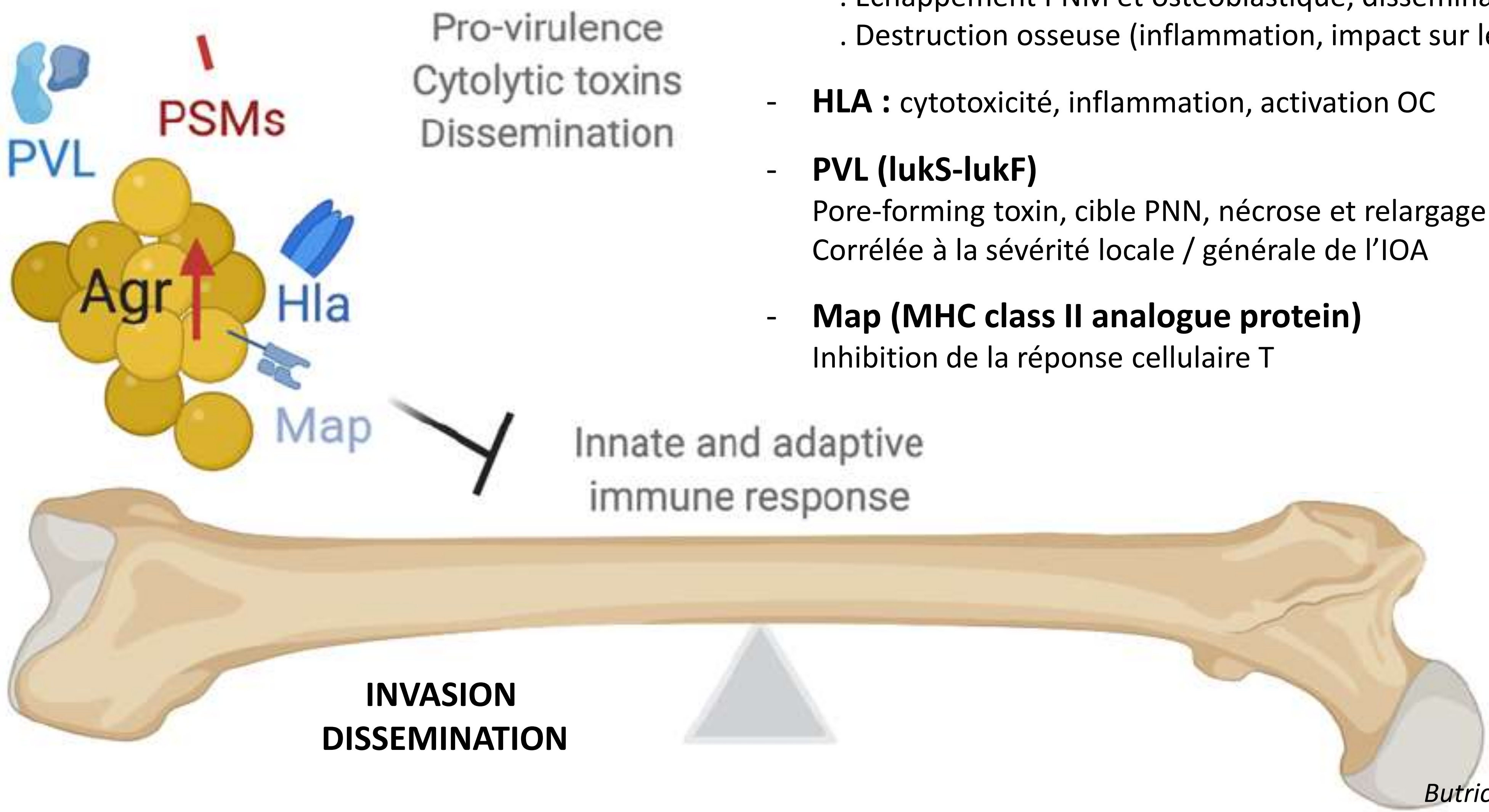
Initiation de l'infection : toxines et adhésines

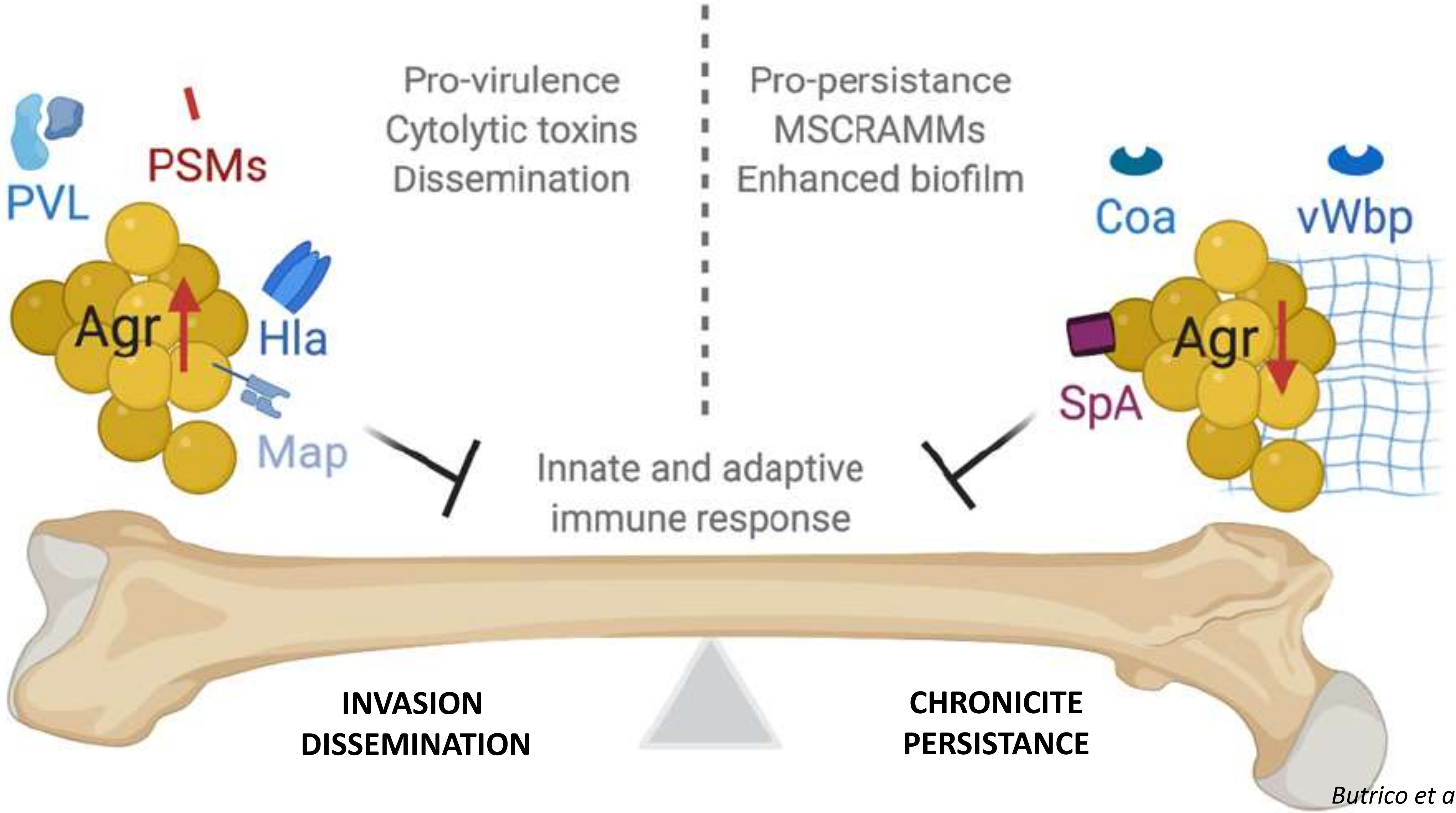

PVL
PSMs
Pro-virulence
Cytolytic toxins
Dissemination
Agr
Hla
Map
Innate and adaptive
immune response

INVASION
DISSEMINATION

- **PSMs : α et β, delta-hémolysine (hld)**
  . Cytotoxicité (incluant cellules osseuses)
  . Activation neutrophilique et relargage cytokinique
  . Echappement PNM et ostéoblastique, dissémination
  . Destruction osseuse (inflammation, impact sur le remodelage)
- **HLA :** cytotoxicité, inflammation, activation OC
- **PVL (lukS-lukF)**
  Pore-forming toxin, cible PNN, nécrose et relargage cytokinique
  Corrélée à la sévérité locale / générale de l'IOA
- **Map (MHC class II analogue protein)**
  Inhibition de la réponse cellulaire T

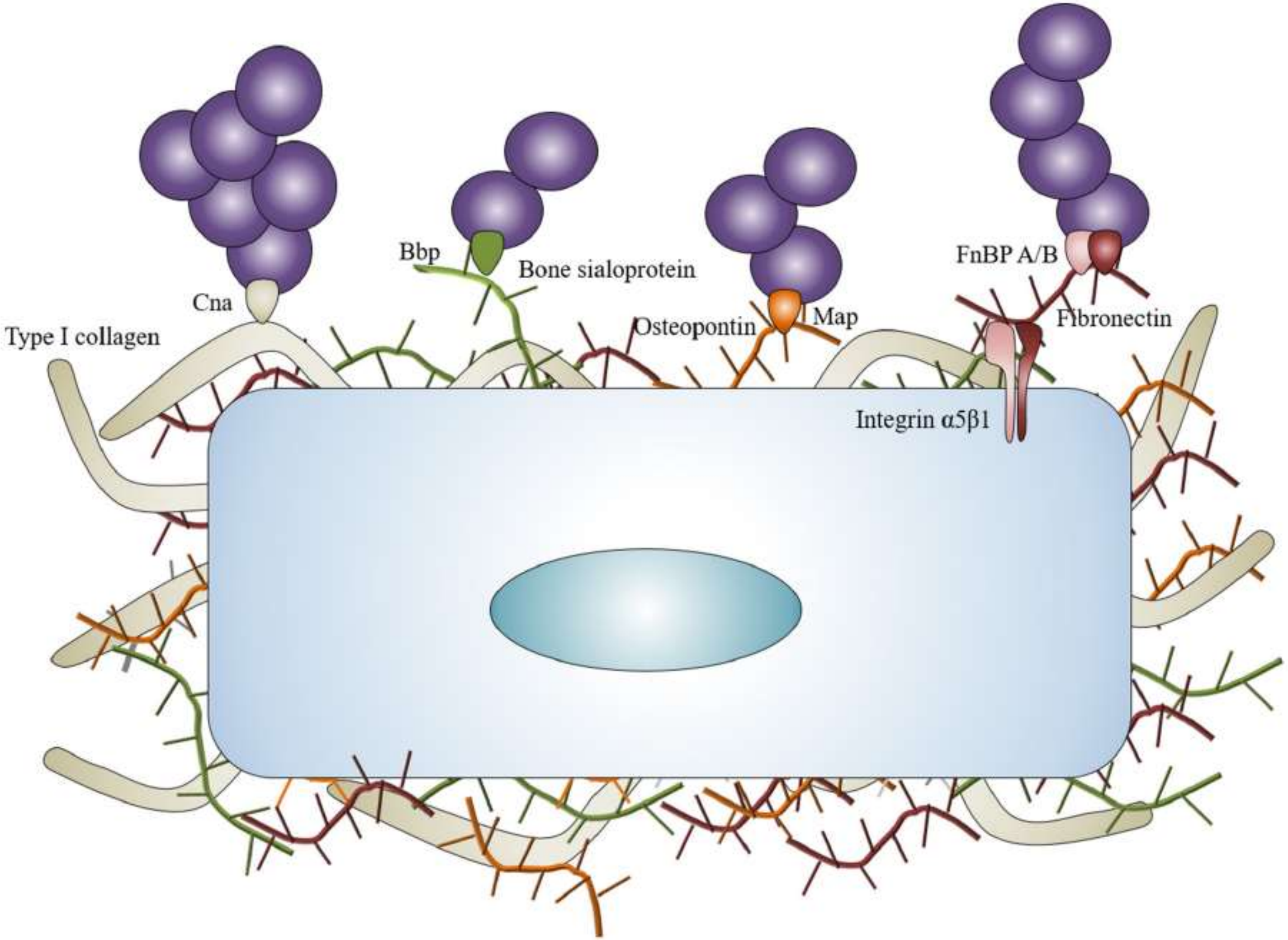
Butrico et al, Toxins 2020

Initiation de l’infection : toxines et adhésines


PVL
PSMs
Agr
Hla
Map
Pro-virulence
Cytolytic toxins
Dissemination
Pro-persistance
MSCRAMMs
Enhanced biofilm
Coa
vWbp
Agr
SpA
Innate and adaptive immune response
INVASION
DISSEMINATION
CHRONICITE
PERSISTANCE

Butrico et al, Toxins 2020

Initiation de l'infection : toxines et adhésines


Bbp
Bone sialoprotein
Cna
Type I collagen
Osteopontin
Map
FnBP A/B
Fibronectin
Integrin α5β1

IOA d'INOCULATION

Quantité de *S. aureus* nécessaire à l'obtention d'une infection / 100 000 en présence de matériel

Idem S. epidermidis, P. acnes

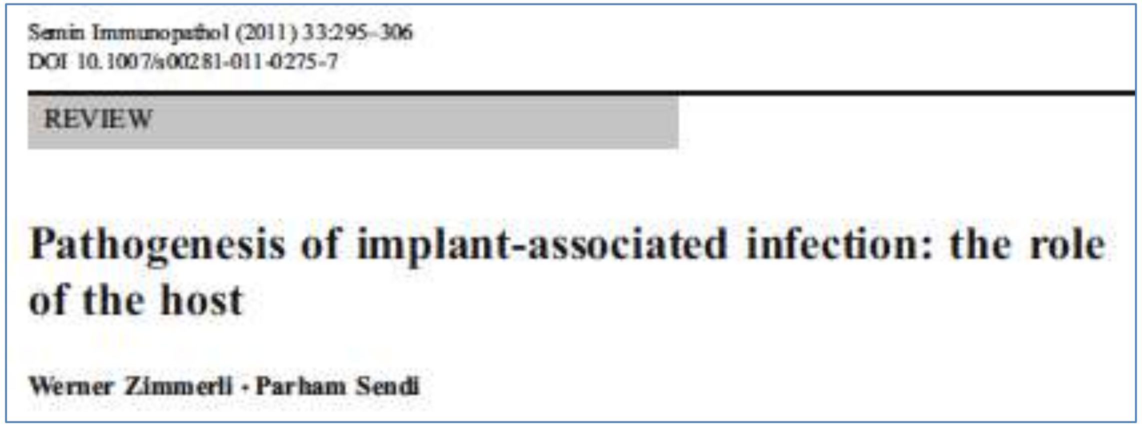
Elek et al 1957 – Zimmerli et al, JID 1982

IOA HEMATOGENES

Risque de greffe bactérienne en cas de bactériémie
S. aureus **= 20-40% vs autres germes = 1-2%**

Josse et al, Front microbiol 2005

Initiation de l’infection : rôle du matériel



Semin Immunopathol (2011) 33:295–306
DOI 10.1007/s00281-011-0275-7

REVIEW

Pathogenesis of implant-associated infection: the role of the host

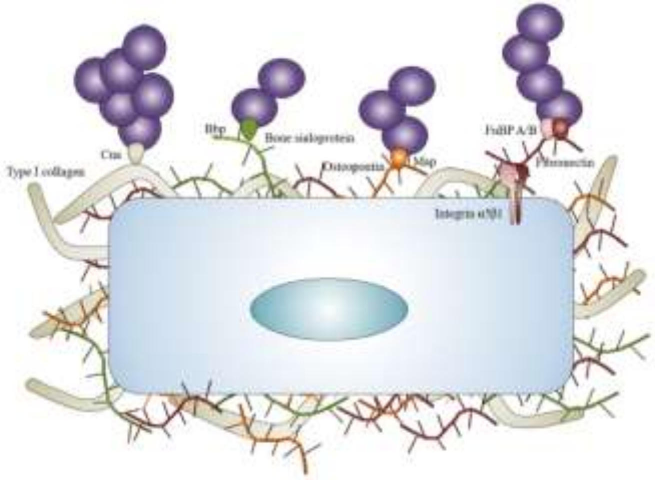
Werner Zimmerli · Parham Sendi

Type de biomatériaux

- Métaux
- Polymères
- Substituts osseux

↓

« BIOCOMPATIBILITE »



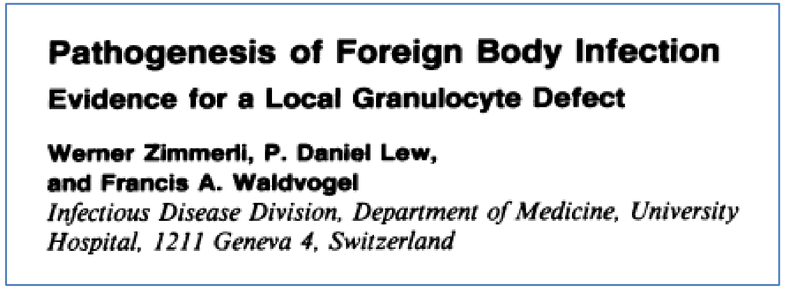
1. Couverture du matériel par matrice extracellulaire
Collagène, fibrine, fibrinogène …

→ Adhésion, initiation de la formation de biofilm

2. Inhibition locale de l’immunité innée

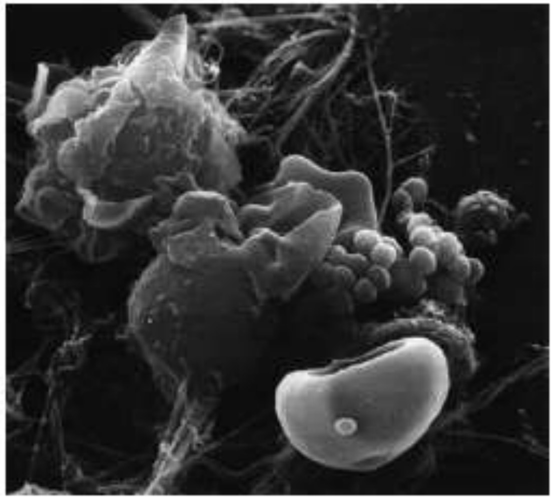
- inhibition de la phagocytose
- dégranulation des PNN
- mort cellulaire
- activation du complément

→ Diminution +++ de la clairance bactérienne
→ Descellement aseptique (?)



Pathogenesis of Foreign Body Infection
Evidence for a Local Granulocyte Defect

Werner Zimmerli, P. Daniel Lew, and Francis A. Waldvogel
Infectious Disease Division, Department of Medicine, University Hospital, 1211 Geneva 4, Switzerland



Chronicité : importance du concept

Inoculation

Diagnostic
Début de prise en charge

3-4 sem

< 3-4 semaines

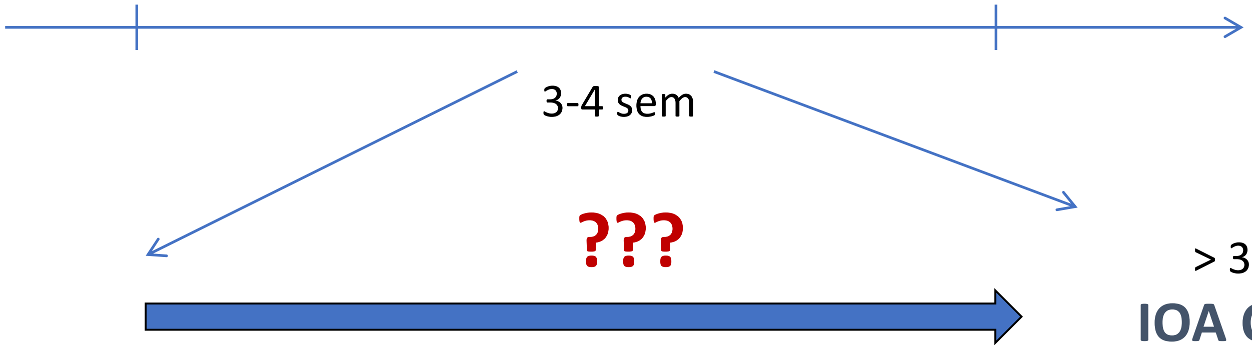
IOA AIGUE

- Réplication bactérienne +++
- Infection suppurative
- Congestion vasculaire

TRAITEMENT CONSERVATEUR

Risque de récidive

Chronicité : importance du concept



Inoculation

Diagnostic
Début de prise en charge

3-4 sem

???

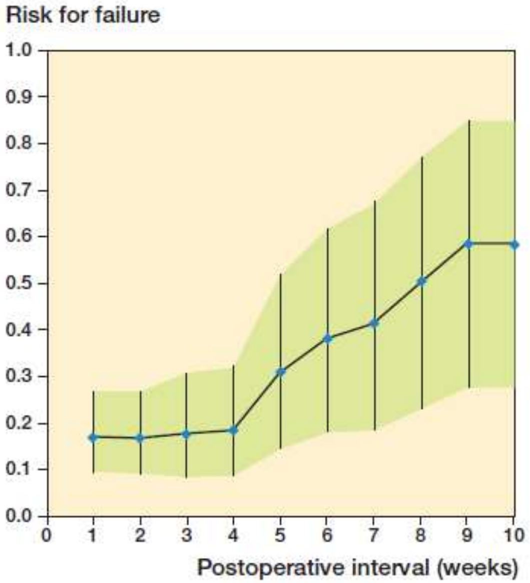
< 3-4 semaines

IOA AIGUE

- Réplication bactérienne +++
- Infection suppurative
- Congestion vasculaire

TRAITEMENT CONSERVATEUR

Risque de récidive



> 3-4 semaines

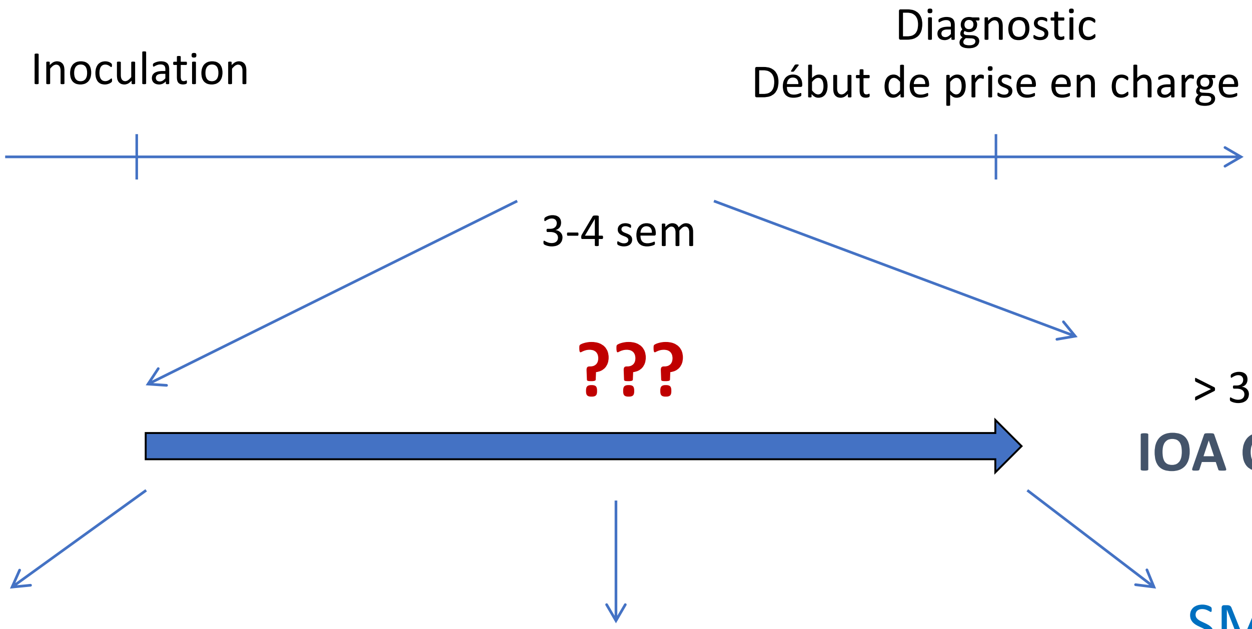
IOA CHRONIQUE

- Tissu osseux ischémique
- Séquestre osseux
- Descellement
- Ralentissement de la croissance bactérienne

ABLATION DES IMPLANTS

Taux élevé d'échec thérapeutique

Chronicité : importance du concept



Inoculation

Diagnostic
Début de prise en charge

3-4 sem

???

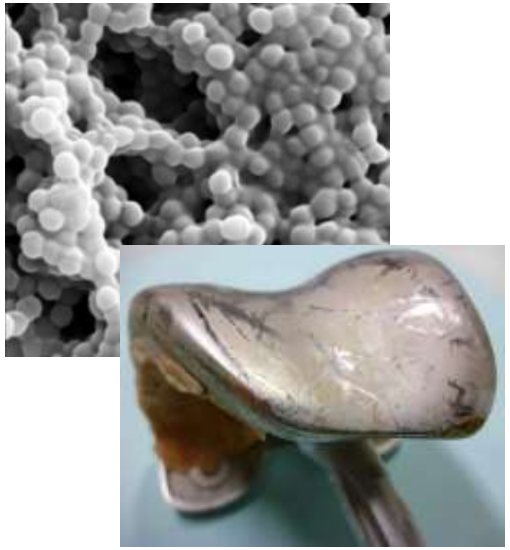
< 3-4 semaines

IOA AIGUE

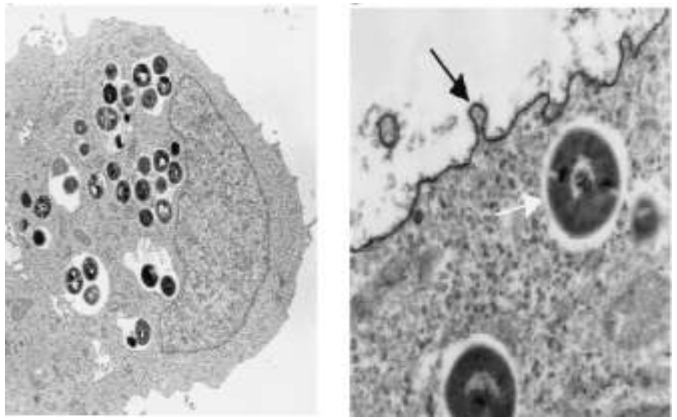
> 3-4 semaines

IOA CHRONIQUE

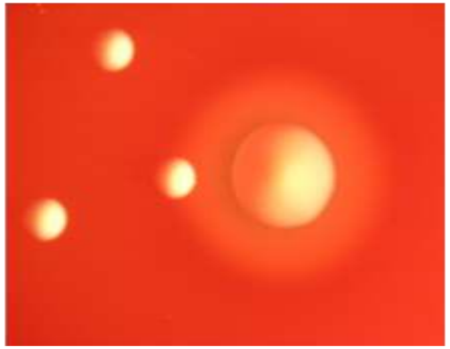
BIOFILM



VIE INTRACELLULAIRE



SMALL COLONY VARIANT (SCV)

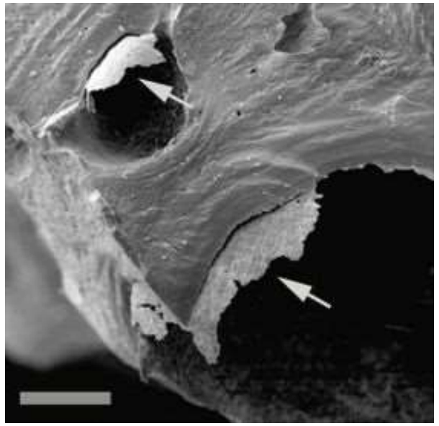


Rôle du biofilm

Formation de biofilm

Des biofilms partout … et depuis longtemps

Stromatolithes
Cyanobactéries
(2,5 milliards d'année)
Australie



Biofilm sur os
de dinosaure

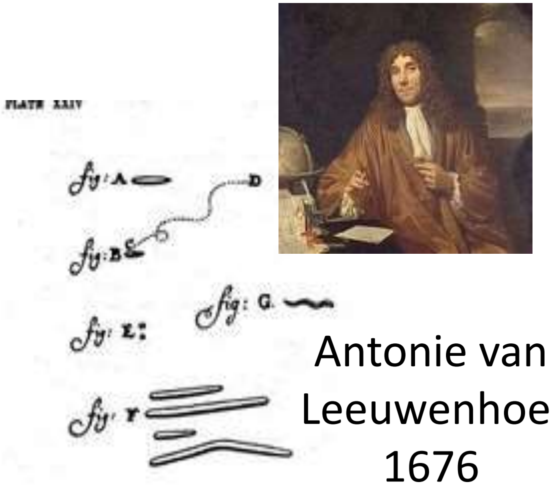
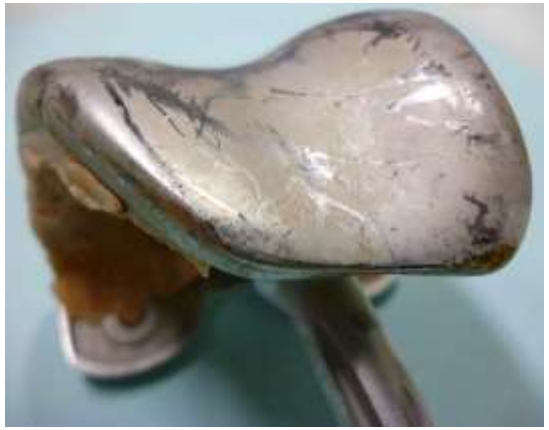


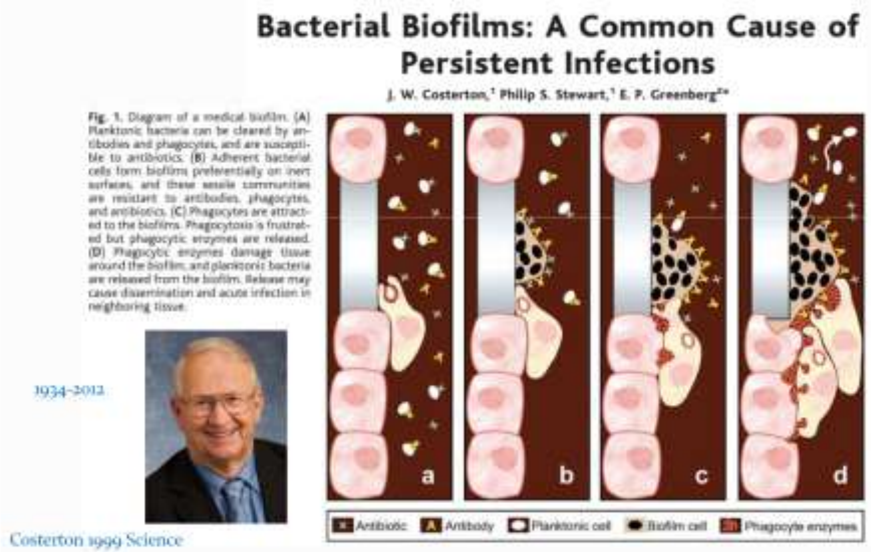
PLATE XXIV
fig: A
D
fig: B
fig: E
fig: G
fig: F

Antonie van
Leeuwenhoek
1676



IOA à *S. epidermidis*
chez Mr M.
Juin 2015
CRIOAc Lyon

COSTERTON
BIOFILM CENTER



Bacterial Biofilms: A Common Cause of Persistent Infections

J. W. Costerton,[1] Philip S. Stewart,[1] E. P. Greenberg[2*]

Fig. 1. Diagram of a medical biofilm. (A) Planktonic bacteria can be cleared by antibodies and phagocytes, and are susceptible to antibiotics. (B) Adherent bacterial cells form biofilms preferentially on inert surfaces, and these sessile communities are resistant to antibodies, phagocytes, and antibiotics. (C) Phagocytes are attracted to the biofilms. Phagocytosis is frustrated but phagocytic enzymes are released. (D) Phagocytic enzymes damage tissue around the biofilm, and planktonic bacteria are released from the biofilm. Release may cause dissemination and acute infection in neighboring tissue.

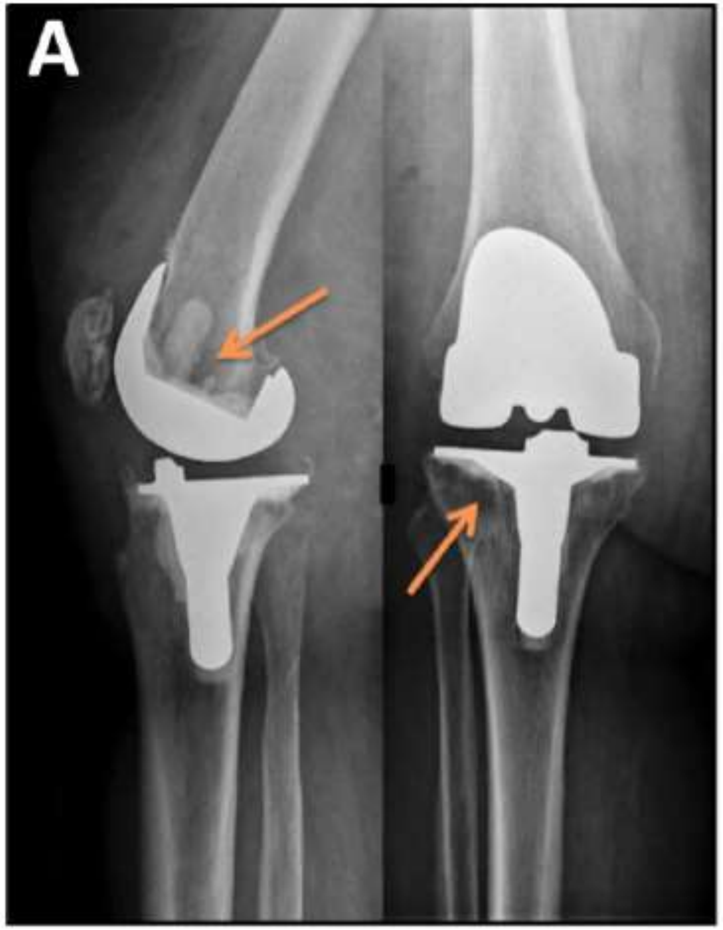
a
b
c
d
Antibiotic
A Antibody
Planktonic cell
Biofilm cell
Phagocyte enzymes

1934-2012

Costerton 1999 Science

Mme M, 80 ans

- PTG posée en 2009
- Douleur progressive importante à la marche
- Sans antécédent particulier
- Allergie connue bêta-lactamines
- Adressé au CRIOAc Lyon



Prise en charge

Service Chirurgie Orthopédique

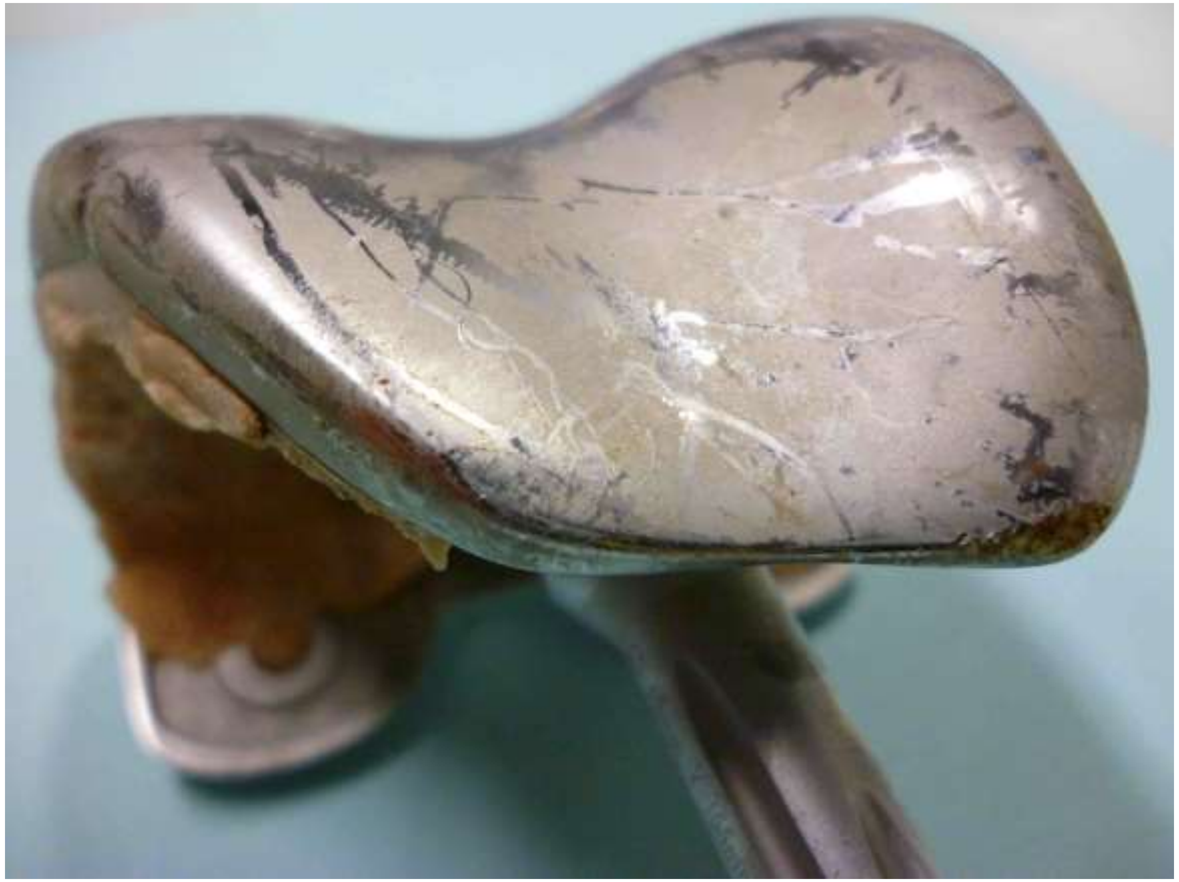
Service MIT Croix Rousse

Service de Bactériologie

Décision changement en deux temps

Mme M, 80 ans

- PTG posée en 2009
- Douleur progressive importante à la marche depuis plusieurs semaines
- Sans antécédent particulier
- Allergie connue bêta-lactamines
- Adressé au CRIOAc Lyon



Décision changement en deux temps

Multiples culture + à *S. epidermidis*

Formation de biofilm

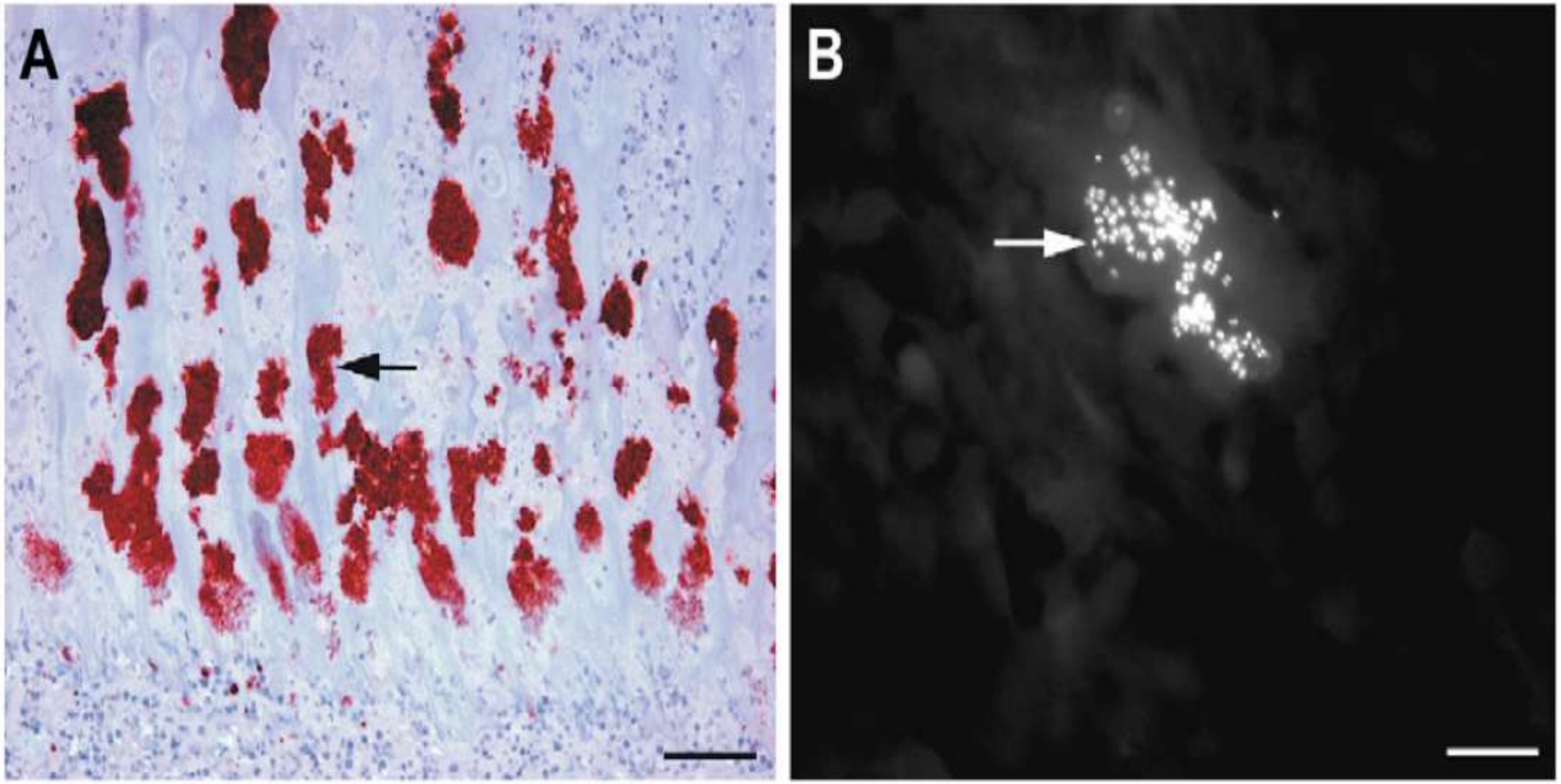
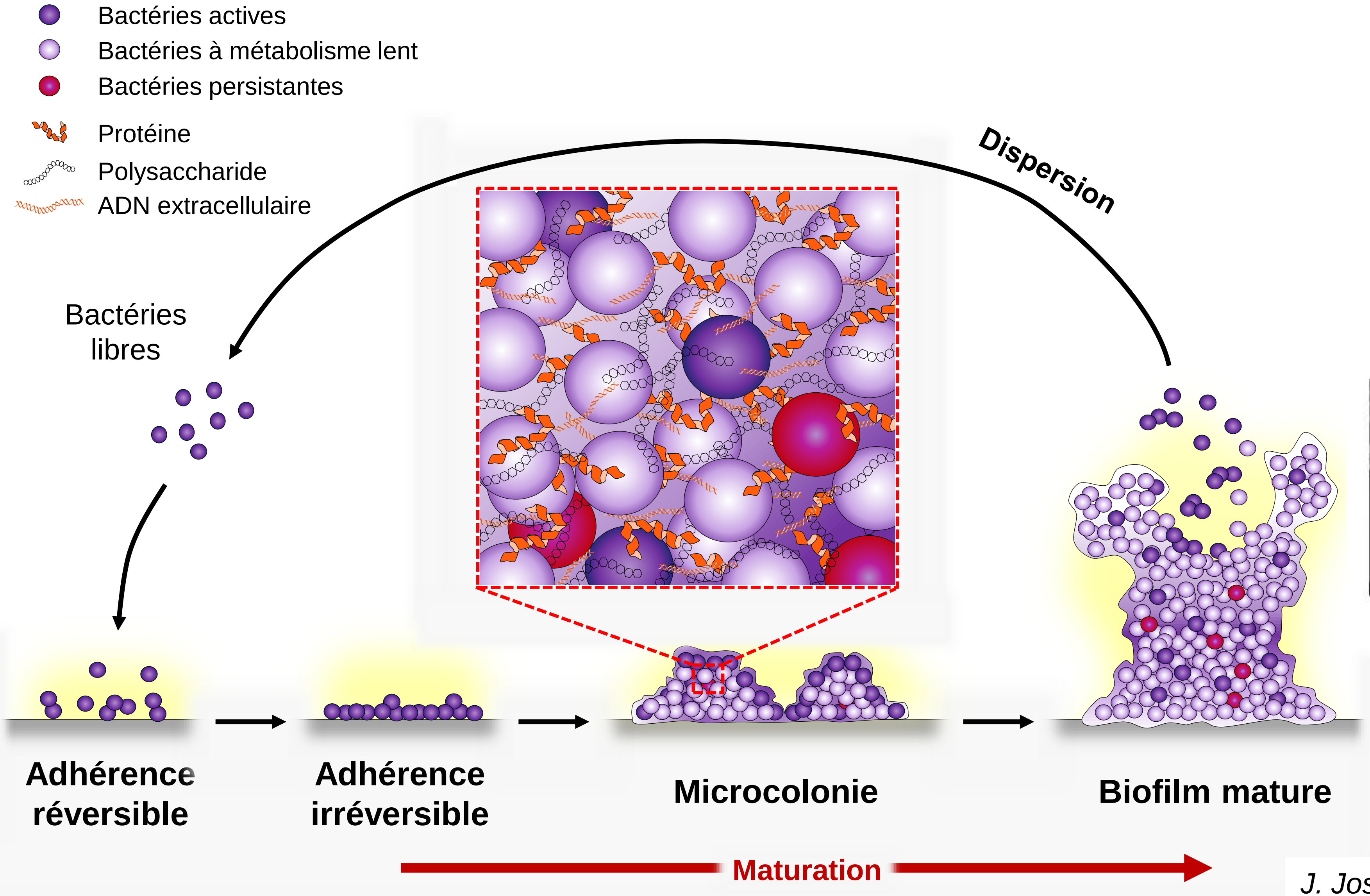
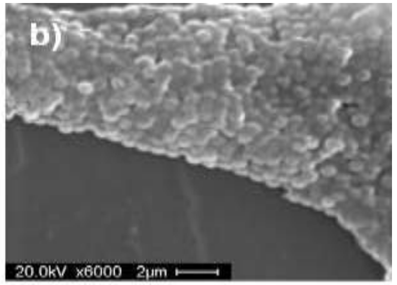


Fig. 5. Lesions caused by *S. aureus* strain S54F9. (A) Identification of *S. aureus* bacteria (arrow) in the abscess shown in Fig. 4F. IHC. Bar, 80 µm. (B) Within the femoral abscess of pig B2 an aggregate of *S. aureus* bacteria (arrow) is embedded in an opaque matrix. PNA FISH. Bar, 10 µm.

Formation de biofilm

1 µm
M2C - GRAM


Bactéries actives
Bactéries à métabolisme lent
Bactéries persistantes
Protéine
Polysaccharide
ADN extracellulaire
Dispersion
Bactéries libres
Adhérence réversible
Adhérence irréversible
Microcolonie
Biofilm mature
Maturation

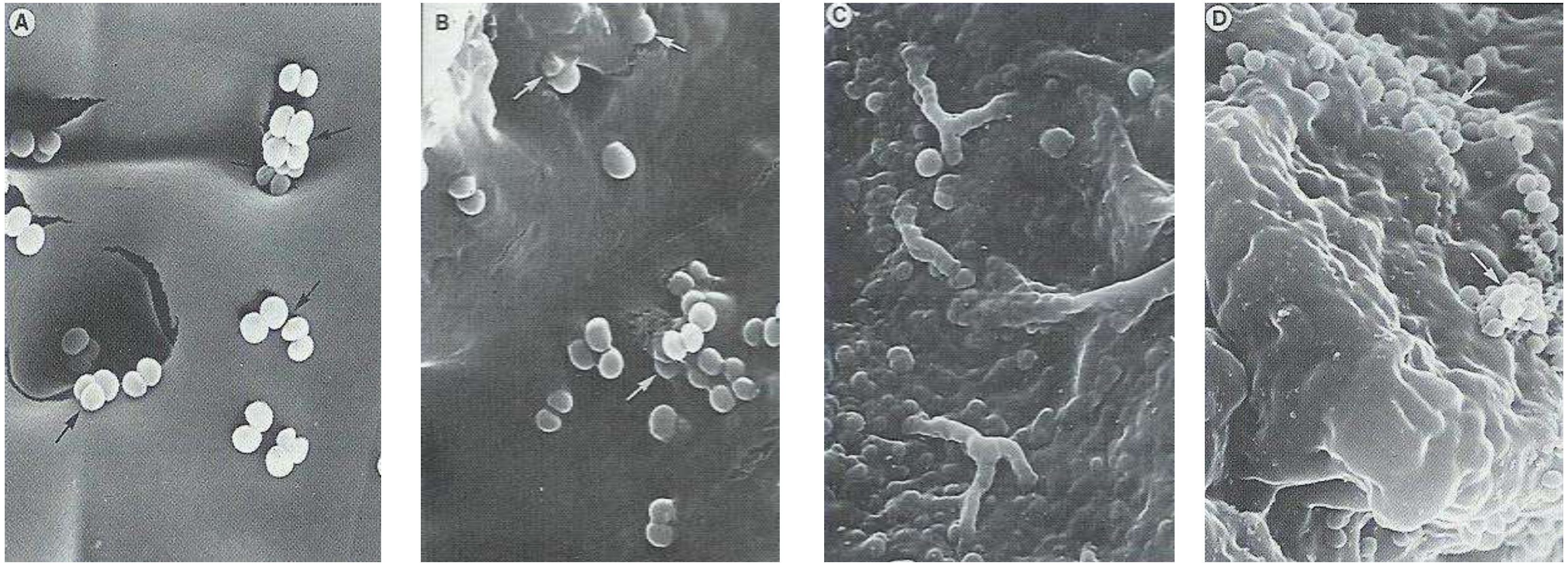

b)
20.0kV x6000 2µm

J. Josse

Biofilm = Structure **dynamique** et **tridimensionnelle** capable d'adhérer à un support vivant ou inerte (séquestre osseux, matériel)

Formation de biofilm

Etapes de la colonisation du matériel par *S. epidermidis*



2 h

Fixation des bactéries sur des irrégularités à la surface du matériel

4 h

Début de fabrication du "slime "

8 h

La surface du matériel est recouverte par une couche épaisse de "slime"

24 h

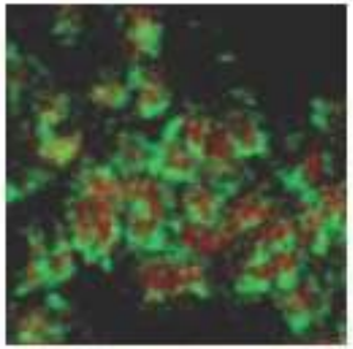
Des bactéries émergent du biofilm, libres et prêtes à se fixer ailleurs

Microphotographies Olson, Ruseska, Costerton J. Biomed Mater Res 1988

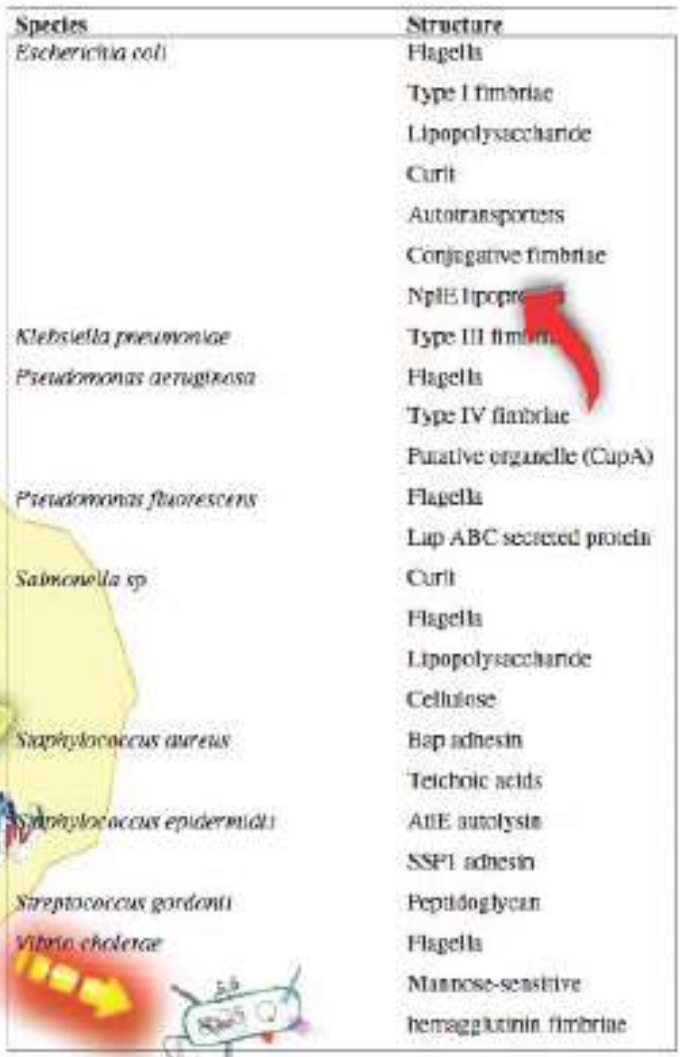
Formation de biofilm

Expression d'adhésines de surface

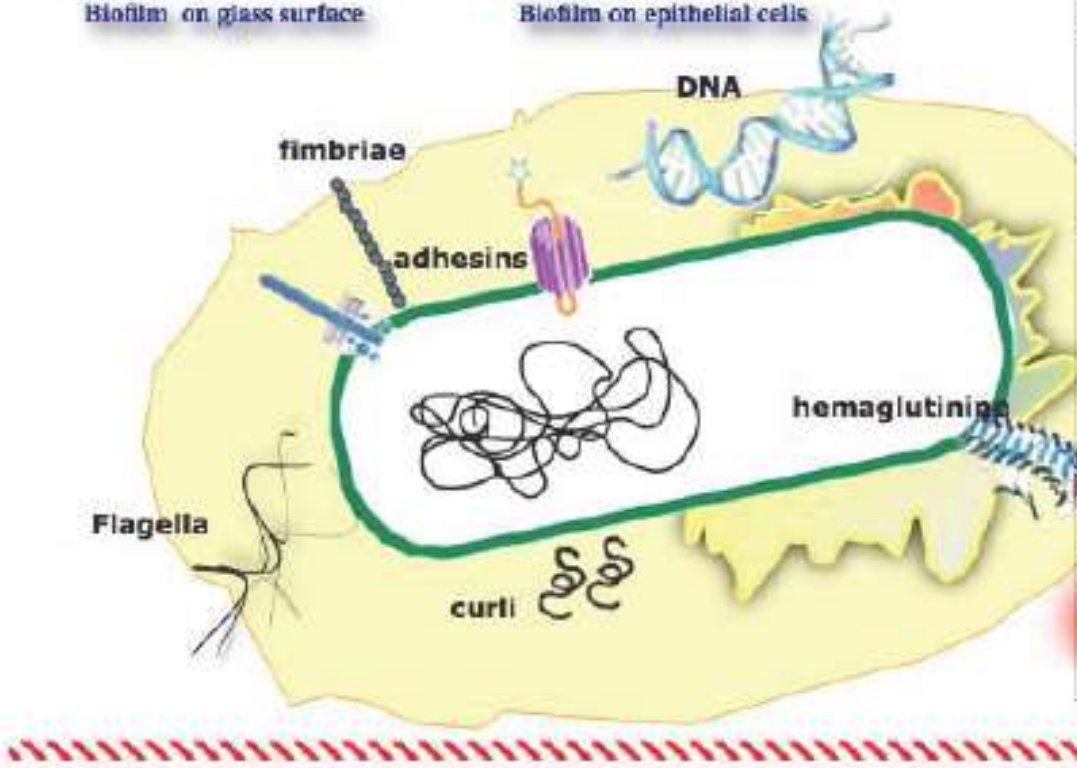
Biofilm on glass surface



Biofilm on epithelial cells



Species	Structure
Escherichia coli	Flagella
	Type I fimbriae
	Lipopolysaccharide
	Curli
	Autotransporters
	Conjugative fimbriae
	NlpE lipopr
Klebsiella pneumoniae	Type III fim
Pseudomonas aeruginosa	Flagella
	Type IV fimbriae
	Putative organelle (CupA)
Pseudomonas fluorescens	Flagella
	Lap ABC secreted protein
Salmonella sp	Curli
	Flagella
	Lipopolysaccharide
	Cellulose
Staphylococcus aureus	Bap adhesin
	Teichoic acids
Staphylococcus epidermidis	AtlE autolysin
	SSP1 adhesin
Streptococcus gordonii	Peptidoglycan
Vibrio cholerae	Flagella
	Mannose-sensitive hemagglutinin fimbriae

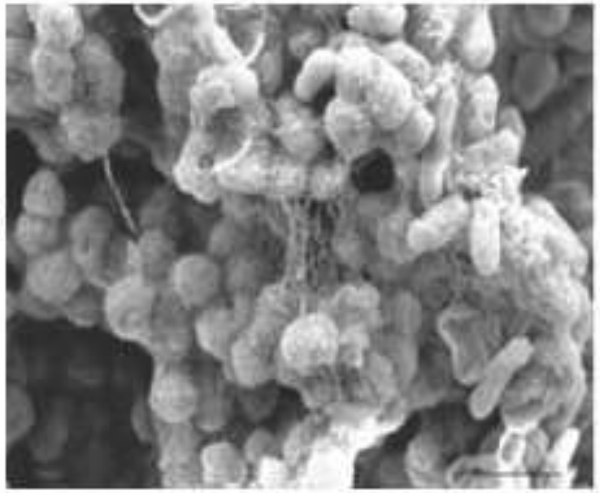


De l’adhérence au biofilm …

Matrice extracellulaire

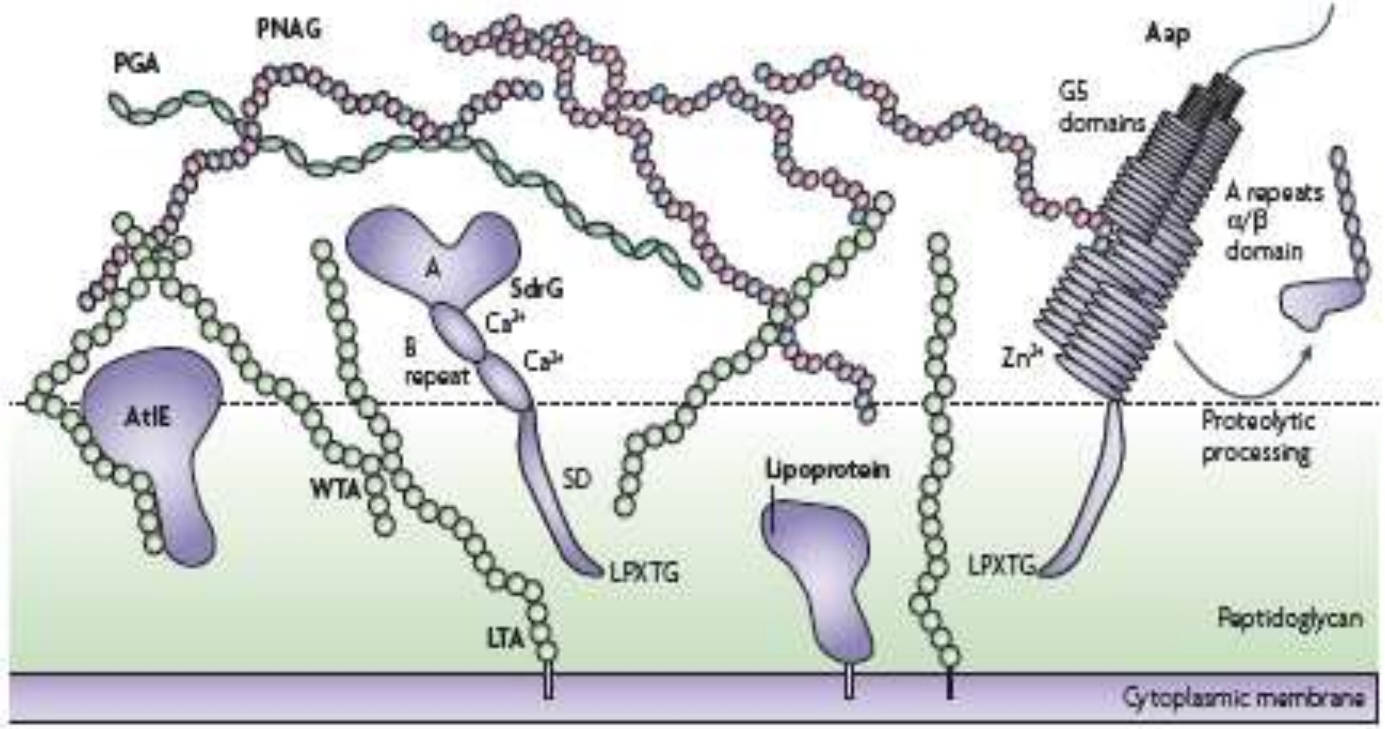
Extracellular polymeric substance (EPS)

Bacteria	Genes	Exopolysaccharide	Composition
E. coli	*wca*	Colanic acid	Glucose, galactose, fucose, glucuronic acid
	pgaABCD	PGA	ß-1,6-GlcNac
	yhjL-N	Cellulose	ß-1,4-Glucosidic polymer
P. aeruginosa	*alg* operon	Alginate	Uronic, mannuronic, guluronic acid
	pelA-G	PEL	Glucose rich
	pslA-O	PSL	Mannose rich
V. cholerae	*vps* genes	VPS	?
Y. pestis	*hms* operon	-	ß-1,6-GlcNac
S. aureus *S. epidemidis*	*icaABCD*	PIA/PNAG	ß-1,6-GlcNac



Formation de biofilm

De l’adhérence au biofilm …


PNAG
PGA
Aap
GS domains
A repeats α/β domain
A
SdrG
Ca2+
B repeat
Ca2+
Zn2+
AtlE
WTA
SD
Lipoprotein
Proteolytic processing
LPXTG
LPXTG
Peptidoglycan
LTA
Cytoplasmic membrane

Aap = Adhesion-associated-protein
AtlE = Autolysine E
Bap = Bone-associated-protein
LTA = Lipo-Teichoic Acid

PGA = Poly-gamma-Glutamyl Acid
PNAG = Poly-N-acetyl-glucosamine ;
Sdr = Serin domain repeat
WTA = Wall Techoic Acid

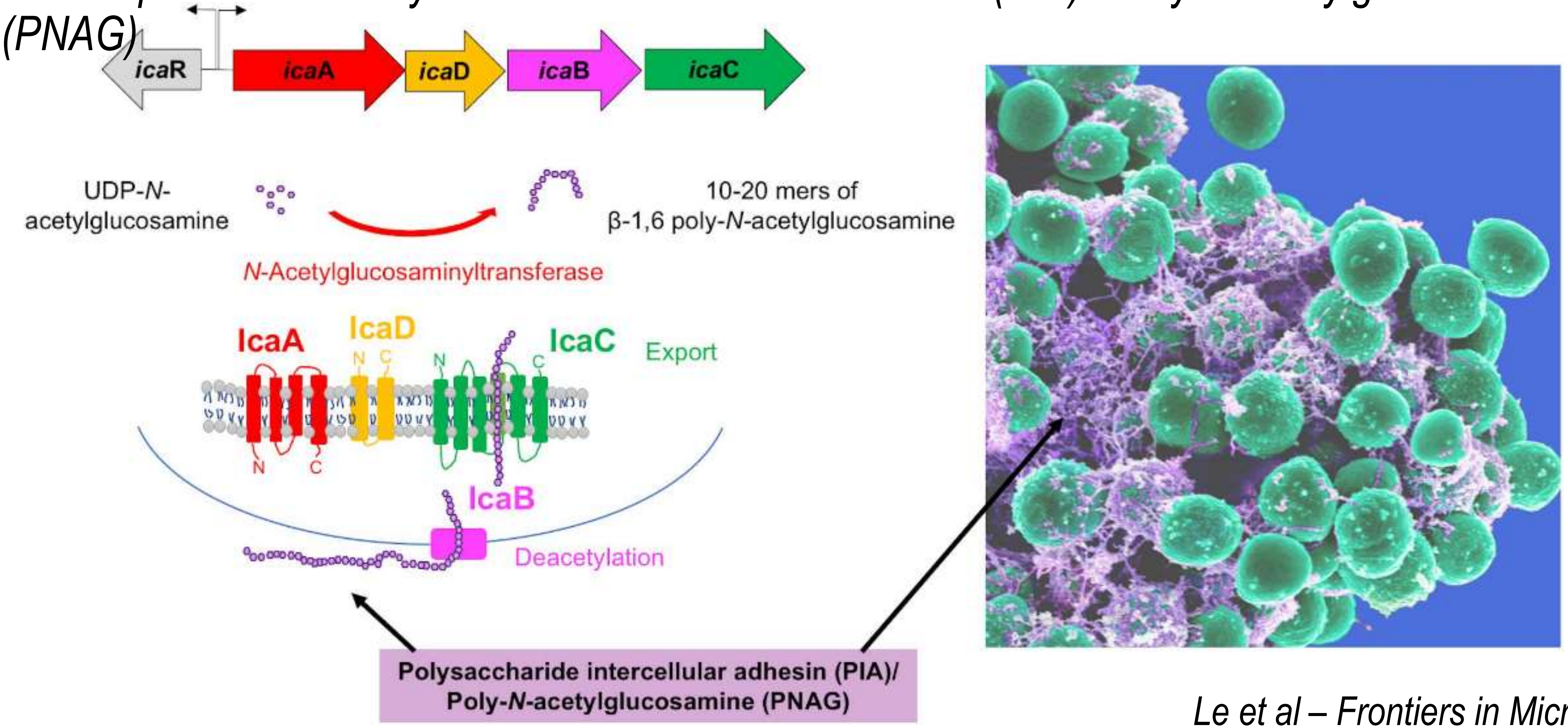
S. epidermidis

Otto, M. Nat Rev Microbiol, 2009.

Formation de biofilm

De l’adhérence au biofilm …

Ex: S. epidermidis - Polysaccharide intercellular adhesin (PIA) / Poly-N-Acetylglucosamine (PNAG)

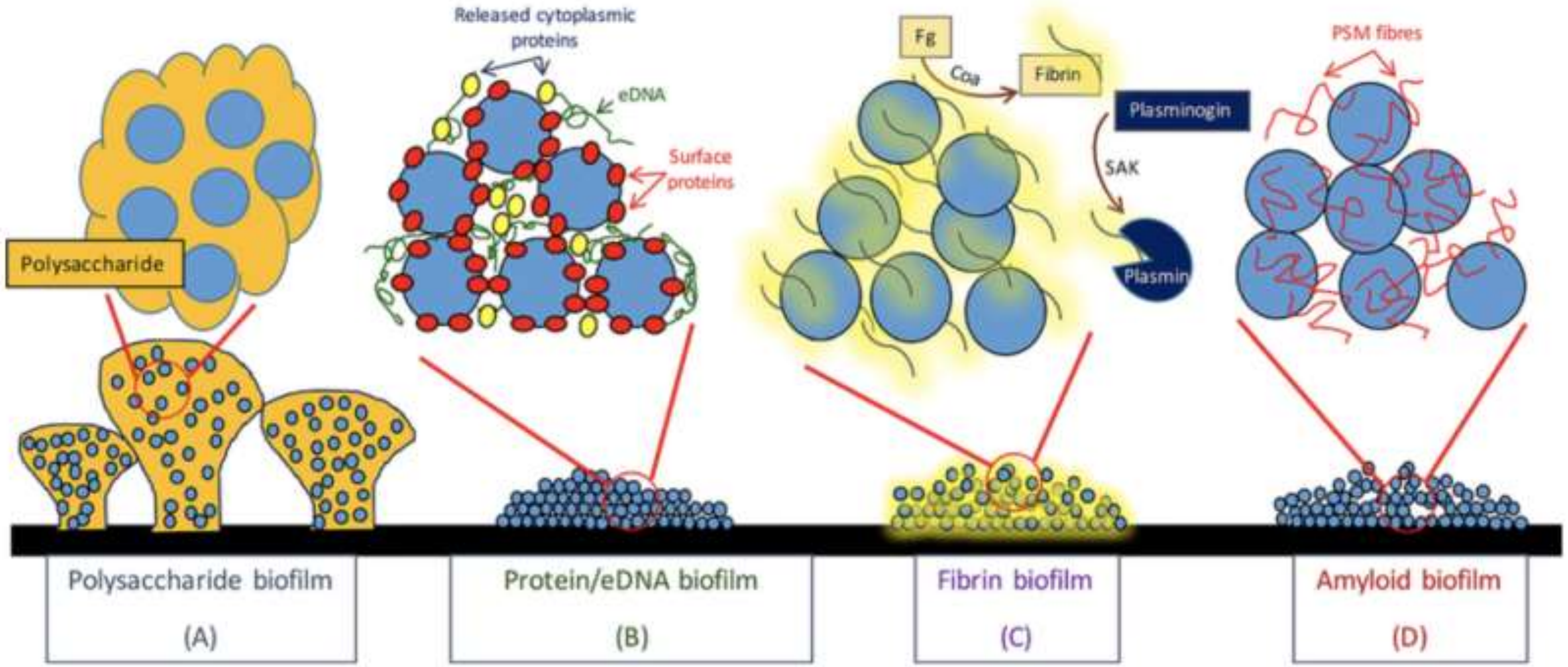

icaR
icaA
icaD
icaB
icaC
UDP-N-acetylglucosamine
10-20 mers of β-1,6 poly-N-acetylglucosamine
N-Acetylglucosaminyltransferase
IcaA
IcaD
IcaC
Export
N
C
IcaB
Deacetylation
Polysaccharide intercellular adhesin (PIA)/ Poly-N-acetylglucosamine (PNAG)

Le et al – Frontiers in Microbiology 2018

Formation de biofilm

De l’adhérence au biofilm …

S. aureus



Polysaccharide
Released cytoplasmic proteins
eDNA
Surface proteins
Fg
Coa
Fibrin
Plasminogin
SAK
Plasmin
PSM fibres
Polysaccharide biofilm
(A)
Protein/eDNA biofilm
(B)
Fibrin biofilm
(C)
Amyloid biofilm
(D)

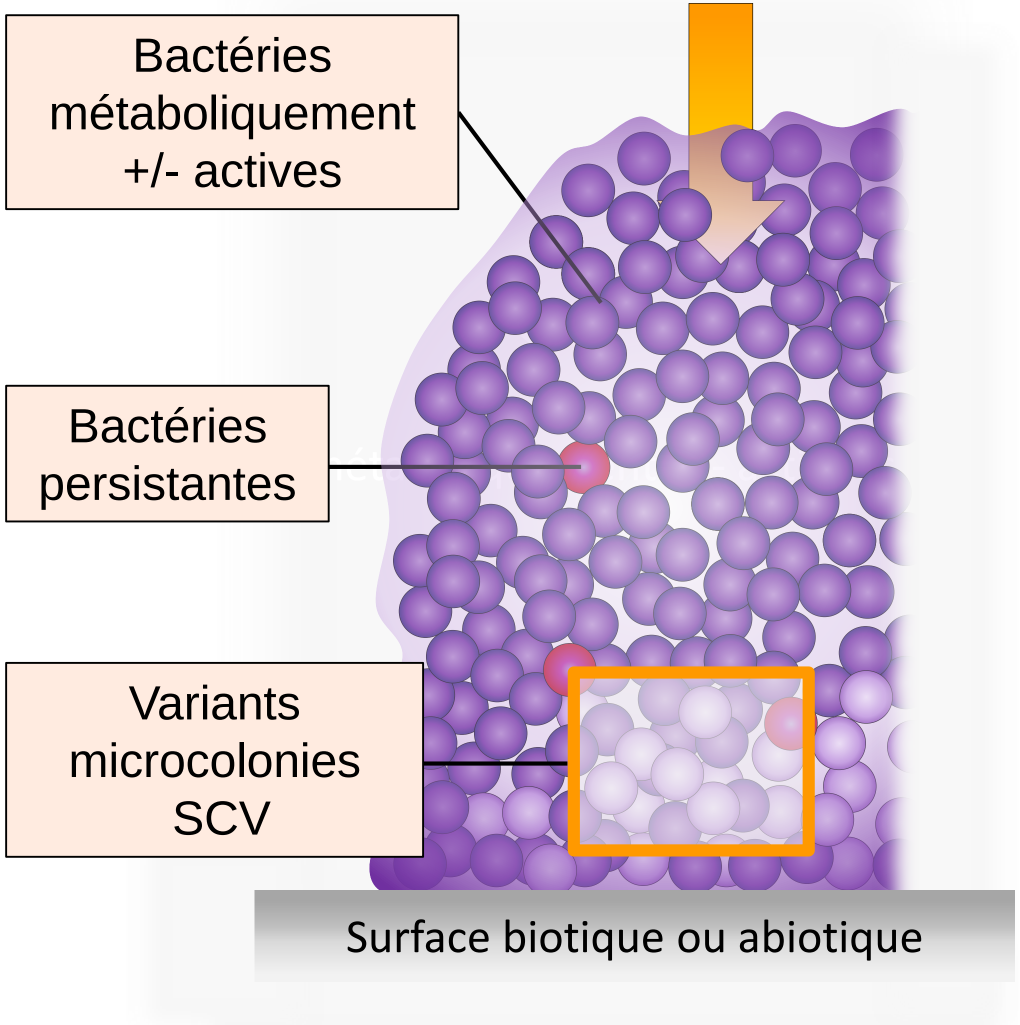
Major mechanisms of biofilm expressed by S. aureus. (A) Polysaccharide-type biofilm is dependent of expression of PIA/PNAG by intracellular adhesion operon (icaADBC)-carrying strains and is common in methicillin-sensitive S. aureus isolates. (B) Surface proteins such as BAP, the FnBPs, and SasG/Aap mediate direct cell-to-cell contact during biofilm accumulation. Cytoplasmic proteins and eDNA released following cell lysis can also act as components of the biofilm matrix. (C) Coagulase-mediated conversion of fibrinogen (Fg) into fibrin, which is recruited into a biofilm scaffold that can be dispersed by plasmin produced following staphylokinase-mediated activation of plasminogen. (D) Phenol-soluble modules have surfactant qualities that can promote biofilm dispersal but can also accumulate as amyloid aggregates that promote biofilm accumulation.

- Composition = variable
- Conséquences de la formation de biofilm = variables

Marta Zapotoczna, et al.
PLoS Pathogens, 2016

Formation de biofilm

Biofilm


Bactéries métaboliquement +/- actives
Bactéries persistantes
Variants microcolonies SCV
Surface biotique ou abiotique

Résistance :

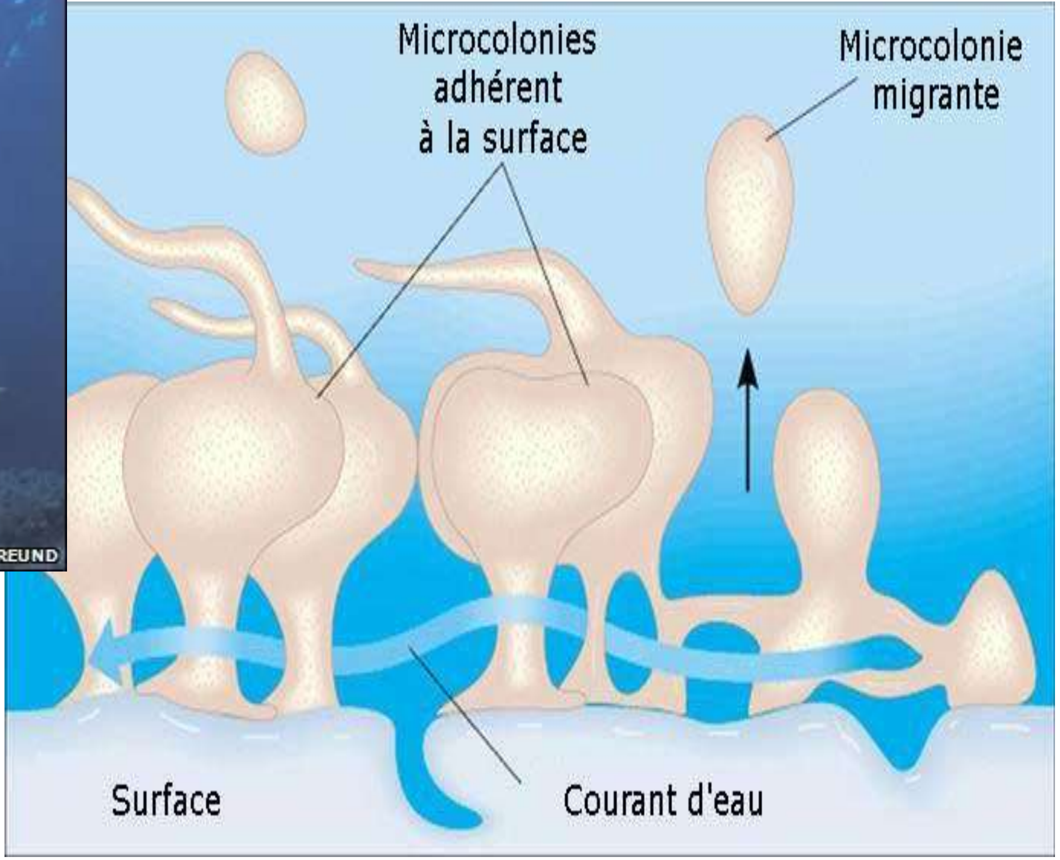
- **Multiplication** en présence d'antibiotique
- Origine génétique

Tolérance :

- **Survie** en présence d'antibiotique
- Origine phénotypique
- Réversible

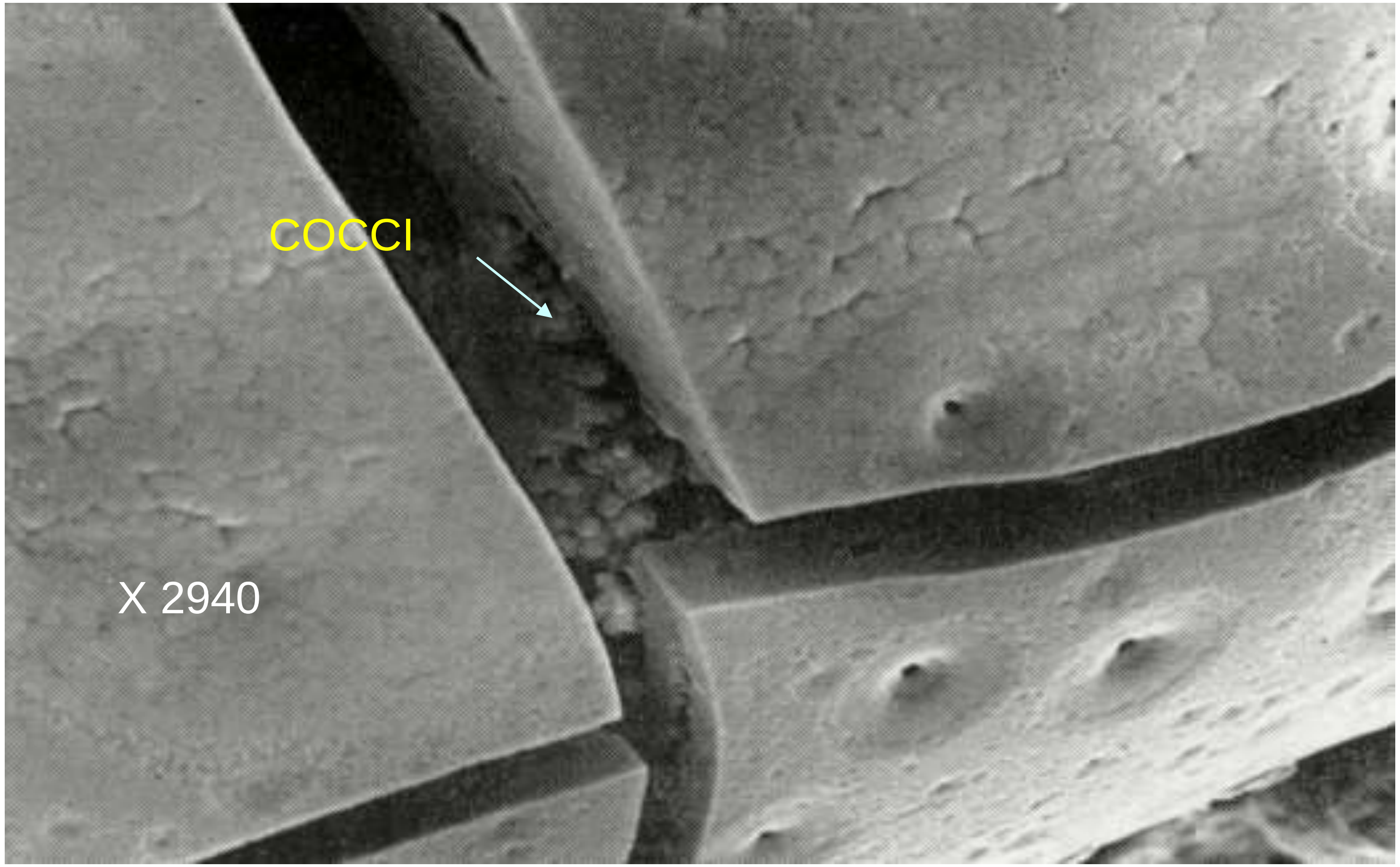
J. Josse

Formation de biofilm


Microcolonies
adhérent
à la surface
Microcolonie
migrante
Surface
Courant d'eau

Each bacteria is in a different microenvironment

Formation de biofilm



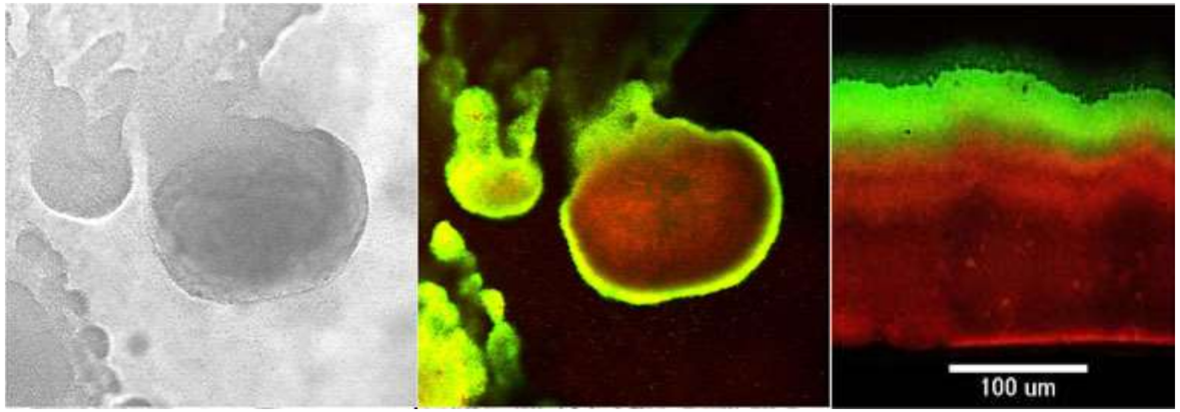
Nombreux cocci visibles au fond d'une cassure d'une couche épaisse de biofilm d'un séquestre infecté
(*From:* Evans : Clin Orthop. 1998, February: 243-249)
Higher power magnification (original magnification, x 2940) of the infected sequestra in prepared by the osmium tetroxide and freeze drying method.

Formation de biofilm

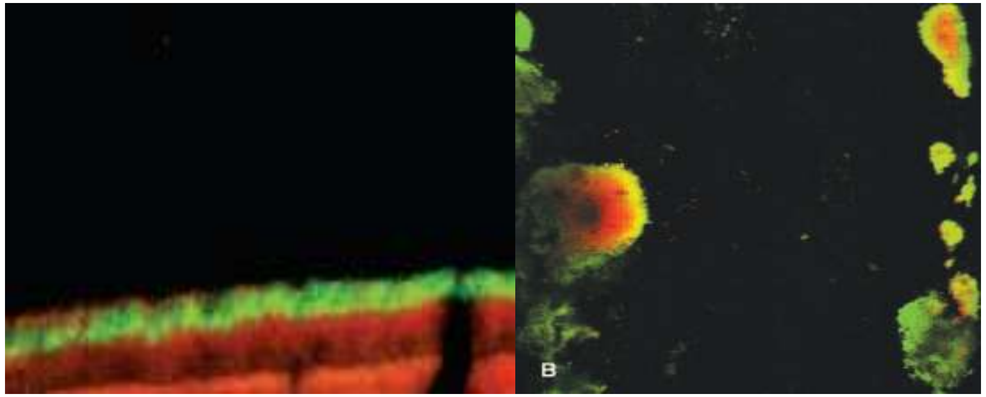
Hétérogénéité du biofilm / micro environnement

État métabolique

Mesure de la synthèse protéique
Expression GFP

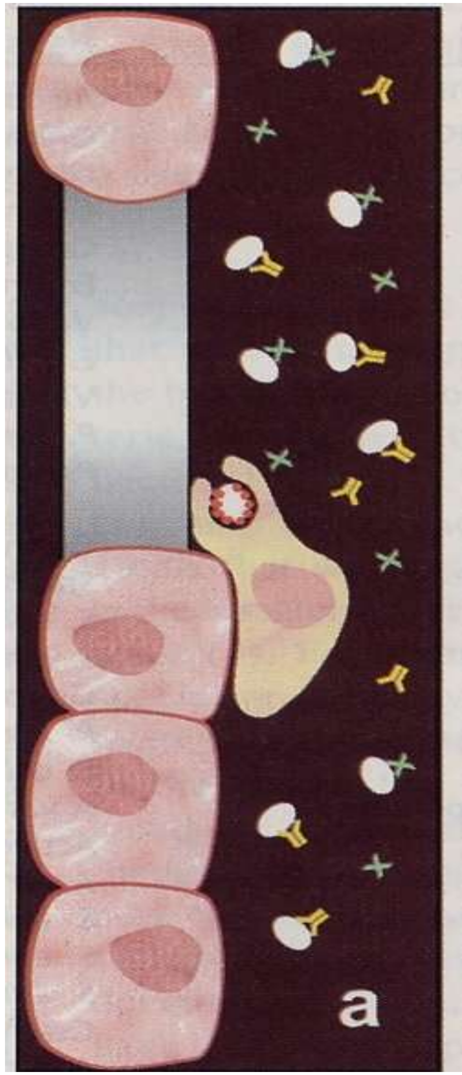


Mesure de la synthèse ADN
5 bromo2-deoxyuridine (BrdU)



Biofilm *S. epidermidis* (*Rani et al.*,, 2007)

Formation de biofilm



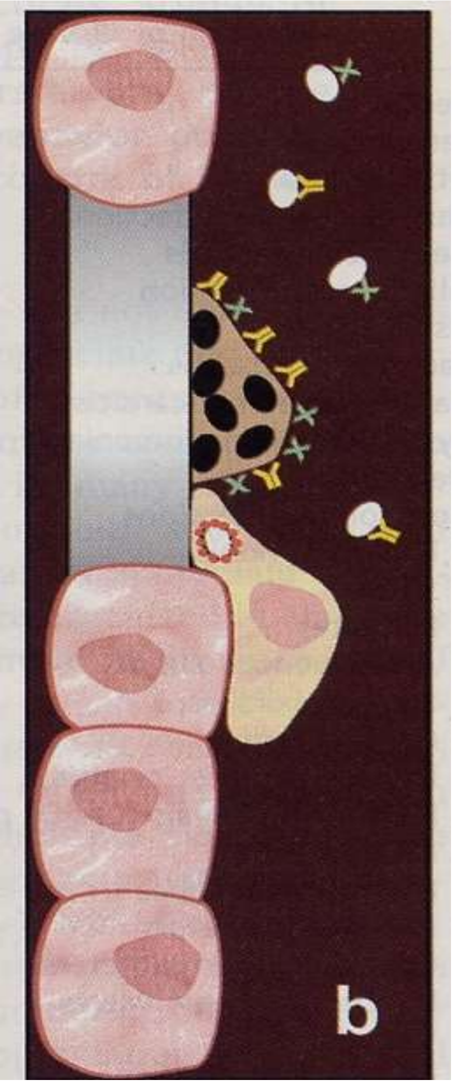
Costerton *et al*. Science 1999

Bactéries planctoniques :

- Elimination par les mécanismes de défense naturels (anticorps, phagocytes…)
- Facilement accessibles aux antibiotiques

Antibiotic
Antibody
Planktonic cell
Biofilm cell
Phagocyte enzymes

Formation de biofilm



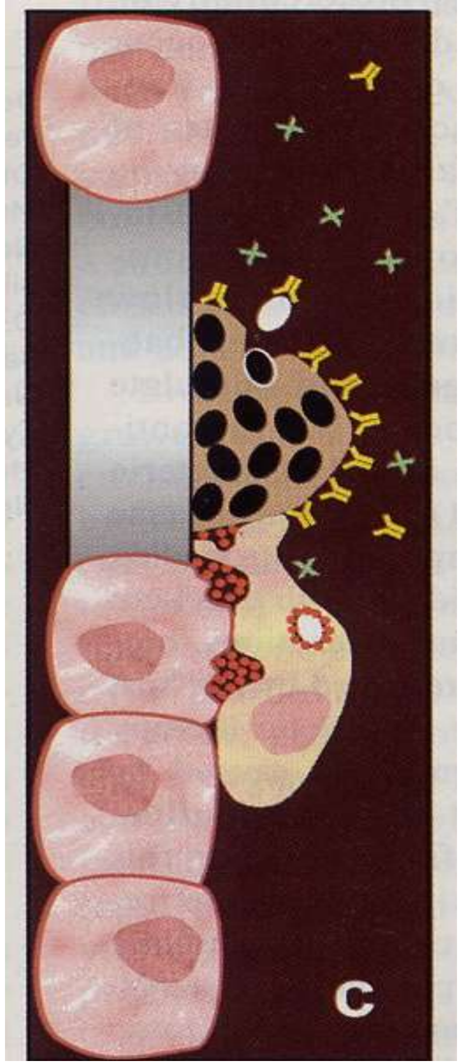
Bactéries sessiles = adhésion sur une surface inerte et entre elles ⇨ biofilm

- échappement au système immunitaire
  - bactéries peu accessibles aux Ac

× Antibiotic
⅄ Antibody
○ Planktonic cell
● Biofilm cell
▨ Phagocyte enzymes

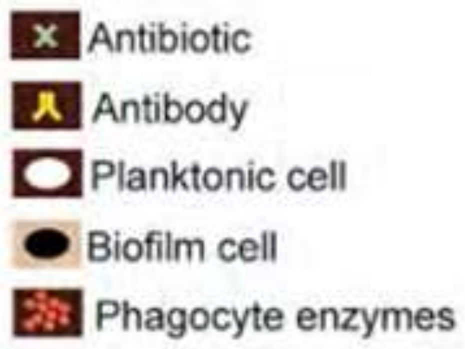
Costerton *et al*. Science 1999

Formation de biofilm

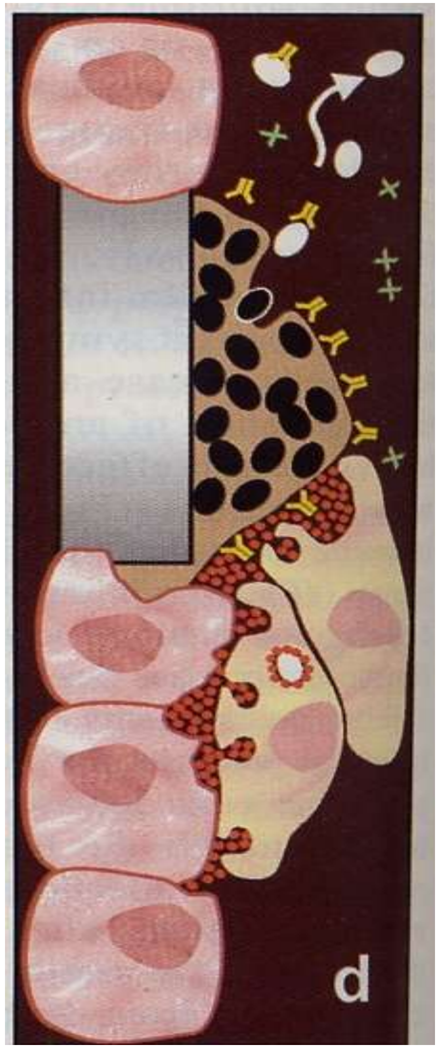


Costerton *et al*. Science 1999

- phagocytes attirés par le biofilm
- phagocytose inopérante
  → enzymes phagocytaires libérées localement


Antibiotic
Antibody
Planktonic cell
Biofilm cell
Phagocyte enzymes

Formation de biofilm



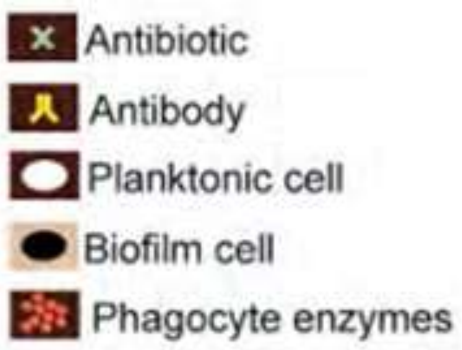
Costerton *et al*. Science 1999

Libération enzymes phagocytaires
= **endommage les tissus autour du biofilm**

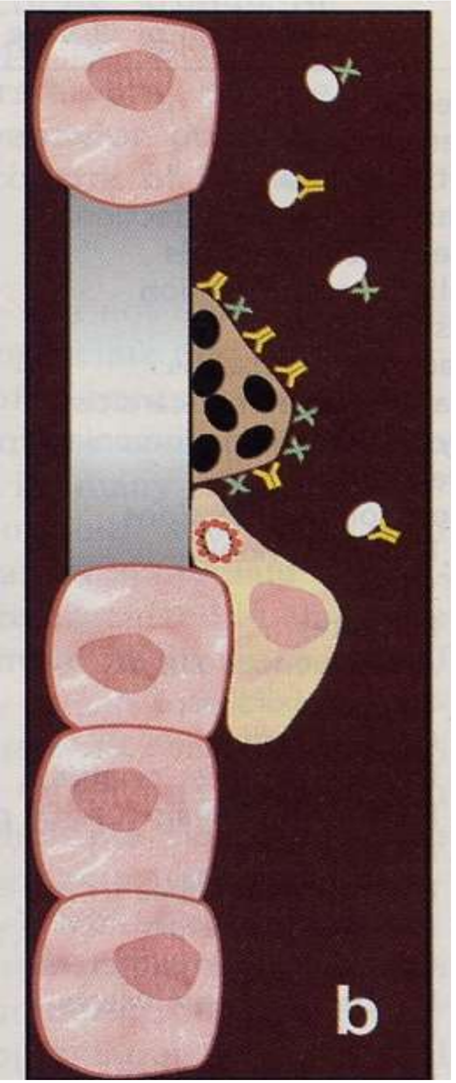
⇨ **descellement**

⇨ **essaimage** de bactéries planctoniques à partir du biofilm :

dissémination de l'infection
infection dans un tissu voisin ou à distance


Antibiotic
Antibody
Planktonic cell
Biofilm cell
Phagocyte enzymes

Formation de biofilm



Costerton *et al*. Science 1999

Bactéries sessiles = adhésion sur une surface inerte et entre elles ⇒ biofilm

- diminution de sensibilité / « tolérance » aux antibiotiques
  - pénétration difficile, ATB piégés/inactivés par des enzymes de la matrice extracellulaire
  - concentrations subinhibitrices d'ATB dans le biofilm, induction de R
  - bactéries sessiles en phase stationnaire de croissance (quiescence) donc moins sensibles aux ATB
  - environnement anaérobie inactivant les aminosides

The Journal of Immunology

Formation de biofilm et physiologie osseuse

Staphylococcus aureus Biofilms Prevent Macrophage Phagocytosis and Attenuate Inflammation In Vivo

Lance R. Thurlow,*[,1] Mark L. Hanke,* Teresa Fritz,* Amanda Angle,* Amy Aldrich,* Stetson H. Williams,* Ian L. Engebretsen,[†] Kenneth W. Bayles,* Alexander R. Horswill,[‡] and Tammy Kielian*

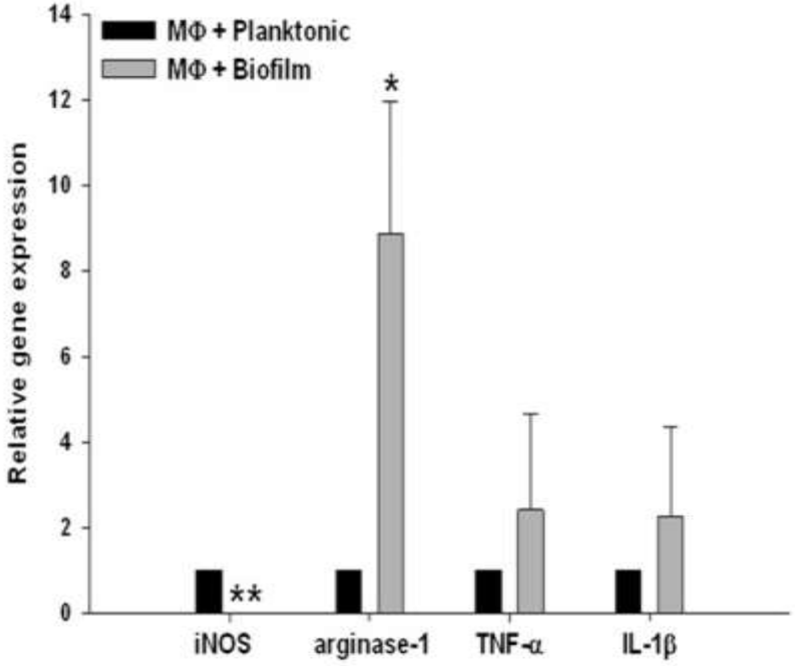


FIGURE 10. *S. aureus* biofilms skew macrophage gene expression toward an alternatively activated M2 phenotype.

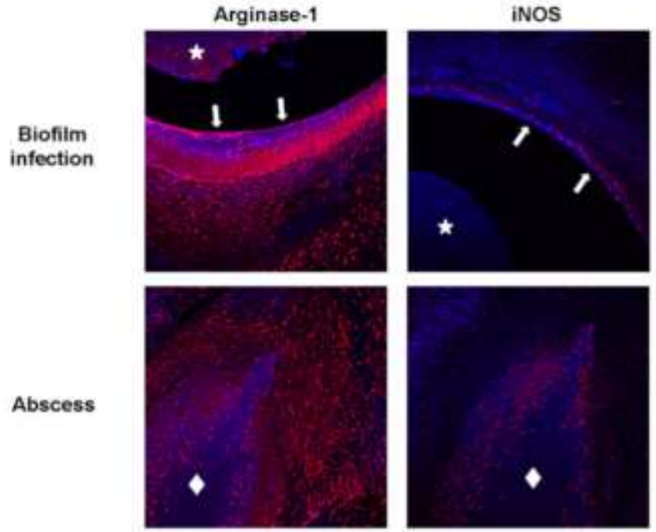
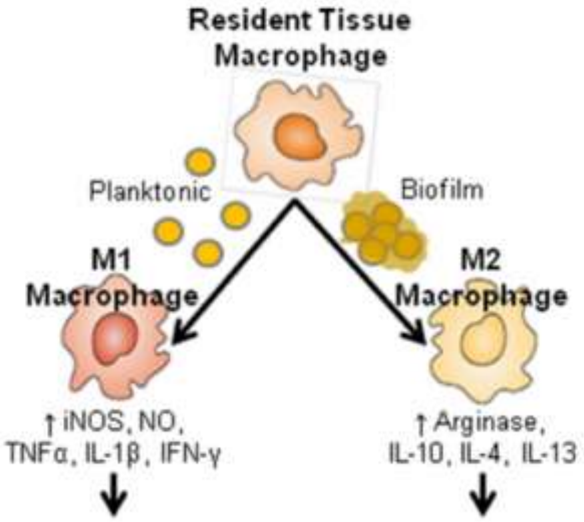

Arginase-1
iNOS
Biofilm infection
Abscess

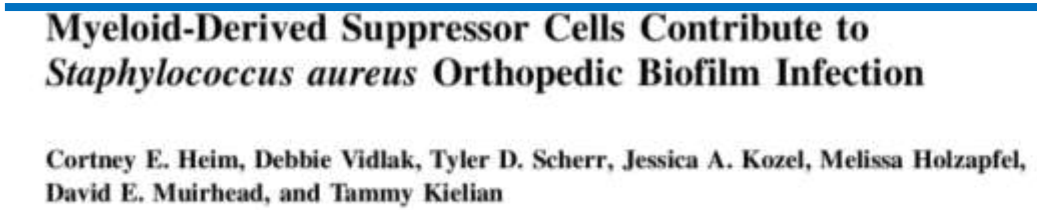
FIGURE 8. *S. aureus* biofilms repress iNOS and augment arginase-1 expression. WT mice were infected with 5×10^5 CFU USA300 LAC::*lux*


Resident Tissue Macrophage
Planktonic
Biofilm
M1 Macrophage
M2 Macrophage
↑ iNOS, NO, TNFα, IL-1β, IFN-γ
↑ Arginase, IL-10, IL-4, IL-13

Pro-inflammatory
Bacterial clearance

Anti-inflammatory
Bacterial persistence

Formation de biofilm et physiologie osseuse

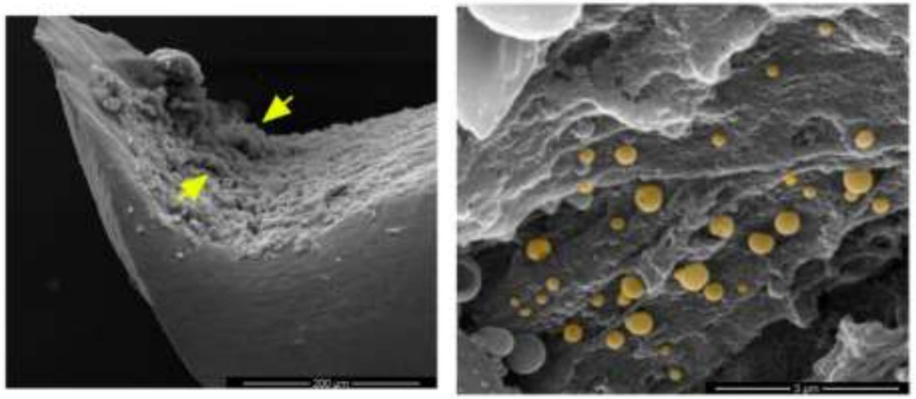


Myeloid-Derived Suppressor Cells Contribute to *Staphylococcus aureus* Orthopedic Biofilm Infection

Cortney E. Heim, Debbie Vidlak, Tyler D. Scherr, Jessica A. Kozel, Melissa Holzapfel,
David E. Muirhead, and Tammy Kielian

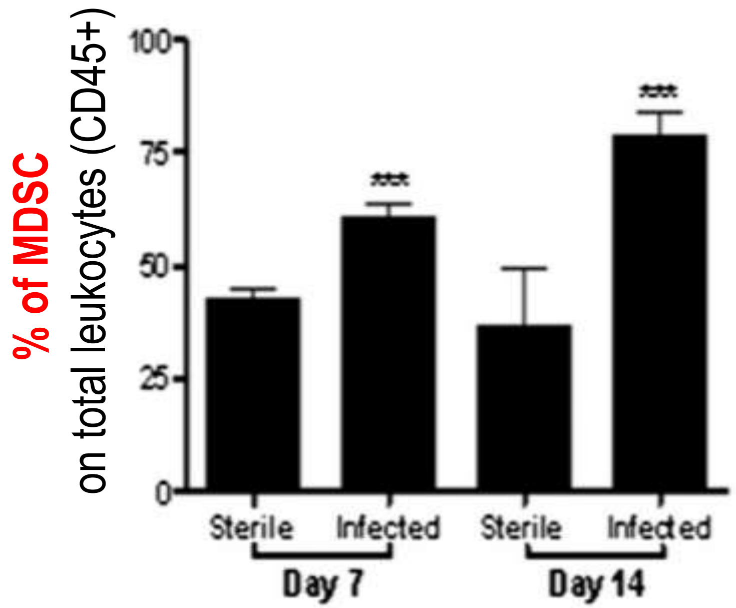
- male C57BL/6 mice (8 wk of age)
- *in vivo* model prosthetic material (nickel-titanium)
- bioluminescent *S. aureus* USA300

S. aureus biofilm on the titanium implant



Myeloid-Derived Supressor cells (MDSC)
= $CD11b^+$ $Gr\text{-}1^+$ cells

Cytometry on cells_tissue
Surrounded infection



Infiltration +++ of MDSCs on the site of biofilm-associated BJI

Formation de biofilm et physiologie osseuse

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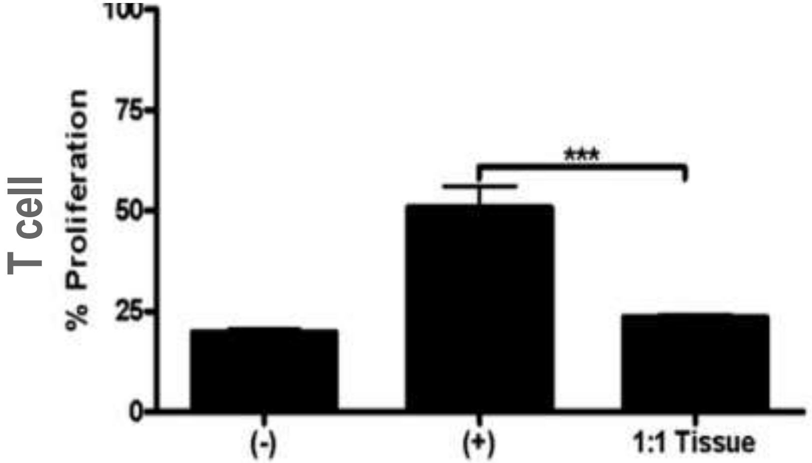
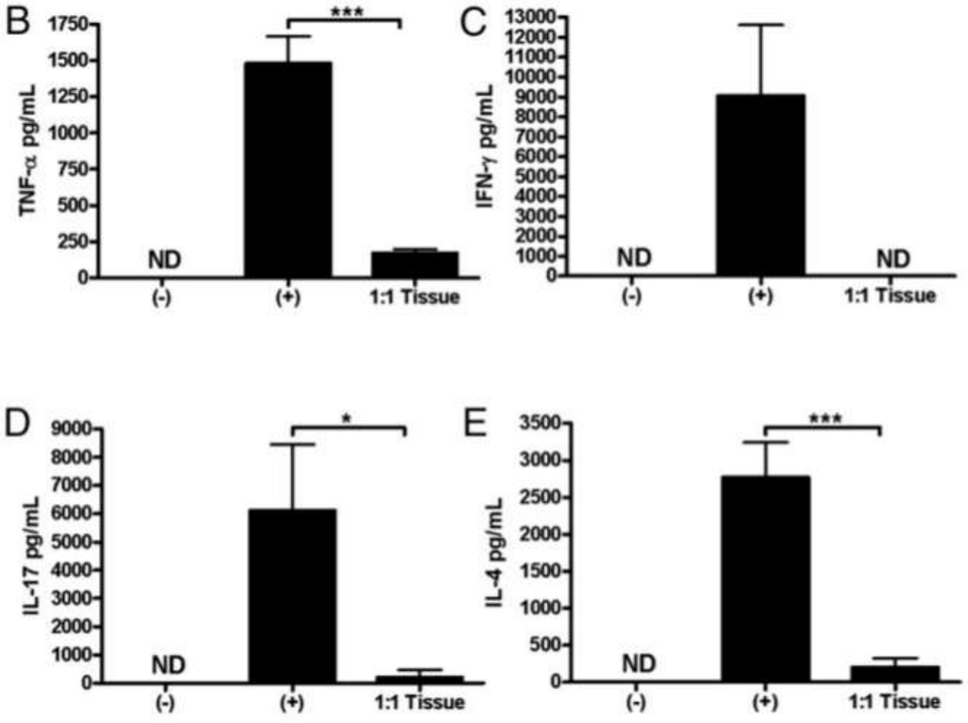


Proinflammatory cytokines

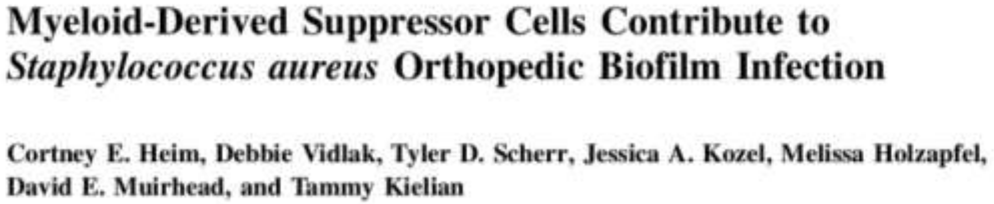


(-), T cells only
(+), T cells + CD3/CD28 Dynabeads
1:1 Tissue, **FACS-purified MDSCs recovered from infected joint tissues at day 14**
ND, not detected.

MDSCs = strong Arg-1 expression, which depletes extracellular arginine, causing T cell dysfunction

MDSCs inhibit . T cells proliferation
. their production of pro-inflammatory cytokines

Formation de biofilm et physiologie osseuse



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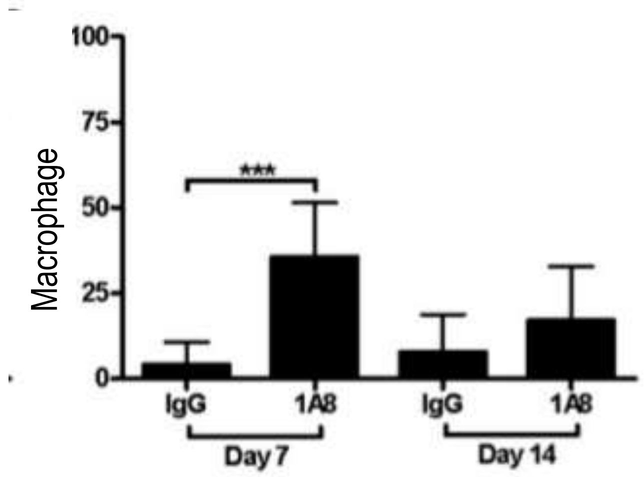
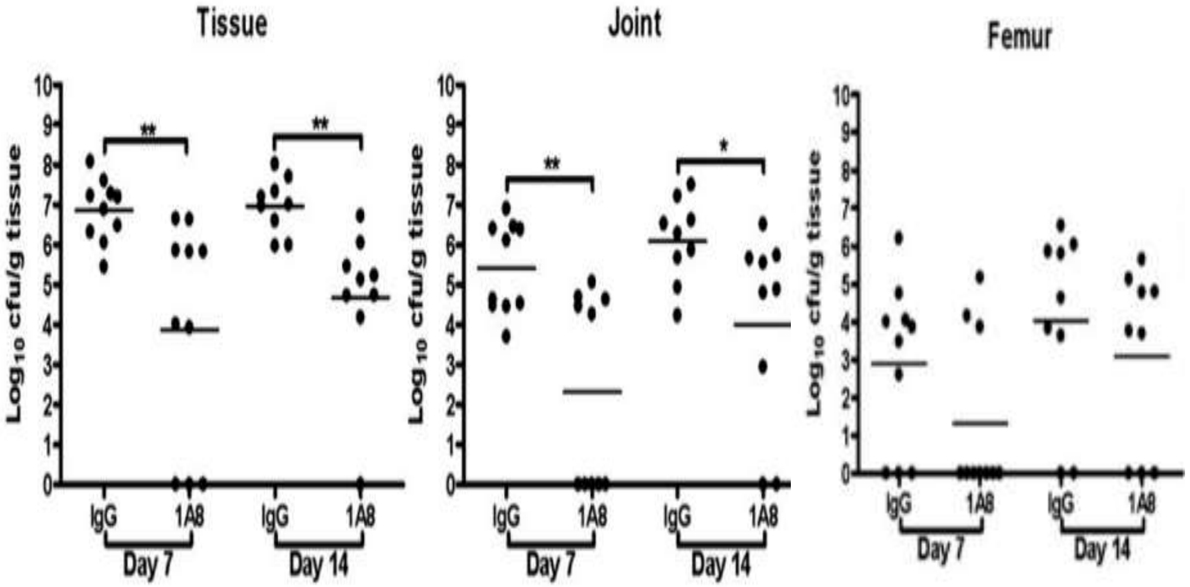
During *S. aureus* orthopedic biofilm infection, **MDSCs depletion** (using 1A8 Ab injection) induces …



… an increase of macrophage/monocyte recruitment



… an improvement of bacterial clearance !

- ➢ Possible development of diagnostic tools and biomarkers

Formation de biofilm et physiologie osseuse

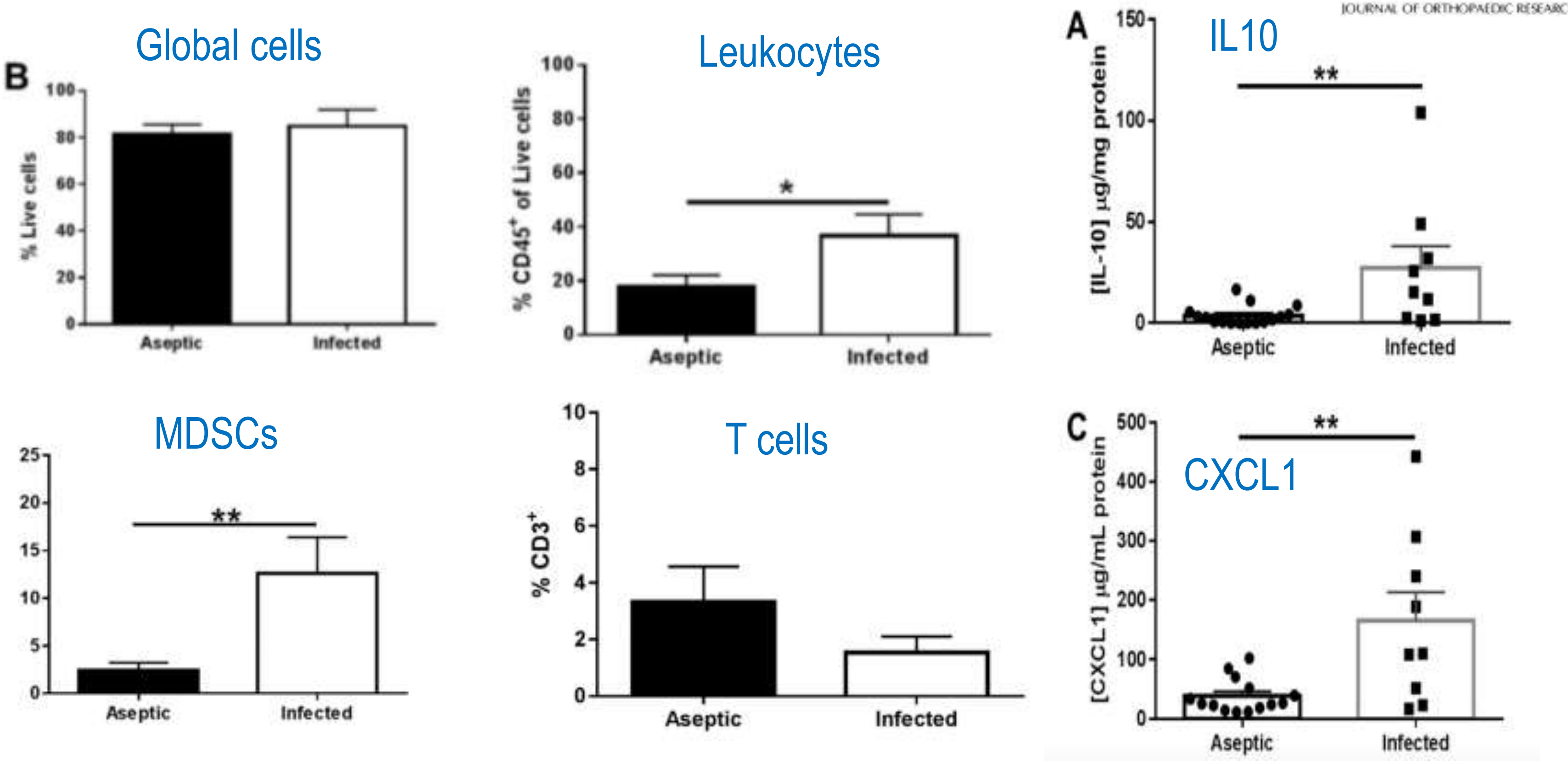
Confirmation in **infected patients vs aseptic patient**

- Use of leukocyte infiltrates associated with soft tissues surrounding the knee joints of aseptic and *S. aureus*–infected implant

Human Prosthetic Joint Infections Are Associated With Myeloid-Derived Suppressor Cells (MDSCs): Implications for Infection Persistence

Cortney E. Heim,[1] Debbie Vidlak,[1] Jessica Odvody,[1] Curtis W. Hartman,[2] Kevin L. Garvin,[2] Tammy Kielian[1]

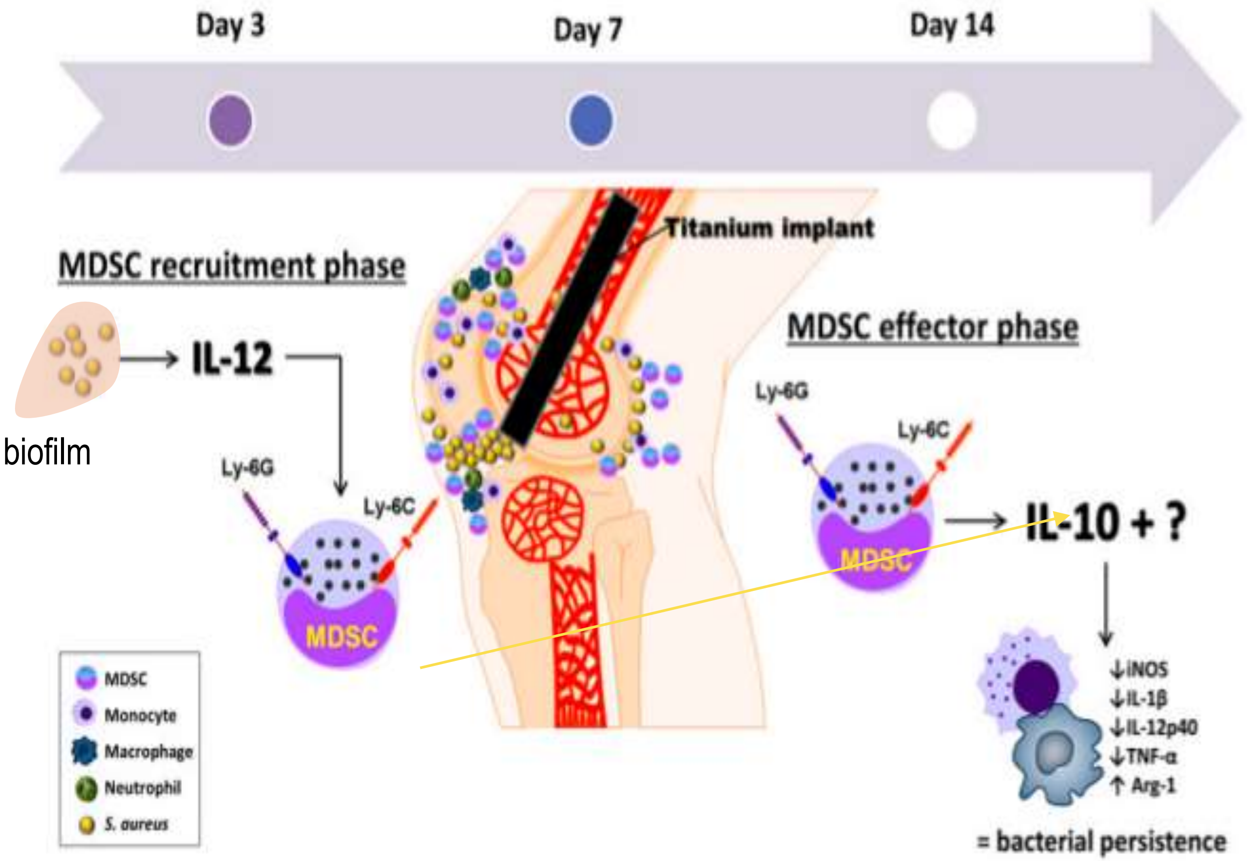
JOURNAL OF ORTHOPAEDIC RESEARCH MONTH 2017



- Possible development of diagnostic tools and biomarkers

Formation de biofilm et physiologie osseuse

Temporal relationship between IL-10 and IL-12 actions during *S. aureus* PJI


Day 3
Day 7
Day 14
Titanium implant
MDSC recruitment phase
biofilm
IL-12
Ly-6G
Ly-6C
MDSC
MDSC effector phase
Ly-6G
Ly-6C
MDSC
IL-10 + ?
↓iNOS
↓IL-1β
↓IL-12p40
↓TNF-α
↑ Arg-1
= bacterial persistence
MDSC
Monocyte
Macrophage
Neutrophil
S. aureus

Heim et al., JBL, 2015

Heim et al., JI, 2015

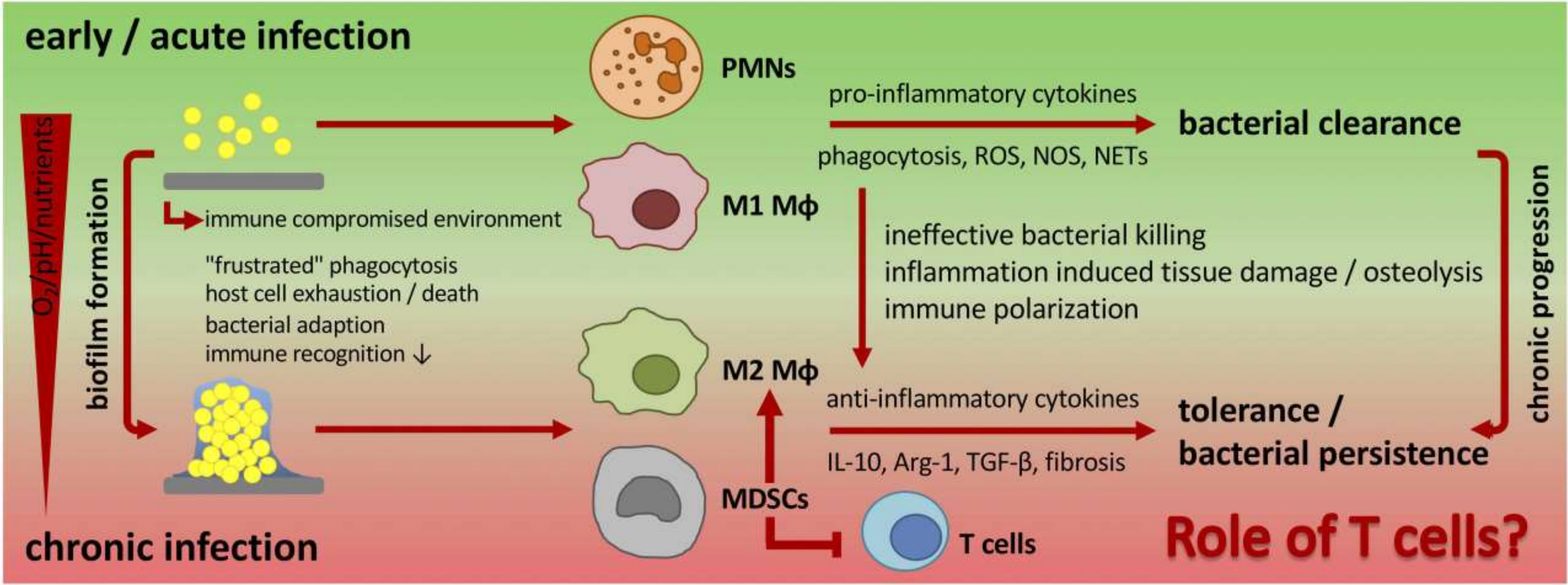
- ✓ M2 macrophage differenciation
- ✓ Decrease of phagocytosis
- ✓ Inhibition of T cell proliferation
- ✓ Anti-inflammatory secretion
- ✓ Induction of local fibrosis

MDSCs = **key contributors** to the chronicity of *S. aureus* biofilms
through their modulation of the host immune response

➢ Inhibitors of MDSCs could be good candidats to prevent/treat biofilm

Chronic Implant-Related Bone Infections—Can Immune Modulation be a Therapeutic Strategy?

Elisabeth Seebach and Katharina F. Kubatzky**



early / acute infection

O_2/pH/nutrients

biofilm formation

immune compromised environment

"frustrated" phagocytosis
host cell exhaustion / death
bacterial adaption
immune recognition ↓

PMNs

M1 Mφ

M2 Mφ

MDSCs

T cells

pro-inflammatory cytokines

phagocytosis, ROS, NOS, NETs

bacterial clearance

ineffective bacterial killing
inflammation induced tissue damage / osteolysis
immune polarization

anti-inflammatory cytokines

IL-10, Arg-1, TGF-β, fibrosis

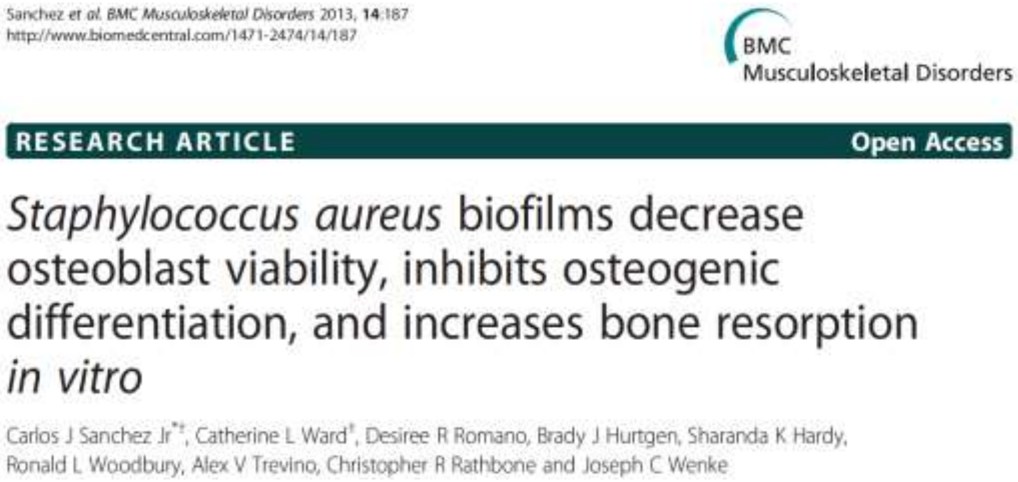
tolerance / bacterial persistence

chronic progression

Role of T cells?

chronic infection

Formation de biofilm et physiologie osseuse



Sanchez *et al. BMC Musculoskeletal Disorders* 2013, **14**:187
http://www.biomedcentral.com/1471-2474/14/187

BMC
Musculoskeletal Disorders

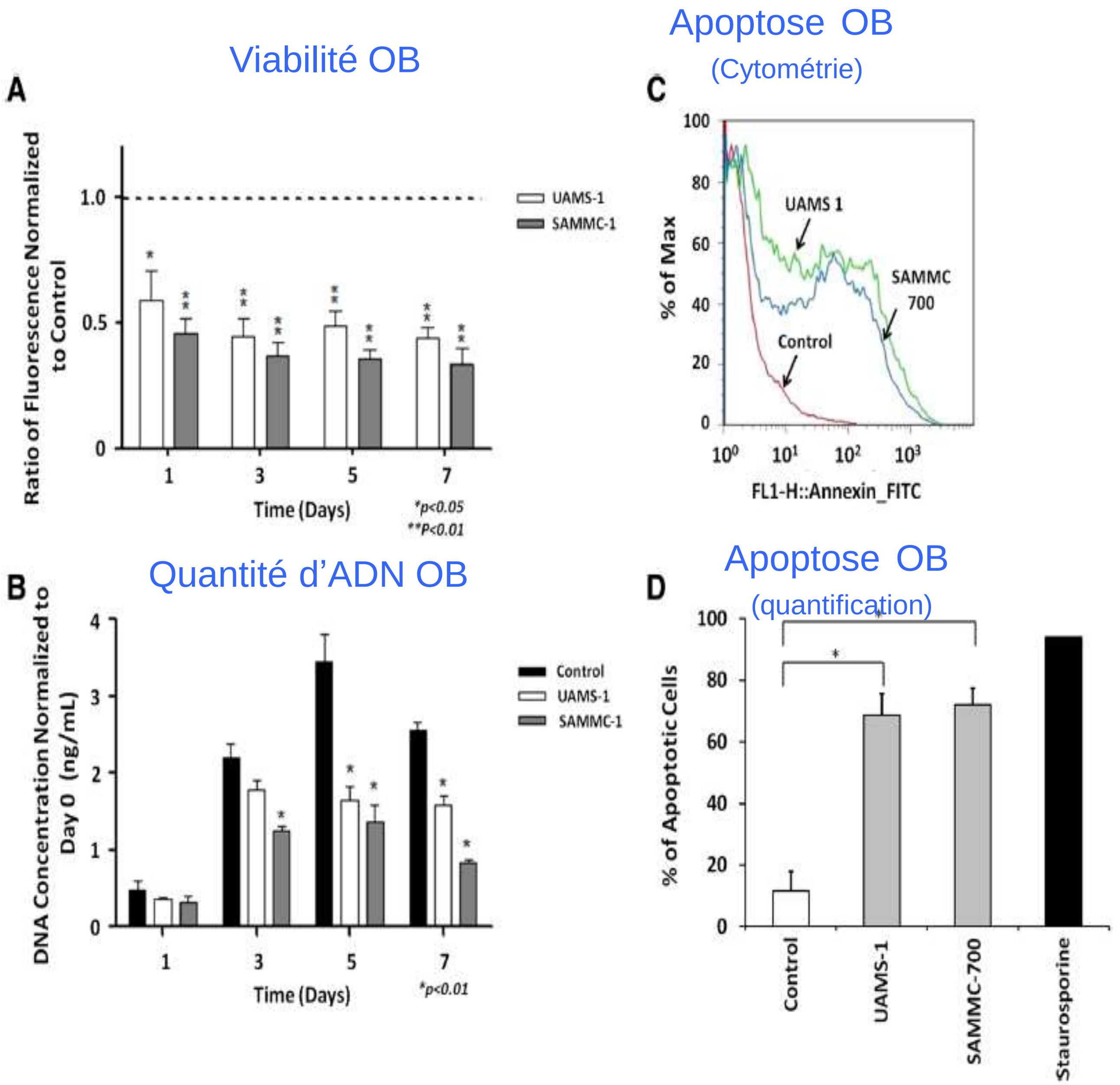
RESEARCH ARTICLE **Open Access**

Staphylococcus aureus biofilms decrease osteoblast viability, inhibits osteogenic differentiation, and increases bone resorption *in vitro*

Carlos J Sanchez Jr[*†], Catherine L Ward[†], Desiree R Romano, Brady J Hurtgen, Sharanda K Hardy, Ronald L Woodbury, Alex V Trevino, Christopher R Rathbone and Joseph C Wenke

Souches *S. aureus* UAMS-1

Souches S. aureus SAMMC-1



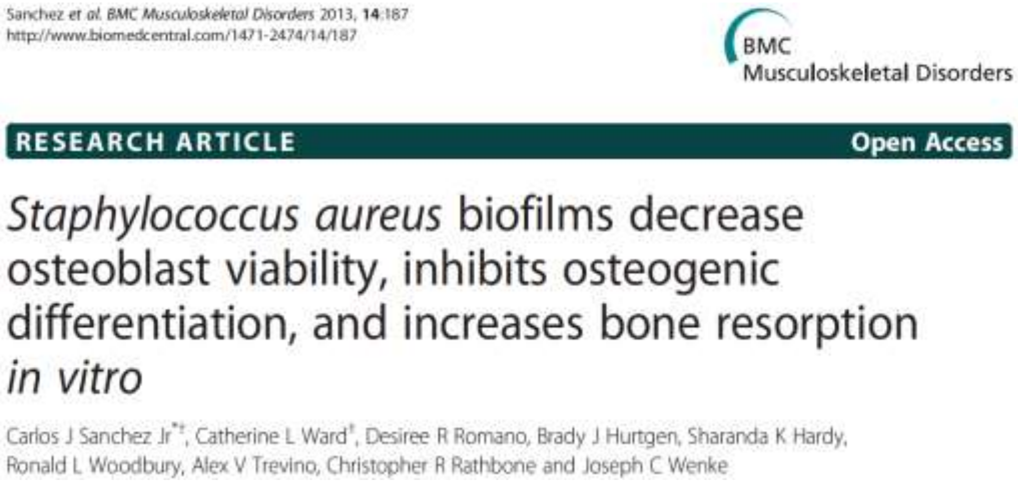
Viabilité OB

Apoptose OB
(Cytométrie)

Quantité d'ADN OB

Apoptose OB
(quantification)

Formation de biofilm et physiologie osseuse



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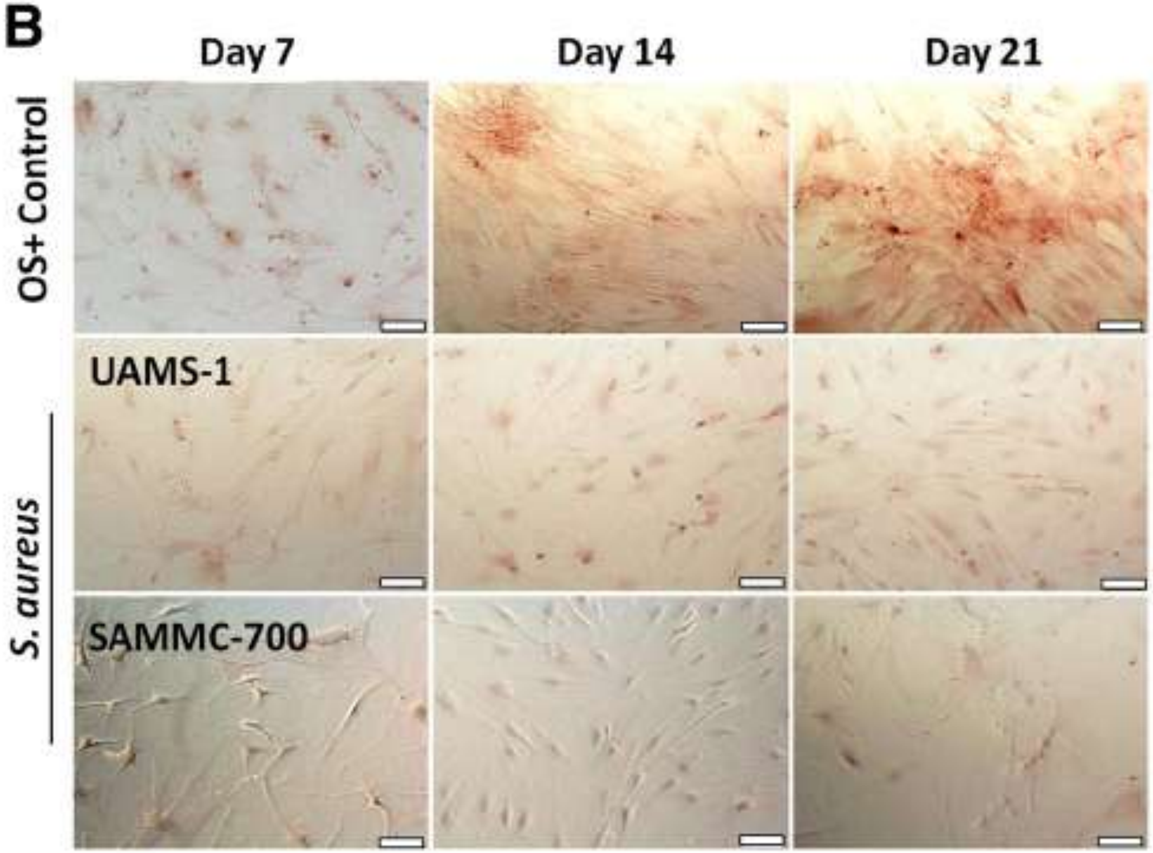
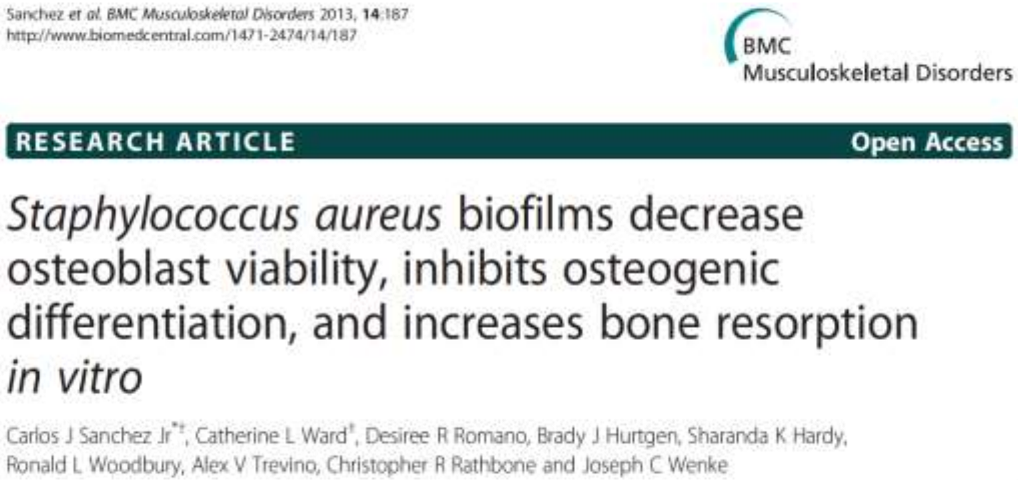
Minéralisation ossuese

+ expression PAL + Osteocalcine



Formation de biofilm et physiologie osseuse



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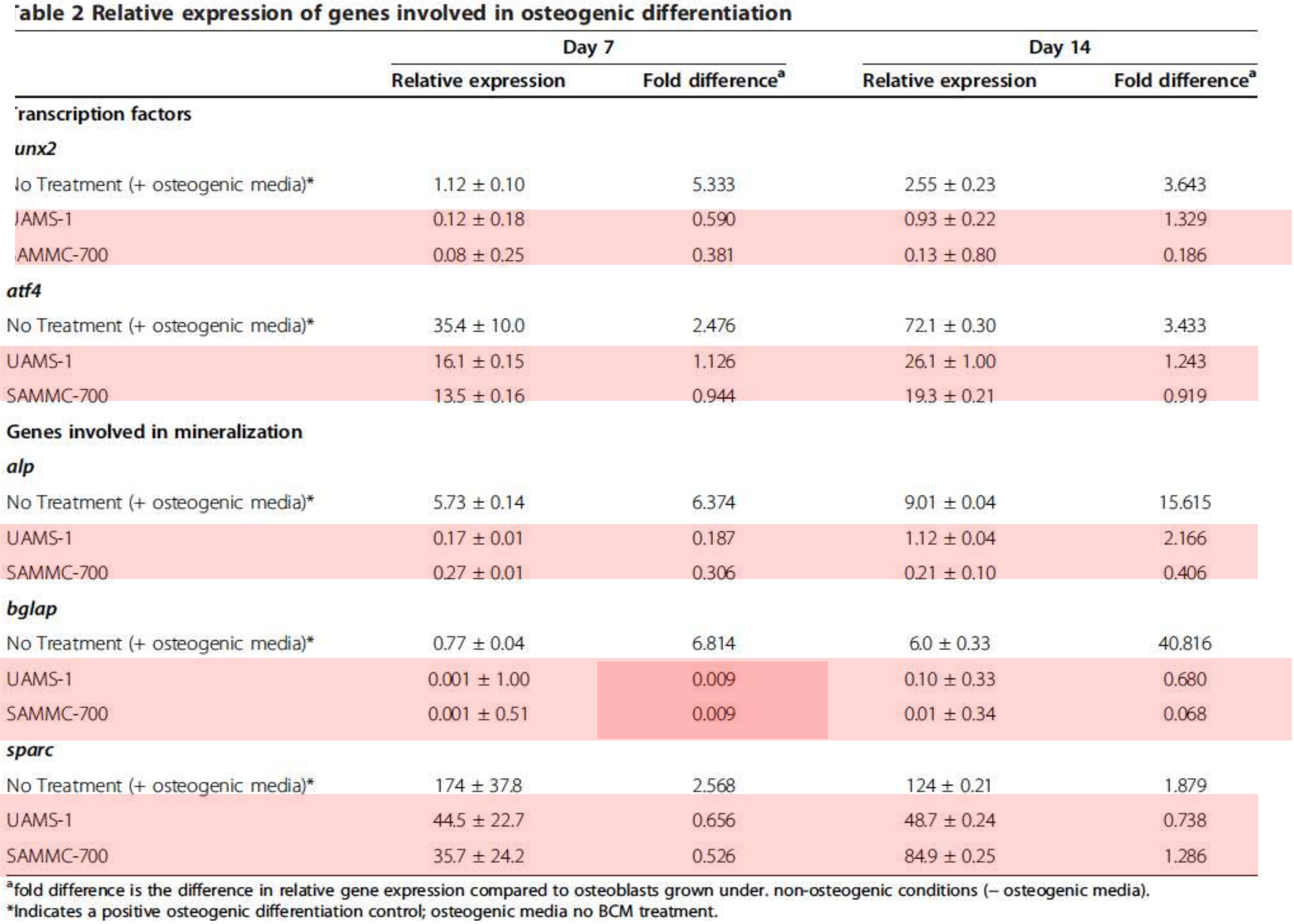
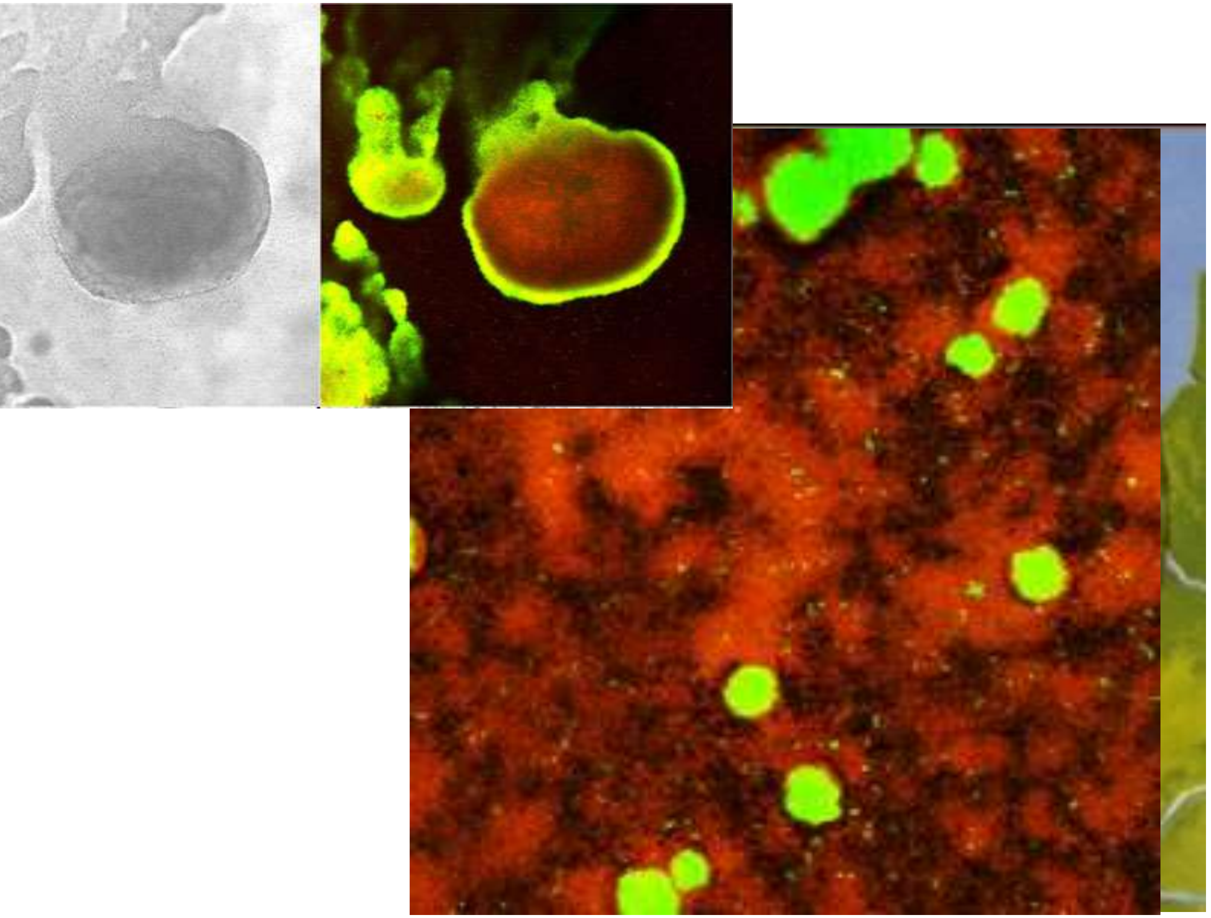


able 2 Relative expression of genes involved in osteogenic differentiation

	Day 7		Day 14	
	Relative expression	Fold difference[a]	Relative expression	Fold difference[a]
ranscription factors				
unx2				
lo Treatment (+ osteogenic media)*	1.12 ± 0.10	5.333	2.55 ± 0.23	3.643
JAMS-1	0.12 ± 0.18	0.590	0.93 ± 0.22	1.329
AMMC-700	0.08 ± 0.25	0.381	0.13 ± 0.80	0.186
atf4				
No Treatment (+ osteogenic media)*	35.4 ± 10.0	2.476	72.1 ± 0.30	3.433
UAMS-1	16.1 ± 0.15	1.126	26.1 ± 1.00	1.243
SAMMC-700	13.5 ± 0.16	0.944	19.3 ± 0.21	0.919
Genes involved in mineralization				
alp				
No Treatment (+ osteogenic media)*	5.73 ± 0.14	6.374	9.01 ± 0.04	15.615
UAMS-1	0.17 ± 0.01	0.187	1.12 ± 0.04	2.166
SAMMC-700	0.27 ± 0.01	0.306	0.21 ± 0.10	0.406
bglap				
No Treatment (+ osteogenic media)*	0.77 ± 0.04	6.814	6.0 ± 0.33	40.816
UAMS-1	0.001 ± 1.00	0.009	0.10 ± 0.33	0.680
SAMMC-700	0.001 ± 0.51	0.009	0.01 ± 0.34	0.068
sparc				
No Treatment (+ osteogenic media)*	174 ± 37.8	2.568	124 ± 0.21	1.879
UAMS-1	44.5 ± 22.7	0.656	48.7 ± 0.24	0.738
SAMMC-700	35.7 ± 24.2	0.526	84.9 ± 0.25	1.286

[a] fold difference is the difference in relative gene expression compared to osteoblasts grown under. non-osteogenic conditions (– osteogenic media).
*Indicates a positive osteogenic differentiation control; osteogenic media no BCM treatment.

Formation de biofilm et antibiotiques



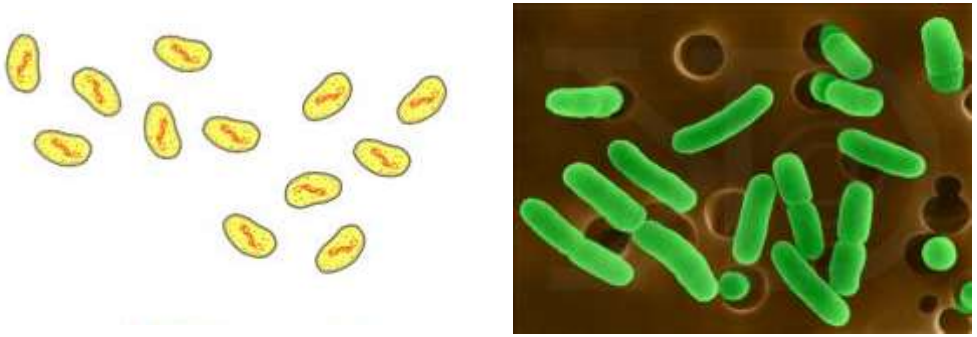
Streptococcus mutans biofilm sur matériel
+ ATB / Baclight
en rouge = bactéries mortes
En vert = bactéries vivantes

CHIC!CHIC!CHIC!
DES Antibiotiques

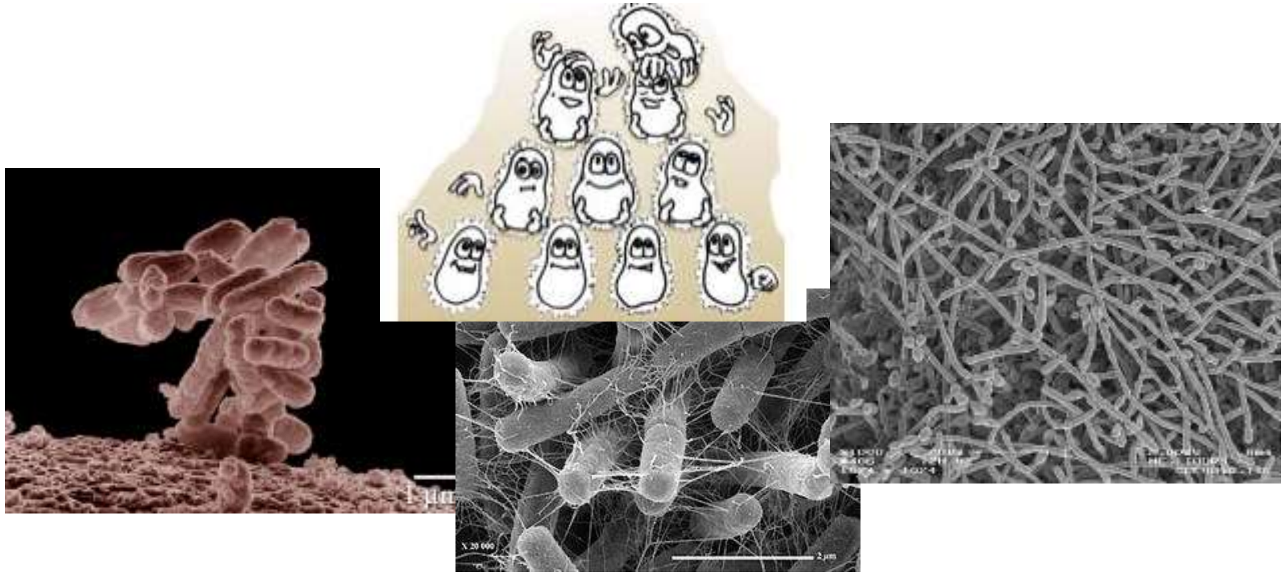
microcolonies biofilm = le village gaulois
antibiotiques = les romains

Formation de biofilm et antibiotiques

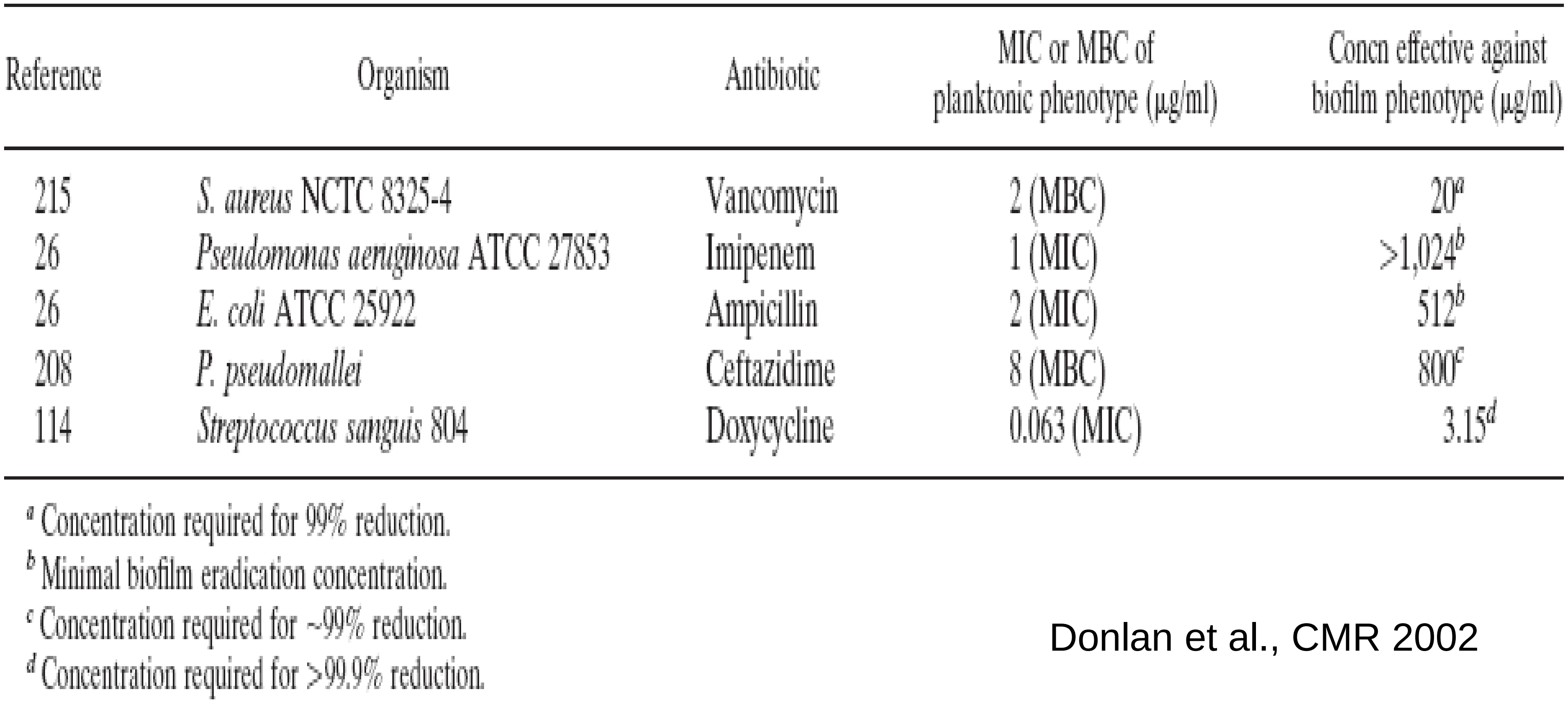
Nous testons :
CMI



Le patient a :
CMEB



Biofilm et antibiotiques

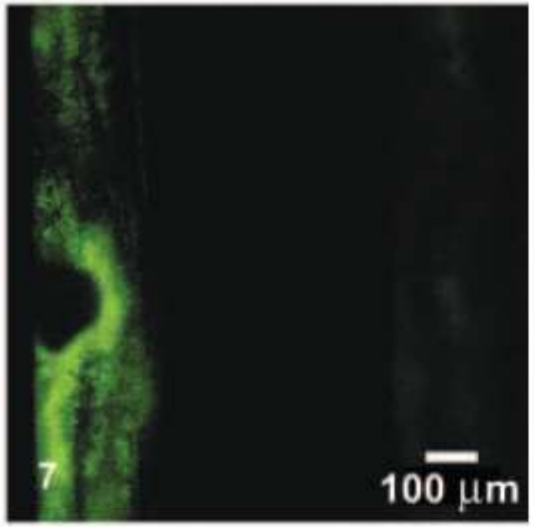


Reference	Organism	Antibiotic	MIC or MBC of planktonic phenotype (μg/ml)	Concn effective against biofilm phenotype (μg/ml)
215	*S. aureus* NCTC 8325-4	Vancomycin	2 (MBC)	20[a]
26	*Pseudomonas aeruginosa* ATCC 27853	Imipenem	1 (MIC)	>1,024[b]
26	*E. coli* ATCC 25922	Ampicillin	2 (MIC)	512[b]
208	*P. pseudomallei*	Ceftazidime	8 (MBC)	800[c]
114	*Streptococcus sanguis* 804	Doxycycline	0.063 (MIC)	3.15[d]

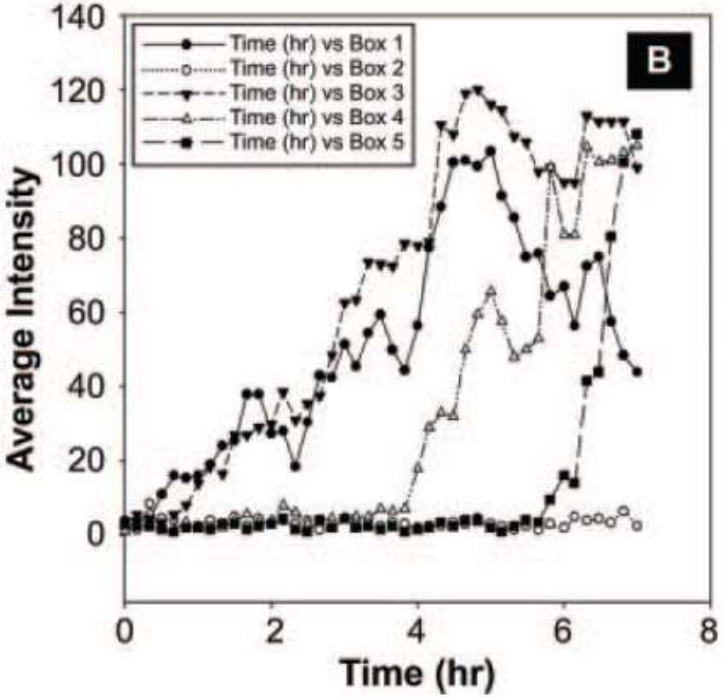
[a] Concentration required for 99% reduction.
[b] Minimal biofilm eradication concentration.
[c] Concentration required for ~99% reduction.
[d] Concentration required for >99.9% reduction.

Donlan et al., CMR 2002

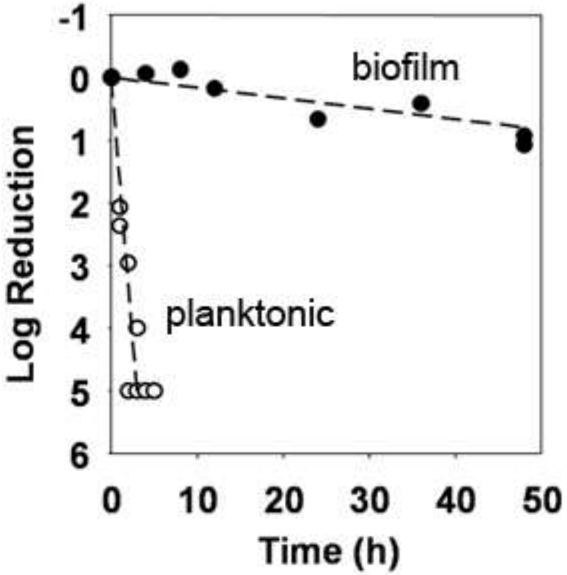
Formation de biofilm et antibiotiques



**Biofilm *S. aureus* GFP
GFP sous contrôle promoteur
Tétracycline dependant**



**Cinétique de fluorescence
(induction GFP sous promoteur *tet*)
après ajout de tétéracycline
en différents points du biofilm**

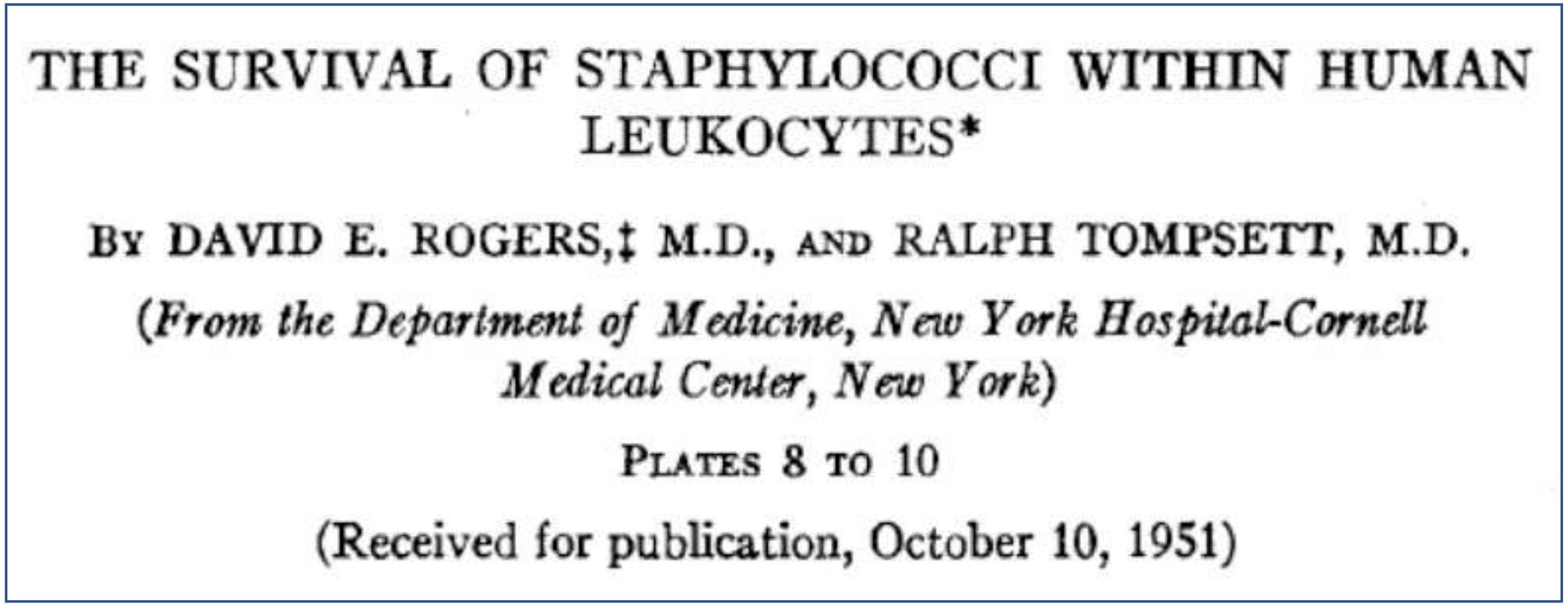


**Cinétique d'activité
de la Rifampicine
sur *S. epidermidis***

Interactions avec les cellules osseuses

Interactions avec les cellules osseuses

Van de Velde H.
Etude sur le mécanisme de la virulence du staphylocoque pyogène.
La Cellule **1894;10**:403-60.



THE SURVIVAL OF STAPHYLOCOCCI WITHIN HUMAN LEUKOCYTES*

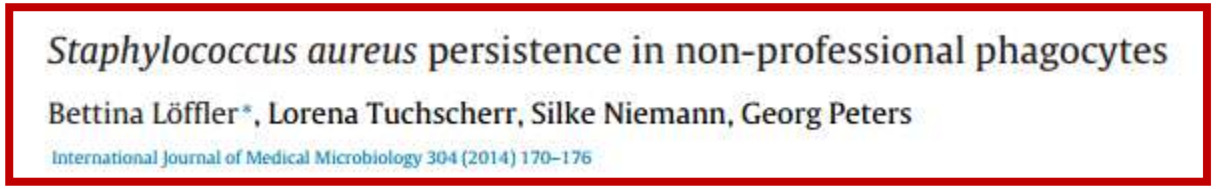
BY DAVID E. ROGERS,‡ M.D., AND RALPH TOMPSETT, M.D.

(From the Department of Medicine, New York Hospital-Cornell Medical Center, New York)

PLATES 8 TO 10

(Received for publication, October 10, 1951)

Interactions avec les cellules osseuses



Staphylococcus aureus persistence in non-professional phagocytes

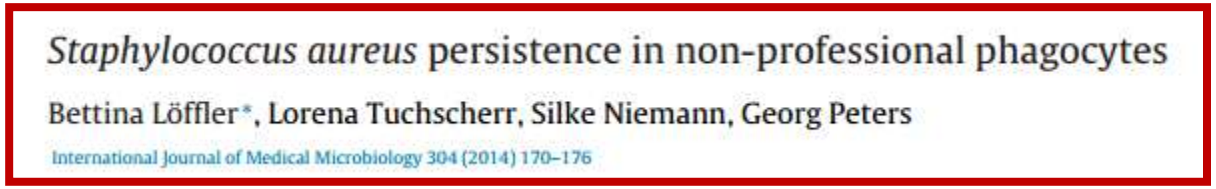
Bettina Löffler*, Lorena Tuchscherr, Silke Niemann, Georg Peters

International Journal of Medical Microbiology 304 (2014) 170–176

Décrit avec de nombreux types cellulaires

- Cellules endothéliales
- Cellules épithéliales
- Kératinocytes
- Fibroblastes
- Leucocytes

Interactions avec les cellules osseuses


Staphylococcus aureus persistence in non-professional phagocytes

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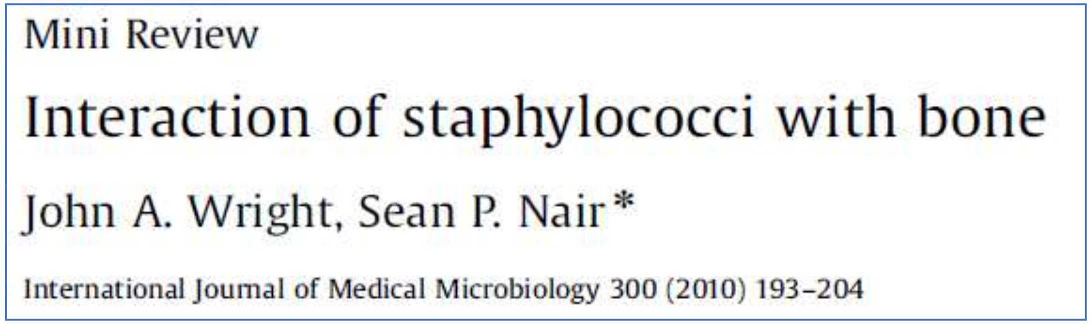
- Fibroblastes
- Leucocytes
- **…. et les cellules osseuses !**


Mini Review

Interaction of staphylococci with bone

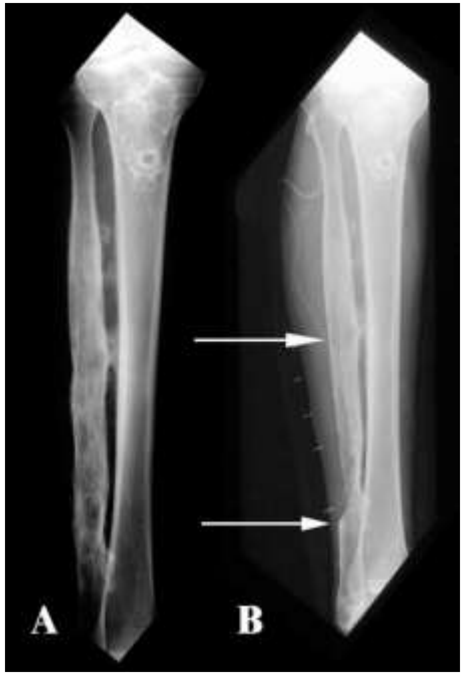
John A. Wright, Sean P. Nair*

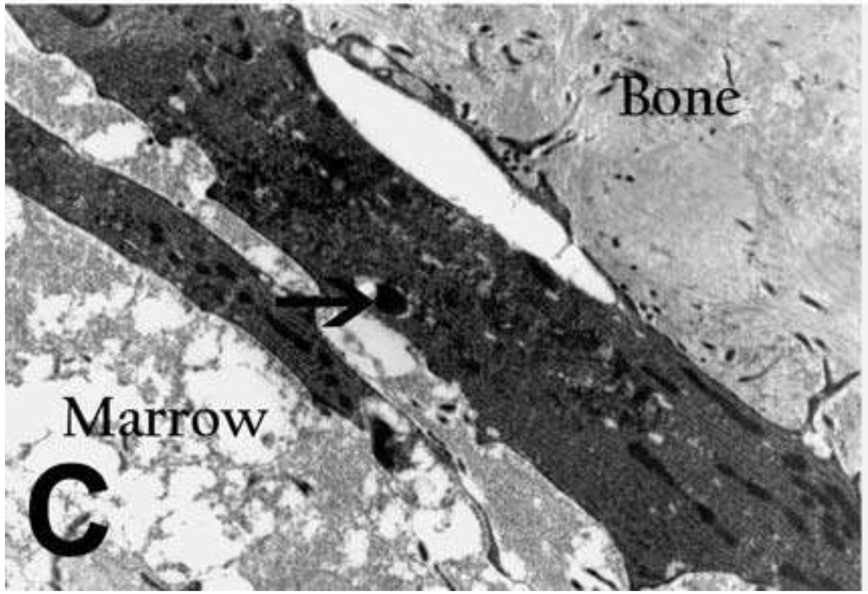
International Journal of Medical Microbiology 300 (2010) 193–204

Bosse, 2005

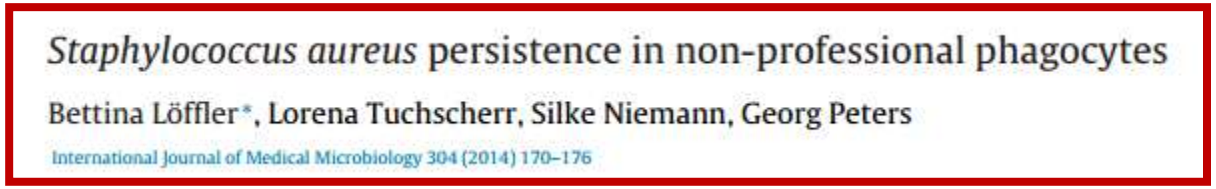
Ostéite chronique

ME





Interactions avec les cellules osseuses



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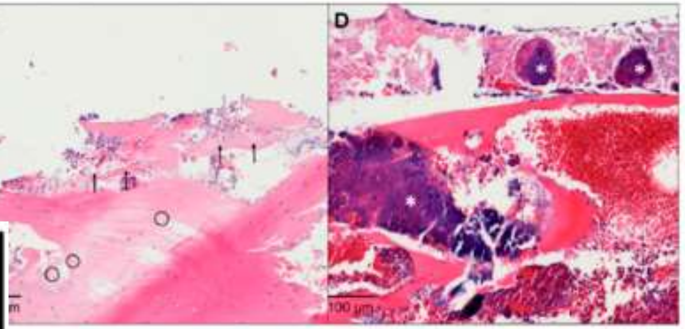
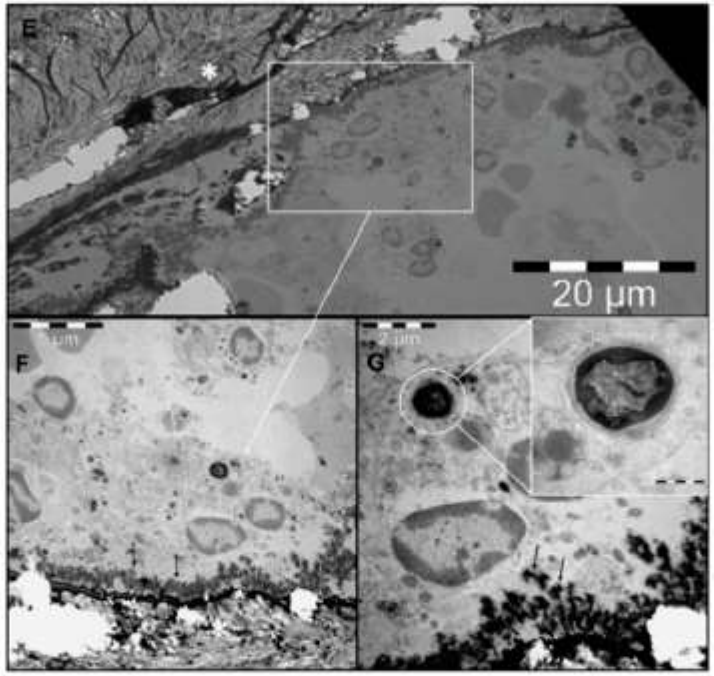
- **…. et les cellules osseuses !**

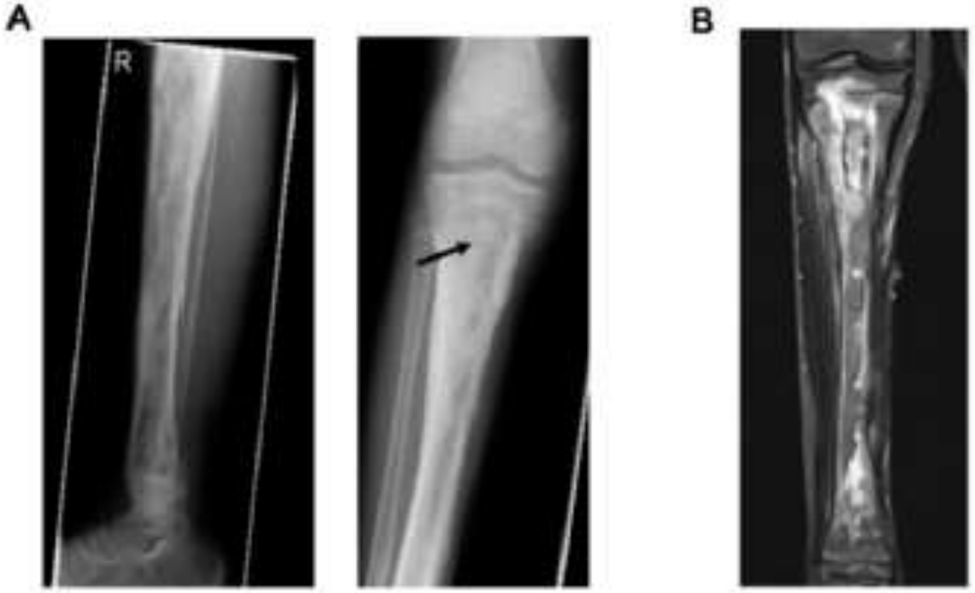
Walter, 2021

Ostéite chronique

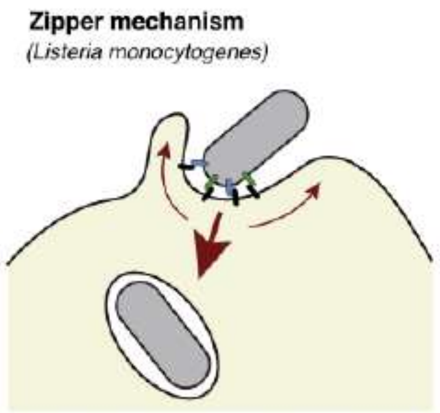
ME



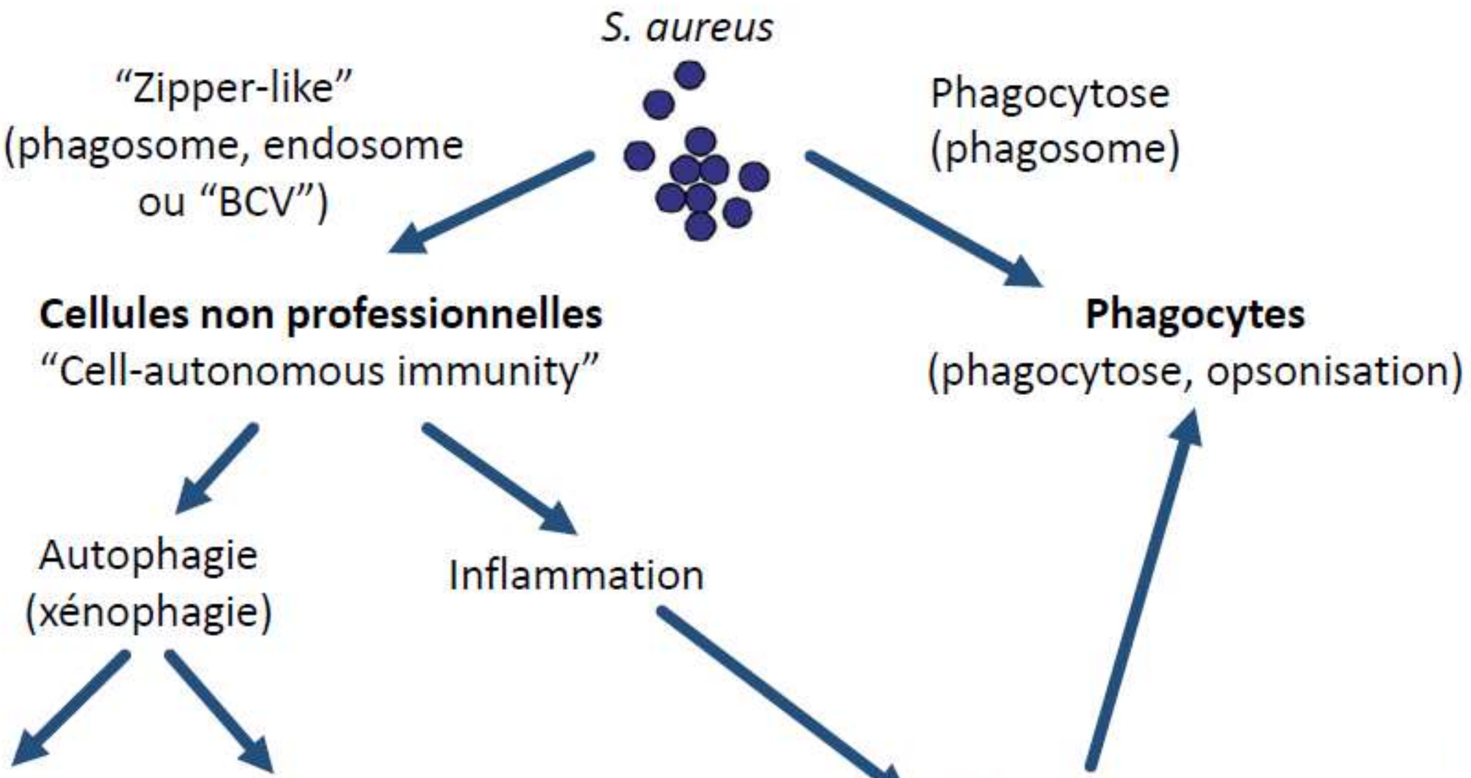




Interactions avec les cellules osseuses


Zipper mechanism
(Listeria monocytogenes)

*BCV : “bacteria-containing vesicle”



S. aureus

“Zipper-like”
(phagosome, endosome
ou “BCV”)

Phagocytose
(phagosome)

Cellules non professionnelles
“Cell-autonomous immunity”

Phagocytes
(phagocytose, opsonisation)

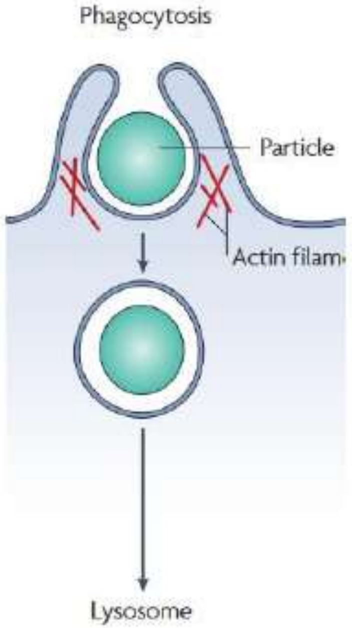
Autophagie
(xénophagie)

Inflammation

Elimination de *S. aureus*
(survie de la cellule hôte)

Réplication intracellulaire
(mort cellulaire)

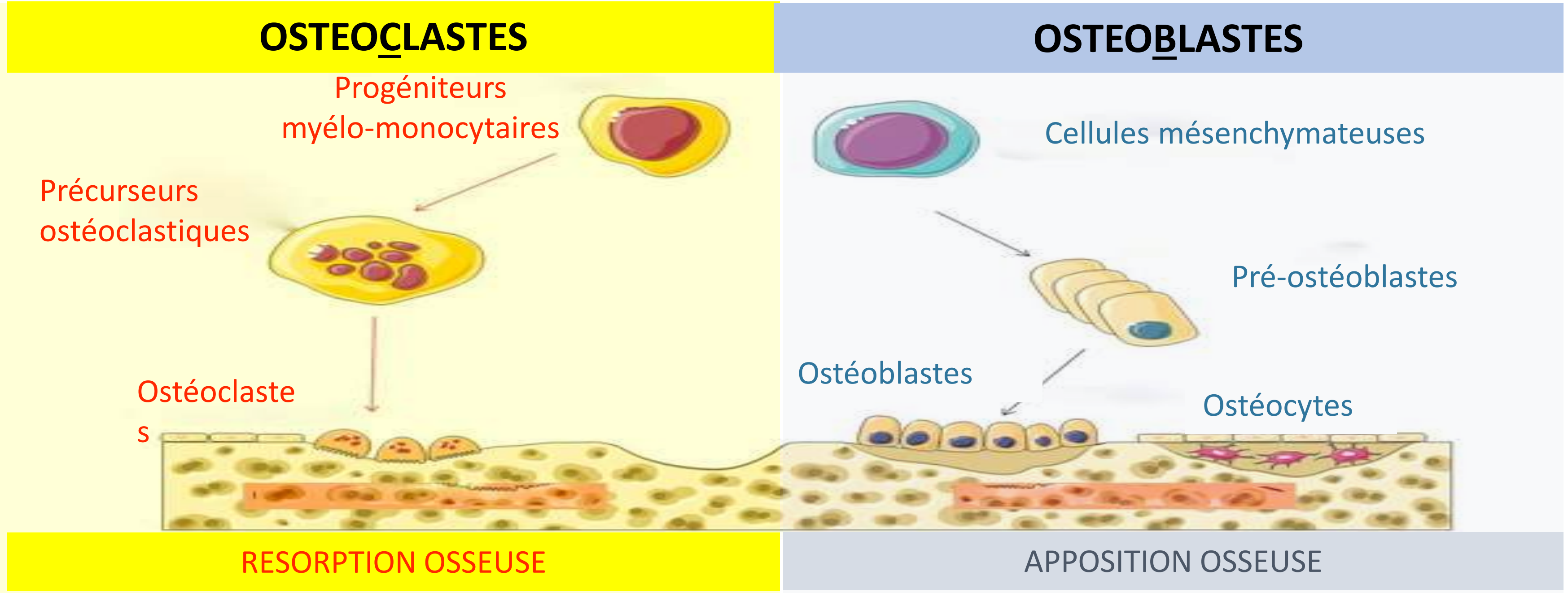
Chimiotactisme


Phagocytosis
Particle
Actin filam
Lysosome

Courtesy of Paul Verhoeven

Ribet, Microbes Infect 2025

Interactions avec les cellules osseuses



OSTEOCLASTES

Progéniteurs myélo-monocytaires

Précurseurs ostéoclastiques

Ostéoclastes

RESORPTION OSSEUSE

OSTEOBLASTES

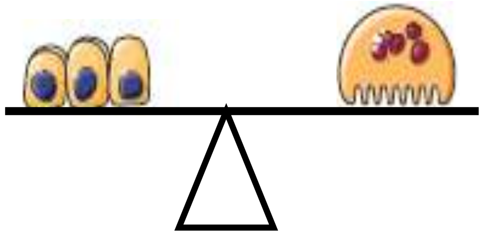
Cellules mésenchymateuses

Pré-ostéoblastes

Ostéoblastes

Ostéocytes

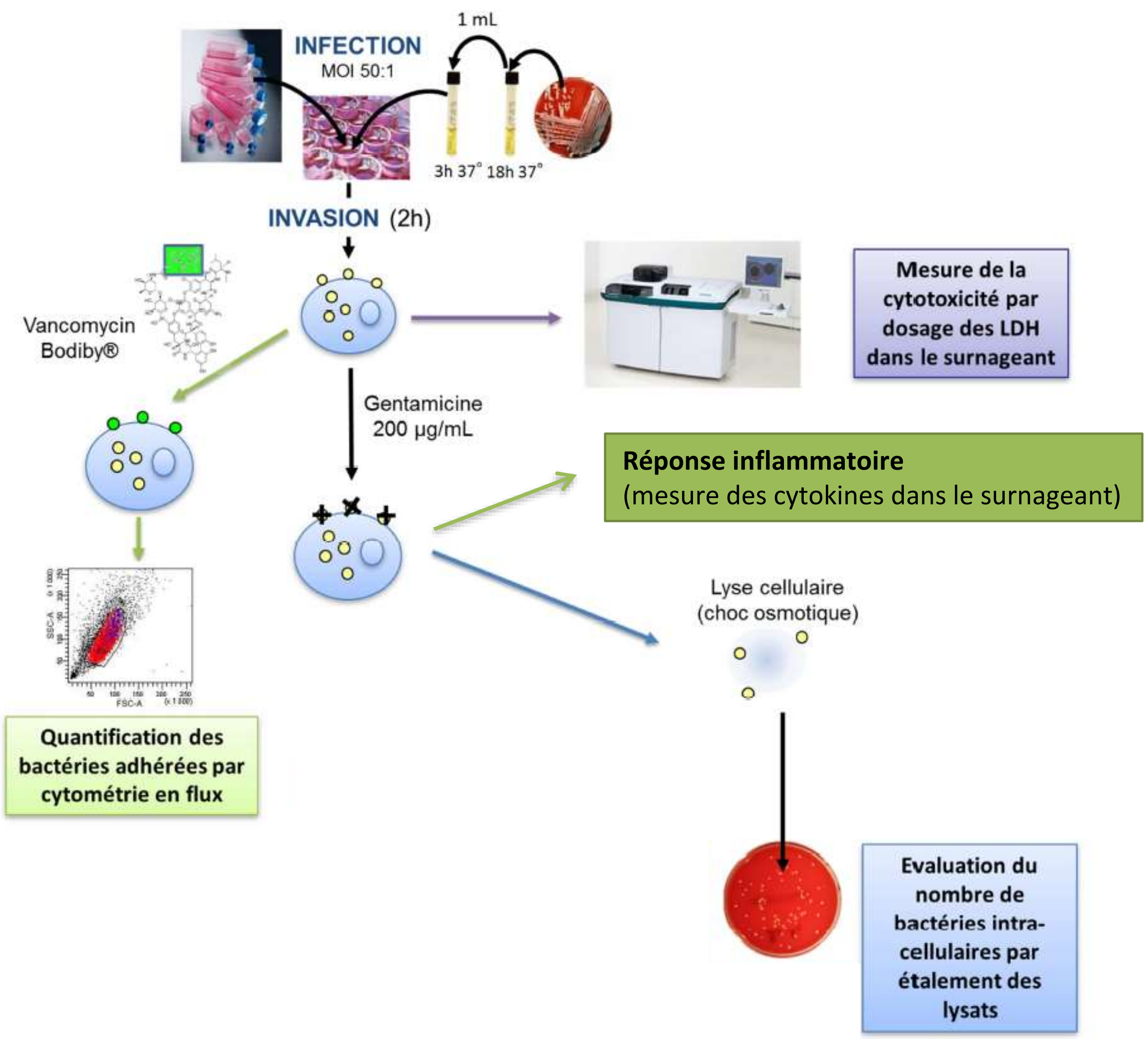
APPOSITION OSSEUSE



Os sain

Equilibre OB / OC

Interactions avec les cellules osseuses : ostéoBlastes

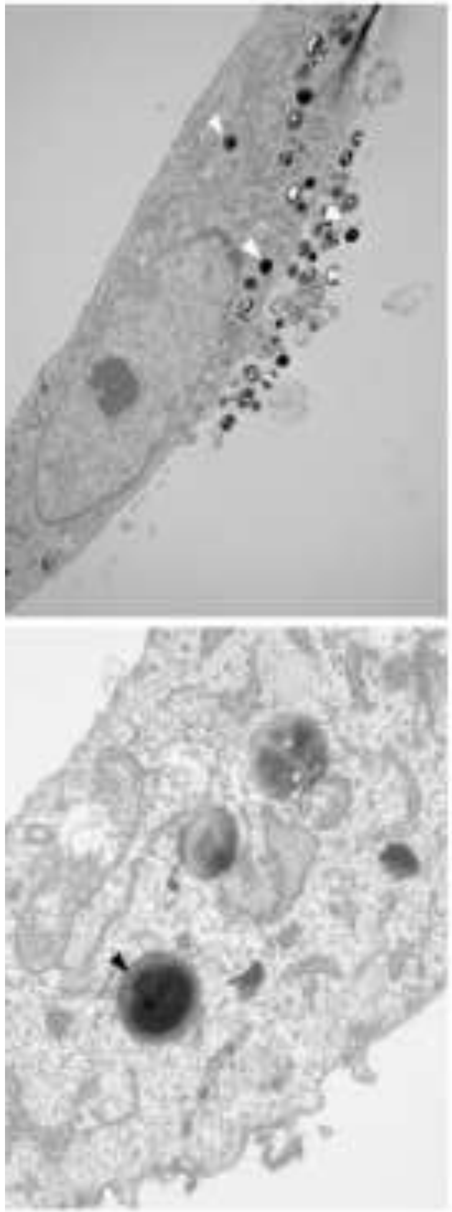

INFECTION
MOI 50:1
1 mL
3h 37° 18h 37°
INVASION (2h)
Vancomycin
Bodiby®
Gentamicine
200 µg/mL
SSC-A
FSC-A
Lyse cellulaire
(choc osmotique)

Mesure de la cytotoxicité par dosage des LDH dans le surnageant

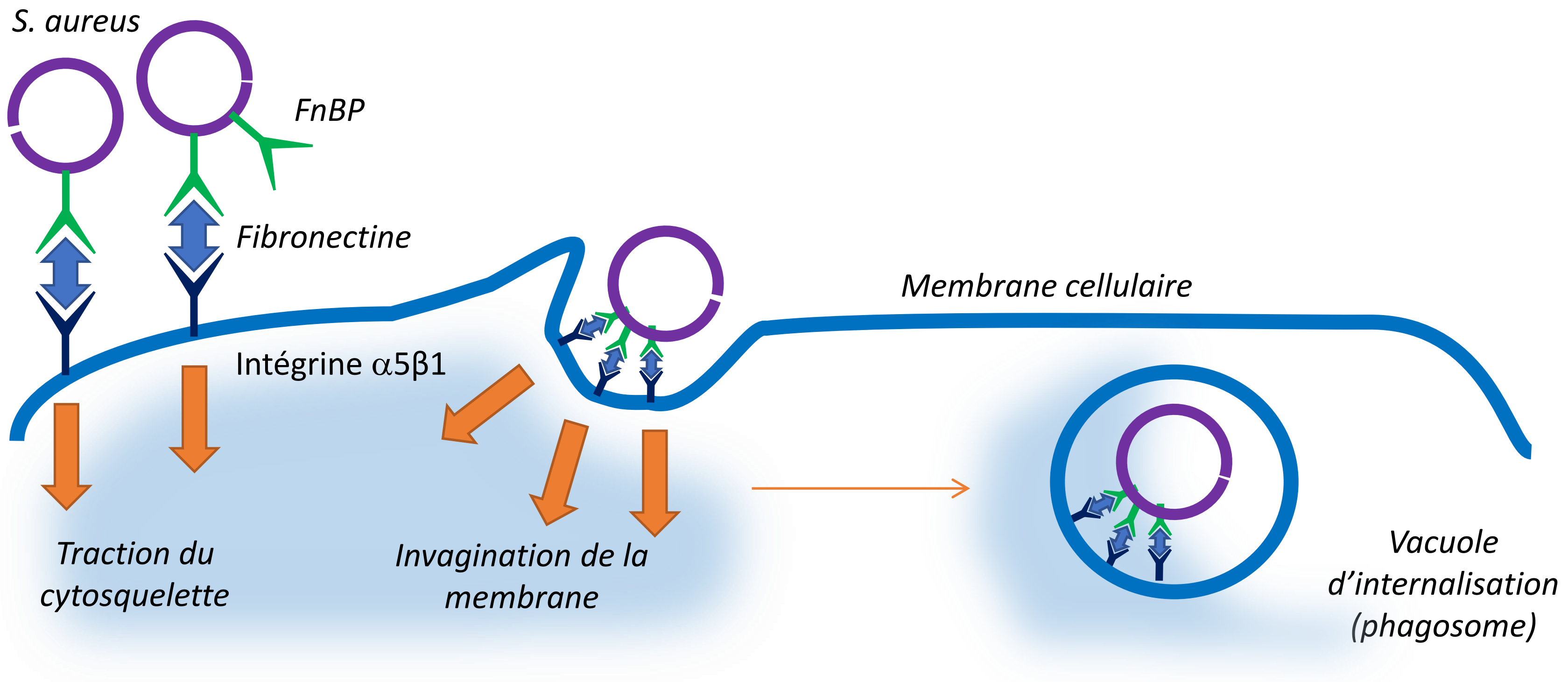
Réponse inflammatoire
(mesure des cytokines dans le surnageant)

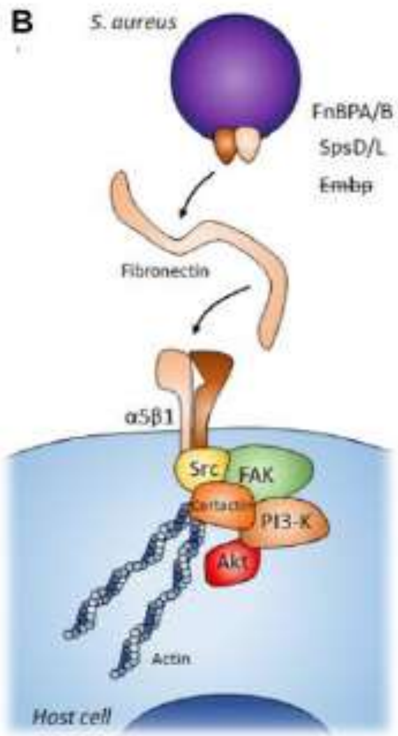
Quantification des bactéries adhérées par cytométrie en flux

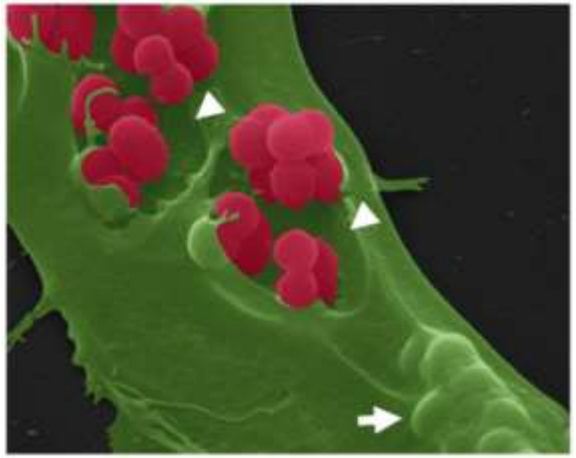
Evaluation du nombre de bactéries intra-cellulaires par étalement des lysats



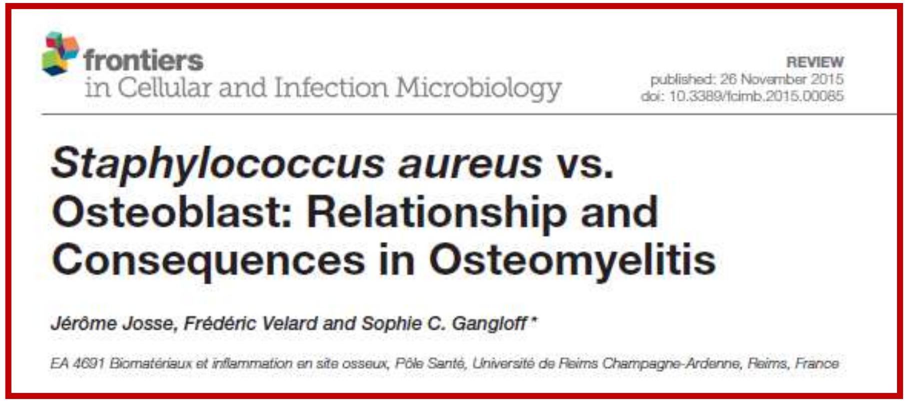
Interactions avec les cellules osseuses : ostéoBlastes


S. aureus
FnBP
Fibronectine
Membrane cellulaire
Intégrine α5β1
Traction du cytosquelette
Invagination de la membrane
Vacuole d'internalisation (phagosome)


B
S. aureus
FnBPA/B
SpsD/L
Embp
Fibronectin
α5β1
Src
FAK
Cortactin
PI3-K
Akt
Actin
Host cell



ME
Hoffmann *et al.*
Eur J Cell Biol 2011



frontiers in Cellular and Infection Microbiology

REVIEW
published: 26 November 2015
doi: 10.3389/fcimb.2015.00085

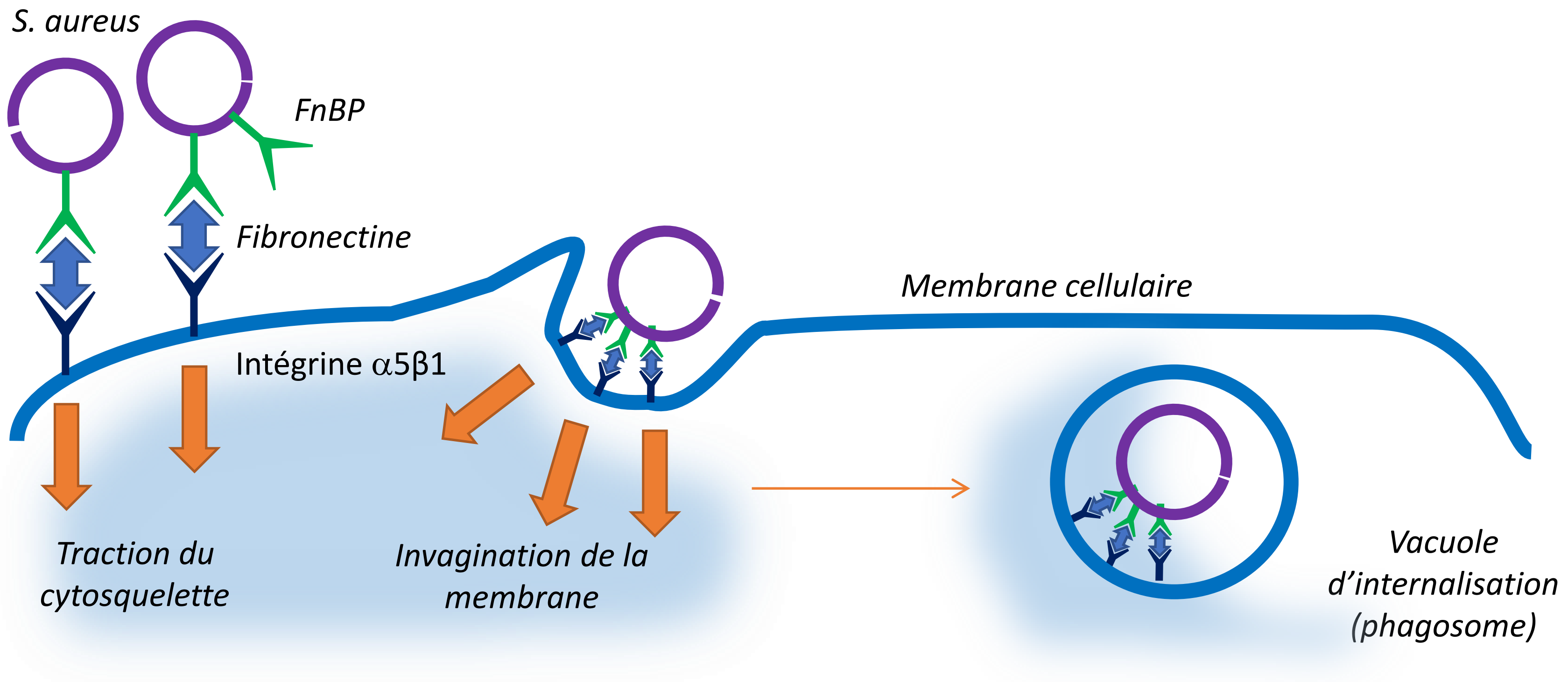
Staphylococcus aureus vs. Osteoblast: Relationship and Consequences in Osteomyelitis

*Jérôme Josse, Frédéric Velard and Sophie C. Gangloff**

EA 4691 Biomatériaux et inflammation en site osseux, Pôle Santé, Université de Reims Champagne-Ardenne, Reims, France

Tribell, Cell Microbiol 2020

Interactions avec les cellules osseuses : ostéoBlastes



S. aureus

FnBP

Fibronectine

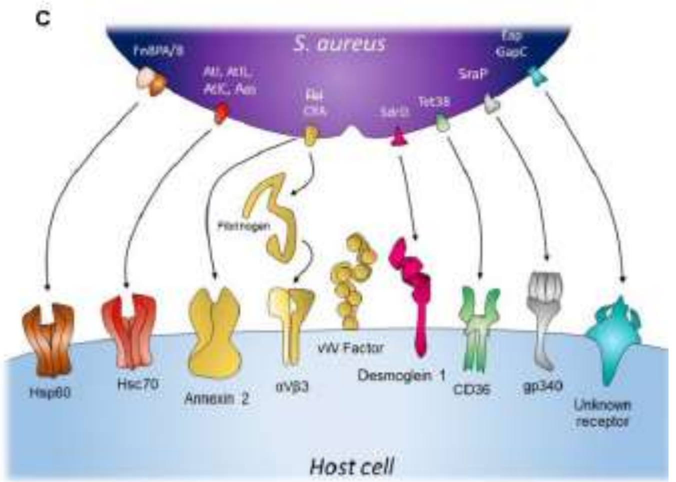
Intégrine $\alpha5\beta1$

Membrane cellulaire

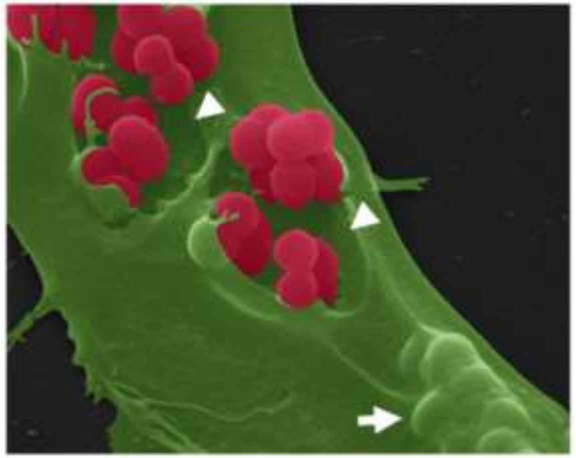
Traction du cytosquelette

Invagination de la membrane

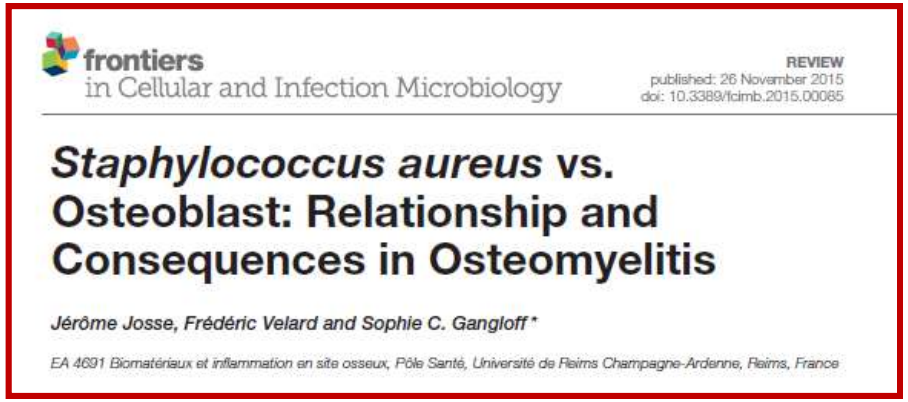
Vacuole d'internalisation (phagosome)


c
S. aureus
FnBPA/B
Atl, AtlL, AtlC, Aas
Fbl ClfA
SdrD
Tet38
SraP
Eap GapC
Fibrinogen
vW Factor
Hsp60
Hsc70
Annexin 2
αVβ3
Desmoglein 1
CD36
gp340
Unknown receptor
Host cell

Voies accessoires



ME
Hoffmann *et al.*
Eur J Cell Biol 2011



frontiers
in Cellular and Infection Microbiology

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Tribell, Cell Microbiol 2020

Interactions avec les cellules osseuses : ostéoBlastes

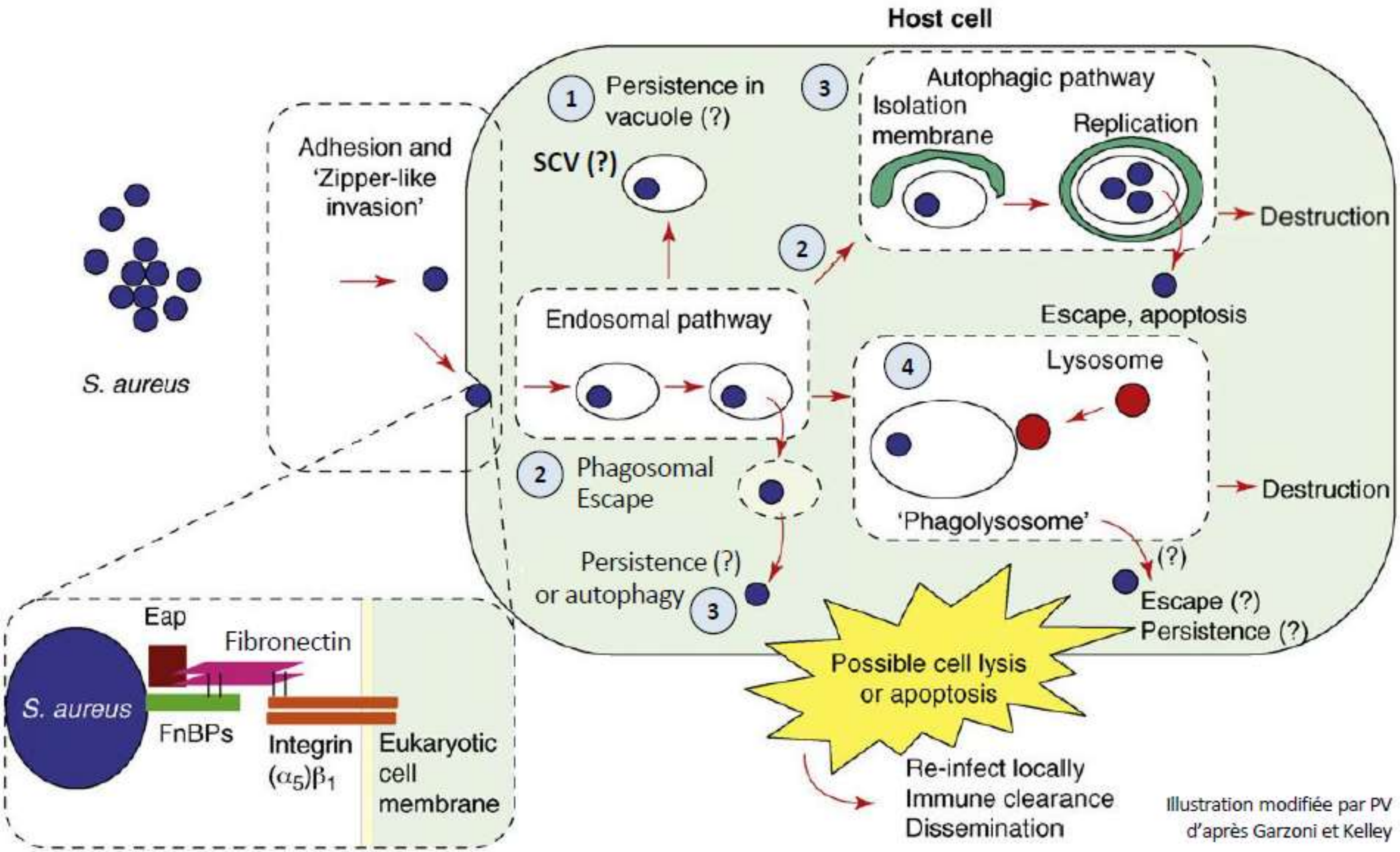
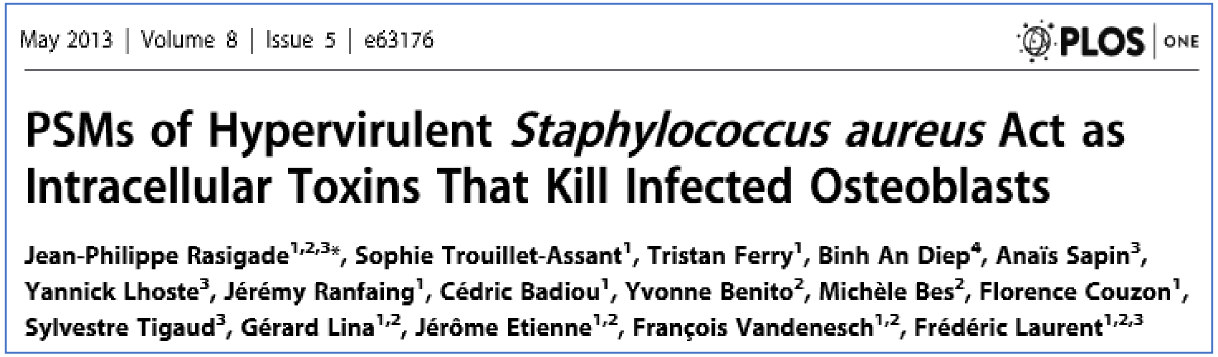

Host cell
S. aureus
Adhesion and
'Zipper-like
invasion'
1
Persistence in
vacuole (?)
SCV (?)
3
Autophagic pathway
Isolation
membrane
Replication
Destruction
2
Escape, apoptosis
Endosomal pathway
4
Lysosome
'Phagolysosome'
Destruction
2
Phagosomal
Escape
Persistence (?)
or autophagy
3
(?)
Escape (?)
Persistence (?)
Possible cell lysis
or apoptosis
Re-infect locally
Immune clearance
Dissemination
Eap
Fibronectin
S. aureus
FnBPs
Integrin
$(\alpha_5)\beta_1$
Eukaryotic
cell
membrane

Illustration modifiée par PV
d'après Garzoni et Kelley

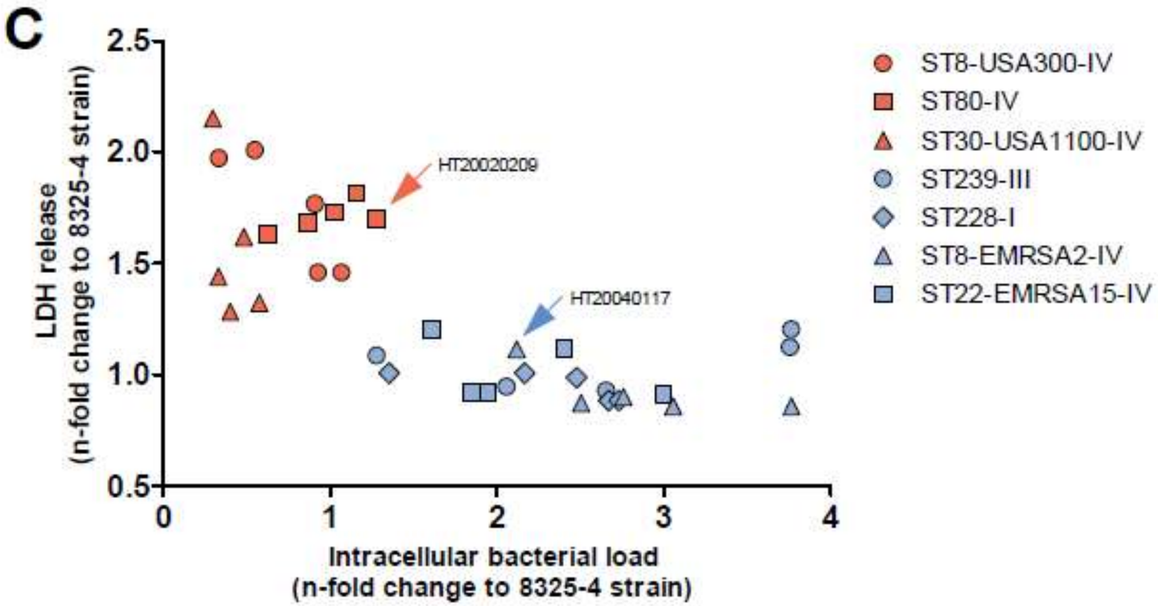
Interactions avec les cellules osseuses : ostéoBlastes



May 2013 | Volume 8 | Issue 5 | e63176 PLOS ONE

PSMs of Hypervirulent *Staphylococcus aureus* Act as Intracellular Toxins That Kill Infected Osteoblasts

Jean-Philippe Rasigade[1,2,3]*, Sophie Trouillet-Assant[1], Tristan Ferry[1], Binh An Diep[4], Anaïs Sapin[3], Yannick Lhoste[3], Jérémy Ranfaing[1], Cédric Badiou[1], Yvonne Benito[2], Michèle Bes[2], Florence Couzon[1], Sylvestre Tigaud[3], Gérard Lina[1,2], Jérôme Etienne[1,2], François Vandenesch[1,2], Frédéric Laurent[1,2,3]



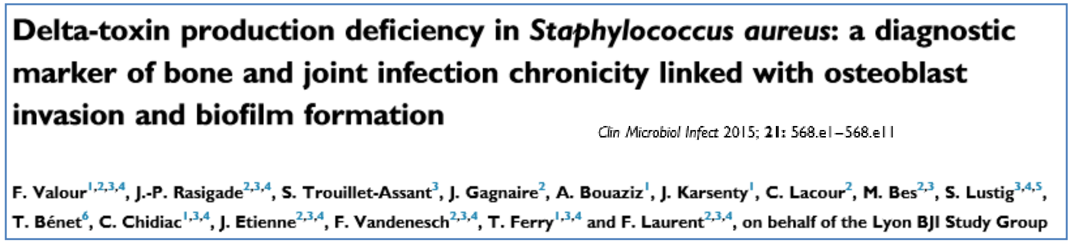
HA-MRSA (n=15)

- Infections post-opératoires
- Sur matériel
- Indolentes : chronicisation, récidive
- **Faible cytotoxicité**
- **Survie intracellulaire +++**

CA-MRSA (n=20)

- Ostéomyélites (enfants) +++
- Progression rapide, inflammation majeure, destruction osseuse, dommages tissulaires
- **Cytotoxicité +++**
- **Faible survie intracellulaire**
- Dommages tissulaires (rôle de la PVL)

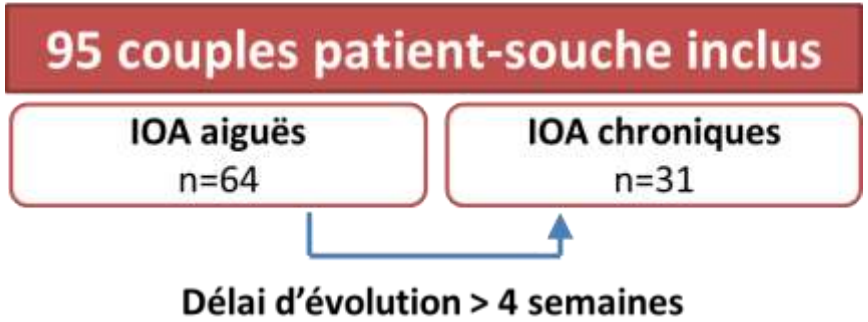
Interactions avec les cellules osseuses : ostéoBlastes



Delta-toxin production deficiency in *Staphylococcus aureus*: a diagnostic marker of bone and joint infection chronicity linked with osteoblast invasion and biofilm formation

Clin Microbiol Infect 2015; **21:** 568.e1–568.e11

F. Valour[1,2,3,4], J.-P. Rasigade[2,3,4], S. Trouillet-Assant[3], J. Gagnaire[2], A. Bouaziz[1], J. Karsenty[1], C. Lacour[2], M. Bes[2,3], S. Lustig[3,4,5], T. Bénet[6], C. Chidiac[1,3,4], J. Etienne[2,3,4], F. Vandenesch[2,3,4], T. Ferry[1,3,4] and F. Laurent[2,3,4], on behalf of the Lyon BJI Study Group

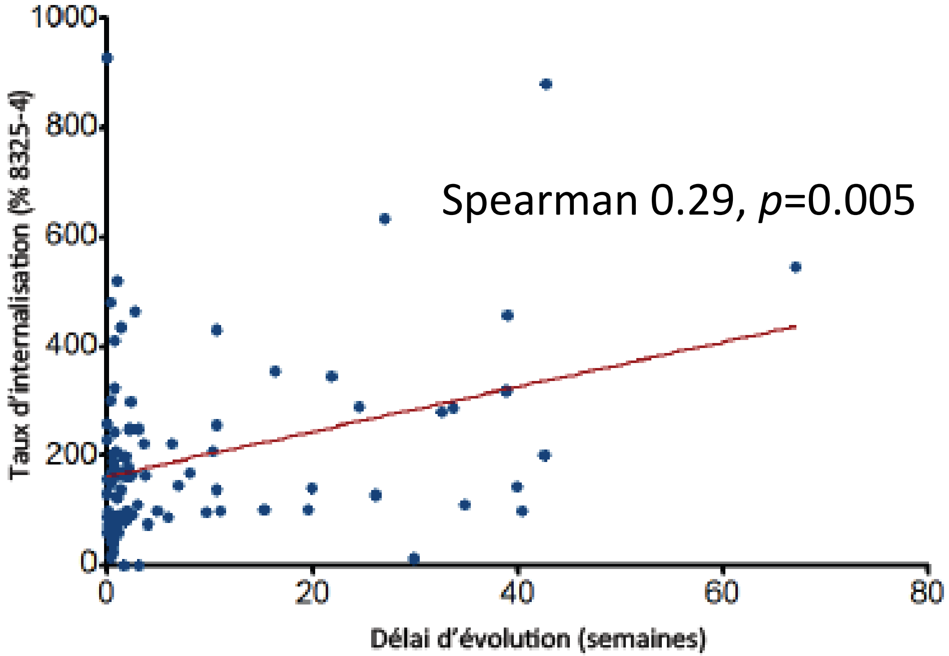


95 couples patient-souche inclus

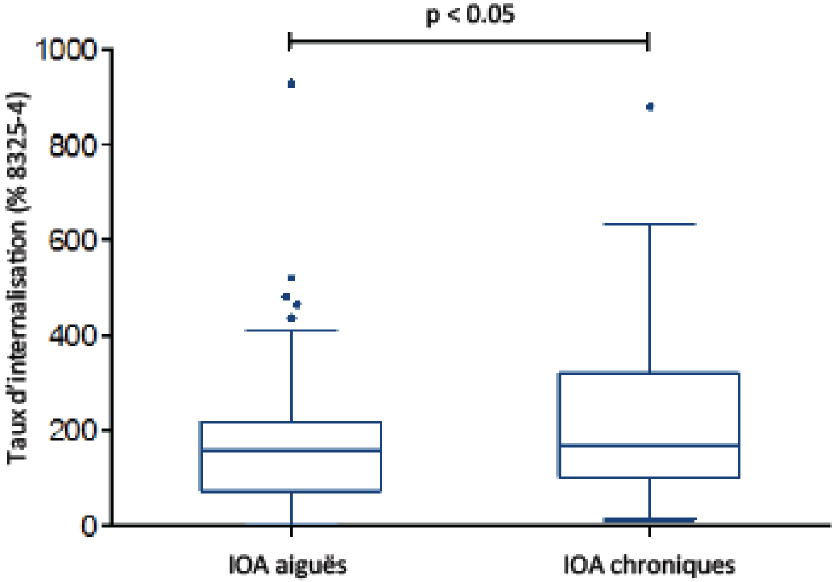
IOA aiguës
n=64

IOA chroniques
n=31

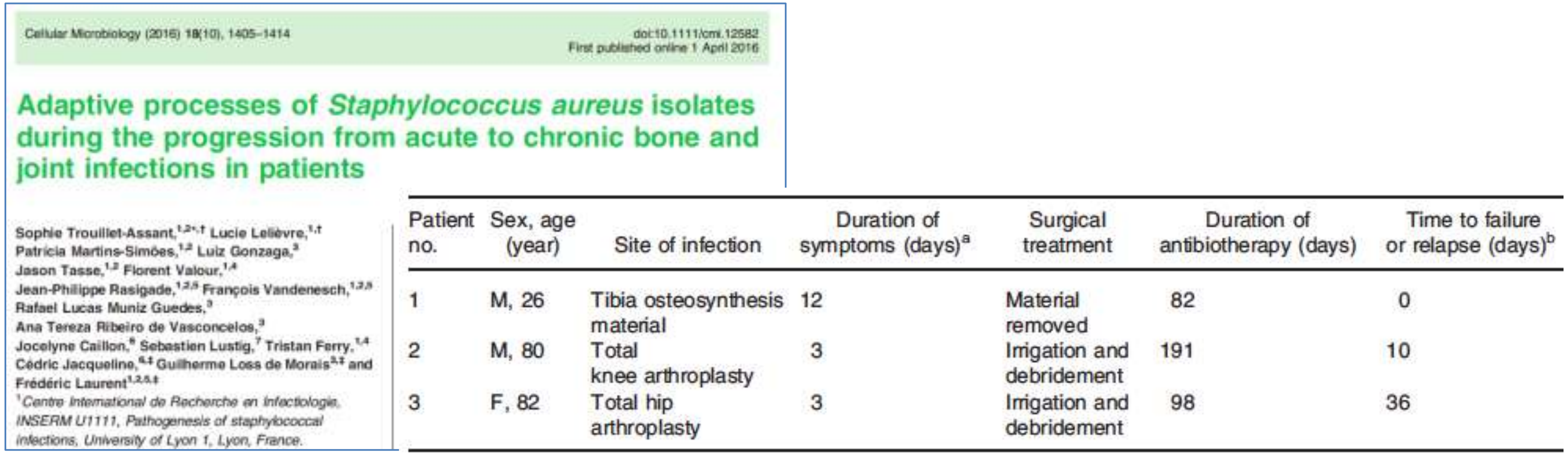
Délai d'évolution > 4 semaines



Spearman 0.29, *p*=0.005



Interactions avec les cellules osseuses : ostéoBlastes

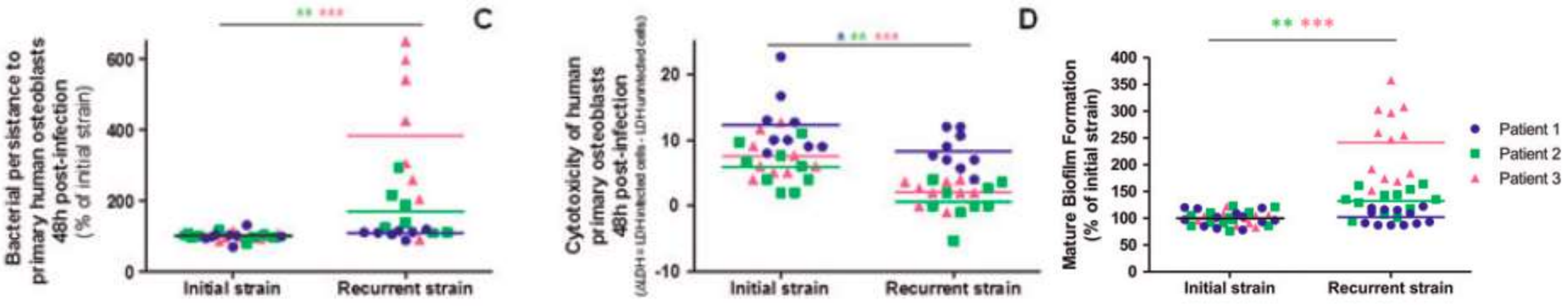


Cellular Microbiology (2016) 18(10), 1405–1414
doi:10.1111/cmi.12582
First published online 1 April 2016

Adaptive processes of *Staphylococcus aureus* isolates during the progression from acute to chronic bone and joint infections in patients

Sophie Trouillet-Assant,[1,2*,†] Lucie Lelièvre,[1,†]
Patricia Martins-Simões,[1,2] Luiz Gonzaga,[3]
Jason Tasse,[1,2] Florent Valour,[1,4]
Jean-Philippe Rasigade,[1,2,5] François Vandenesch,[1,2,5]
Rafael Lucas Muniz Guedes,[3]
Ana Tereza Ribeiro de Vasconcelos,[3]
Jocelyne Caillon,[6] Sebastien Lustig,[7] Tristan Ferry,[1,4]
Cédric Jacqueline,[6,‡] Guilherme Loss de Morais[3,‡] and
Frédéric Laurent[1,2,5,‡]
[1]*Centre International de Recherche en Infectiologie, INSERM U1111, Pathogenesis of staphylococcal infections, University of Lyon 1, Lyon, France.*

Patient no.	Sex, age (year)	Site of infection	Duration of symptoms (days)[a]	Surgical treatment	Duration of antibiotherapy (days)	Time to failure or relapse (days)[b]
1	M, 26	Tibia osteosynthesis material	12	Material removed	82	0
2	M, 80	Total knee arthroplasty	3	Irrigation and debridement	191	10
3	F, 82	Total hip arthroplasty	3	Irrigation and debridement	98	36



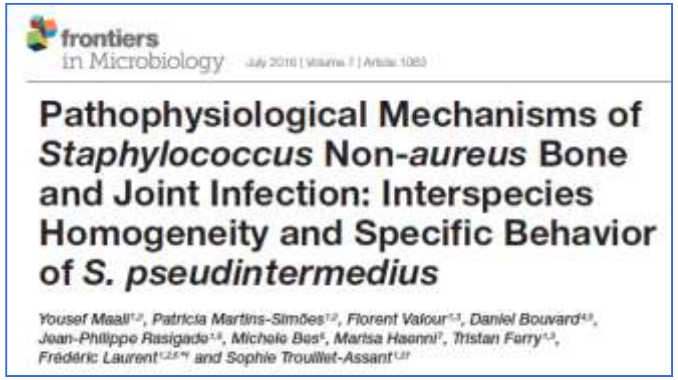
↑ **Persistance**

↓ **Cytotoxicité**

↑ **Biofilm**

PHENOMENES ADAPTATIFS

Interactions avec les cellules osseuses : ostéoBlastes



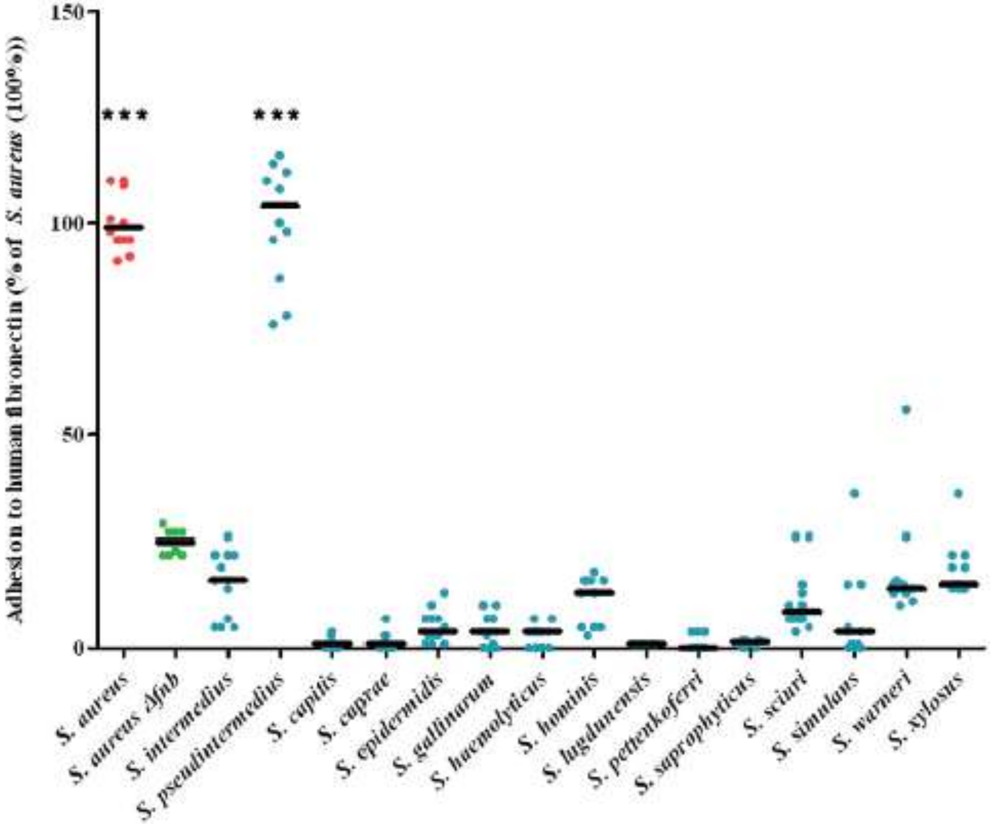
frontiers in Microbiology July 2016 | Volume 7 | Article 1083

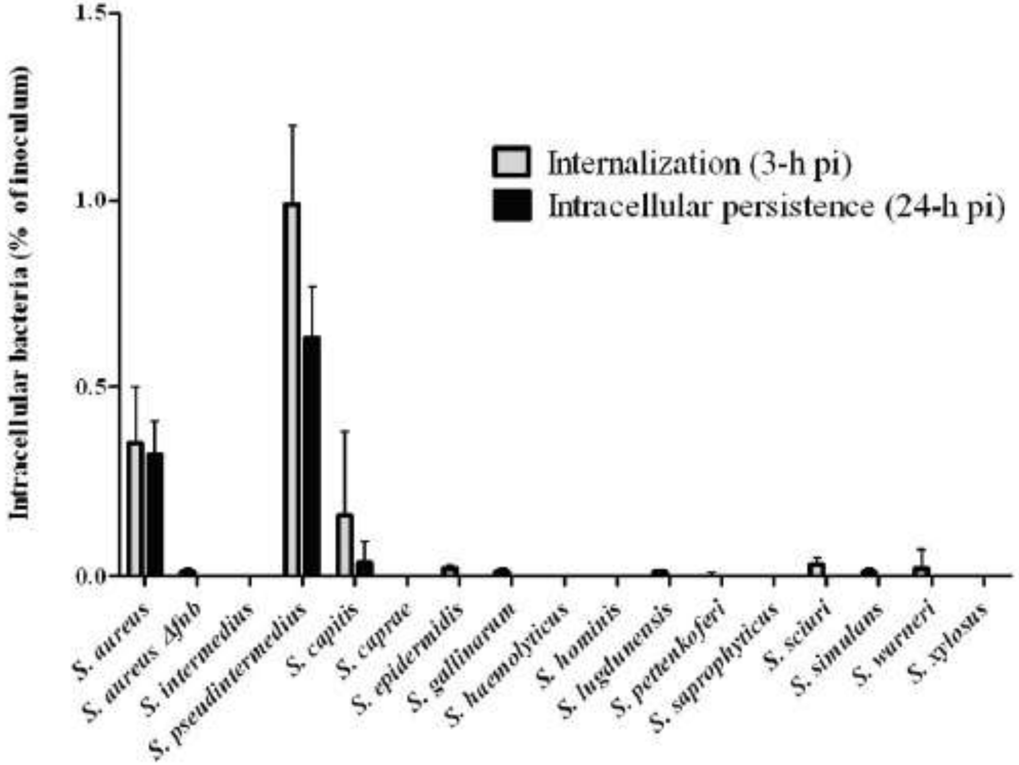
Pathophysiological Mechanisms of *Staphylococcus* Non-*aureus* Bone and Joint Infection: Interspecies Homogeneity and Specific Behavior of *S. pseudintermedius*

Yousef Maali[1,2], Patricia Martins-Simões[1,2], Florent Valour[1,3], Daniel Bouvard[4,5], Jean-Philippe Rasigade[1,6], Michele Bes[6], Marisa Haenni[7], Tristan Ferry[1,3], Frédéric Laurent[1,2,6] and Sophie Trouillet-Assant[1,2]*

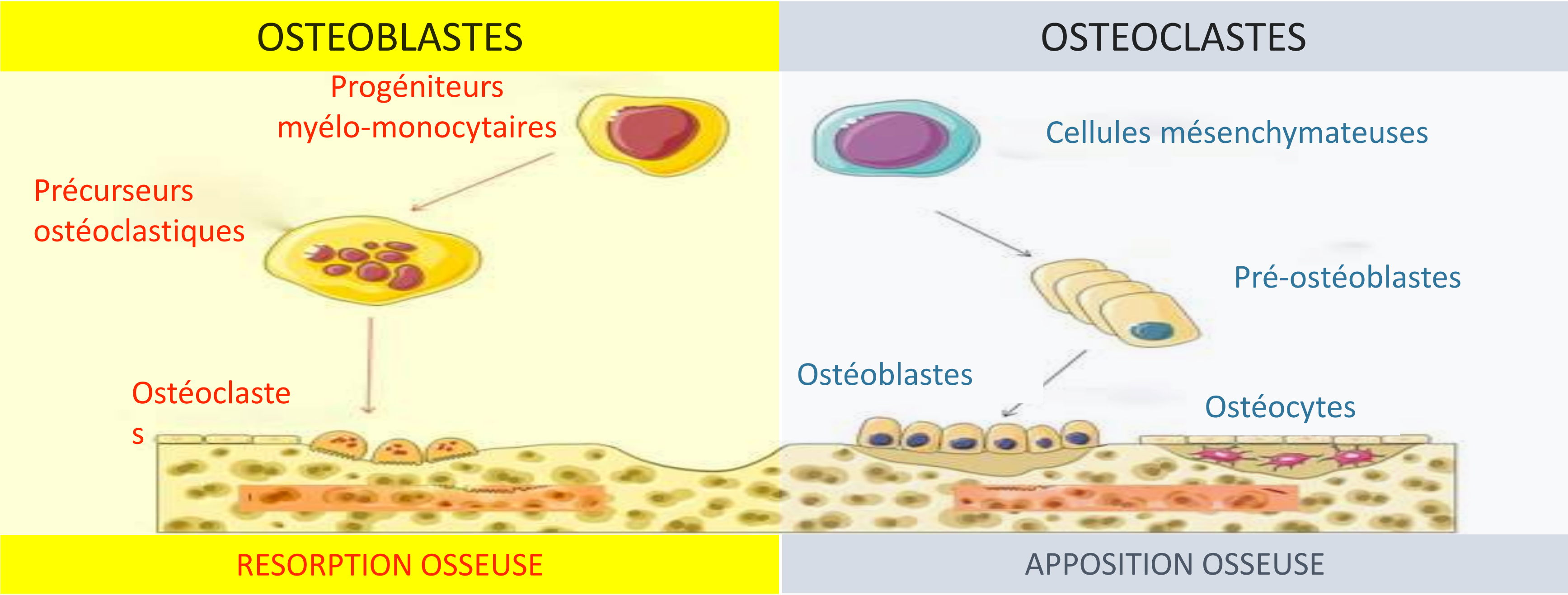
Evaluation de l'adhésion et internalisation dans les ostéoblastes de 16 espèces de SCN (souches cliniques responsables d'IOA)

Faible capacité d'internalisation et de persistance des SCN à l'exception de *S. pseudintermedius*





Interactions avec les cellules osseuses : ostéoClastes



OSTEOBLASTES

Progéniteurs myélo-monocytaires

Précurseurs ostéoclastiques

Ostéoclastes

RESORPTION OSSEUSE

OSTEOCLASTES

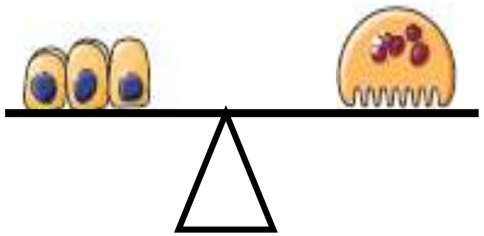
Cellules mésenchymateuses

Pré-ostéoblastes

Ostéoblastes

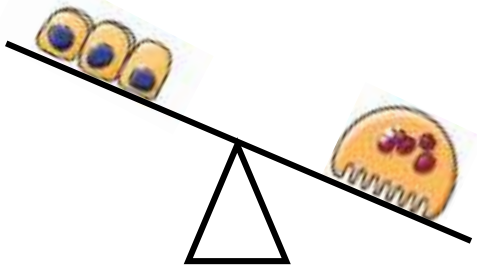
Ostéocytes

APPOSITION OSSEUSE



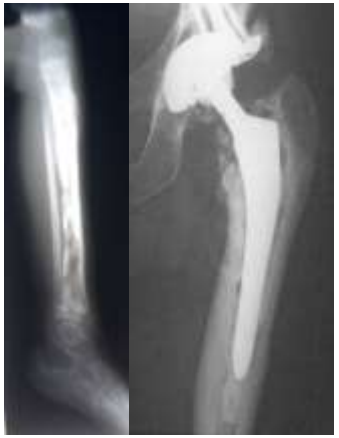
Os sain

Equilibre OB / OC

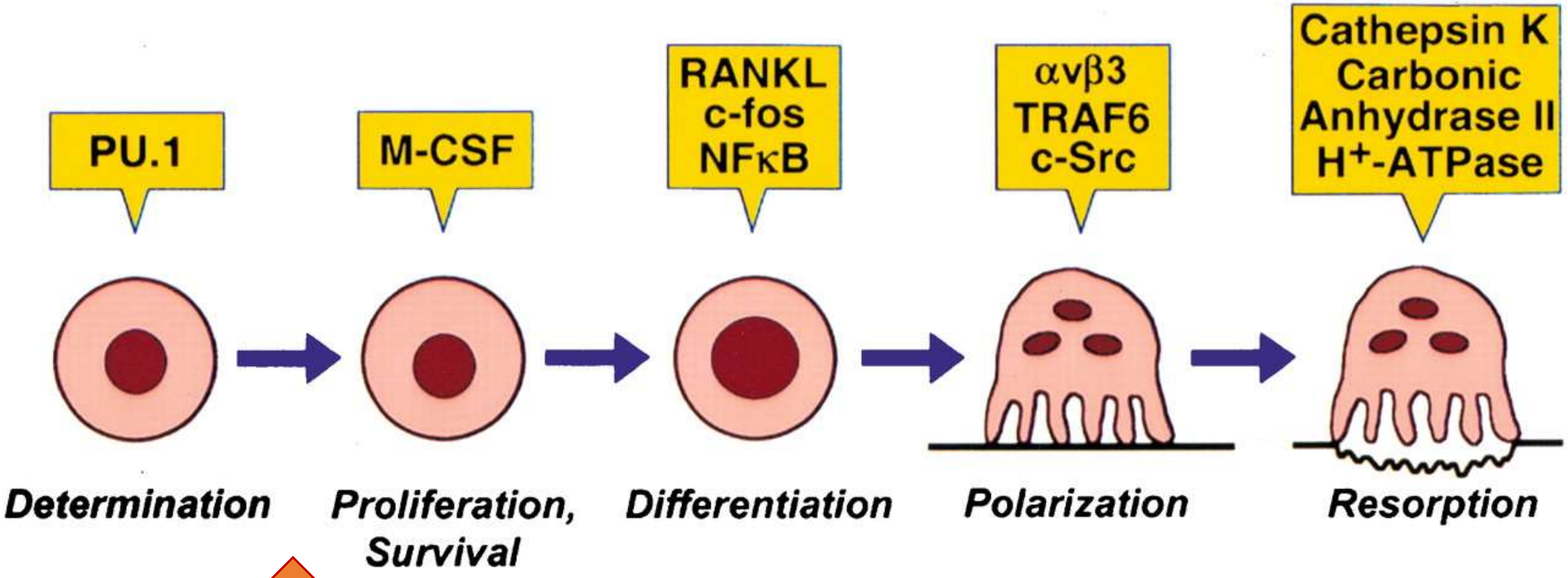


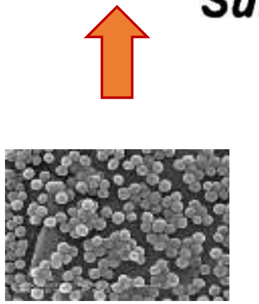
IOA

Ostéolyse progressive
- Descellement prothétique
- Séquestres

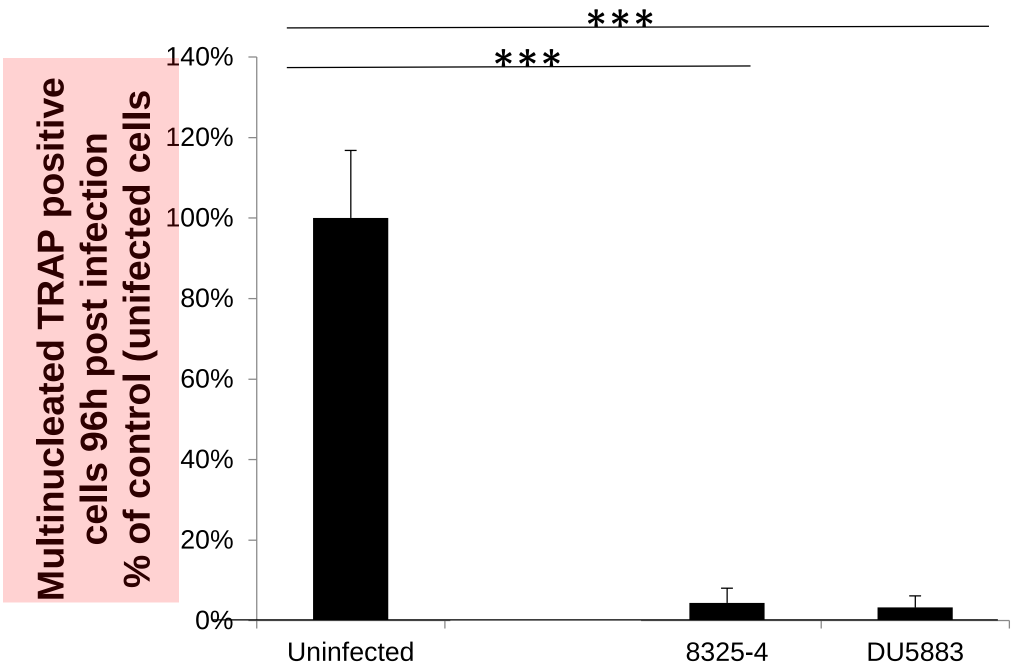


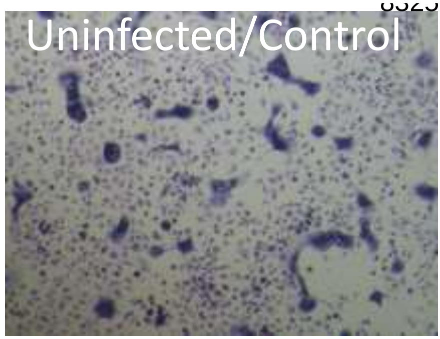
Interactions avec les cellules osseuses : ostéoClastes

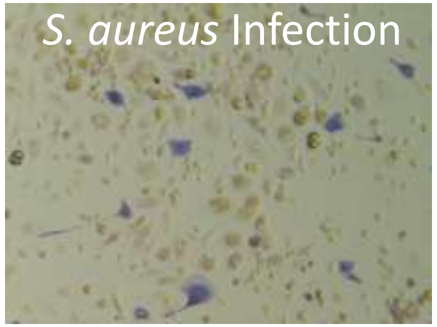

PU.1
M-CSF
RANKL
c-fos
NFκB
αvβ3
TRAF6
c-Src
Cathepsin K
Carbonic
Anhydrase II
H+-ATPase
Determination
Proliferation,
Survival
Differentiation
Polarization
Resorption



Interactions avec les cellules osseuses : ostéoClastes



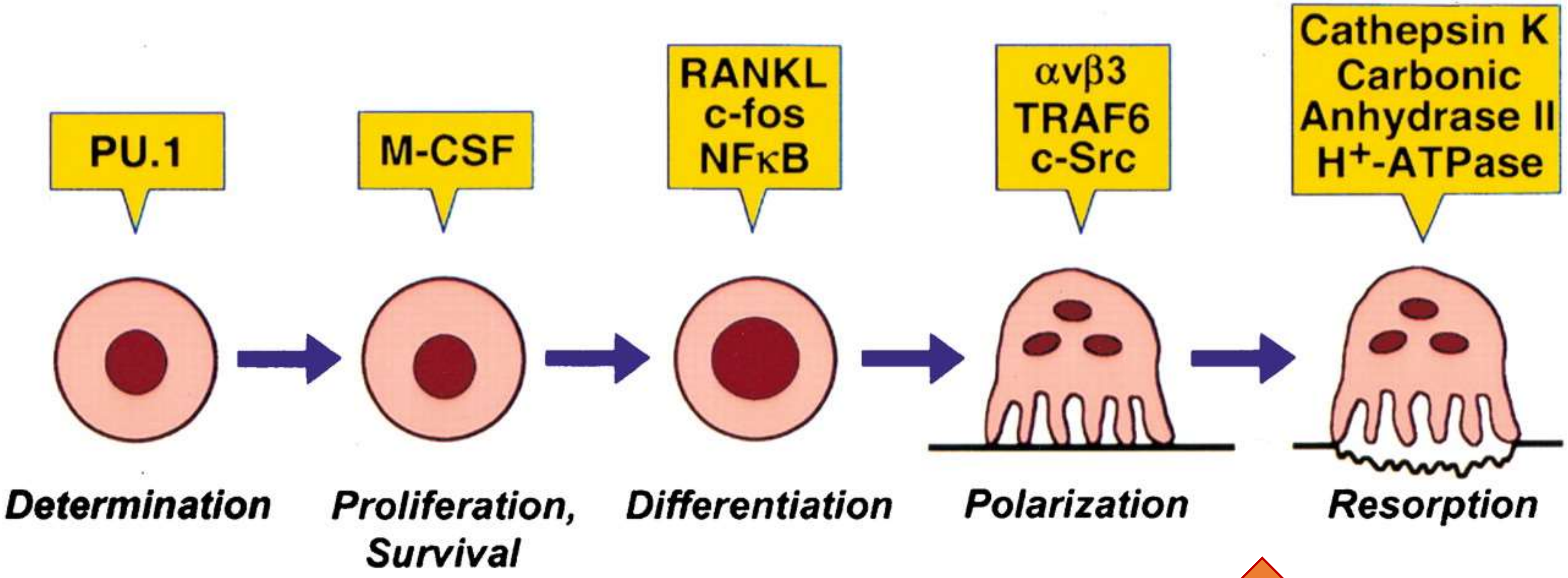

Uninfected/Control

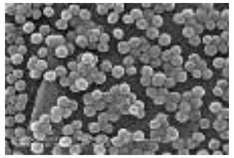

S. aureus Infection

Infection par *S. aureus*
Inhibition de l'ostéoclastogenèse

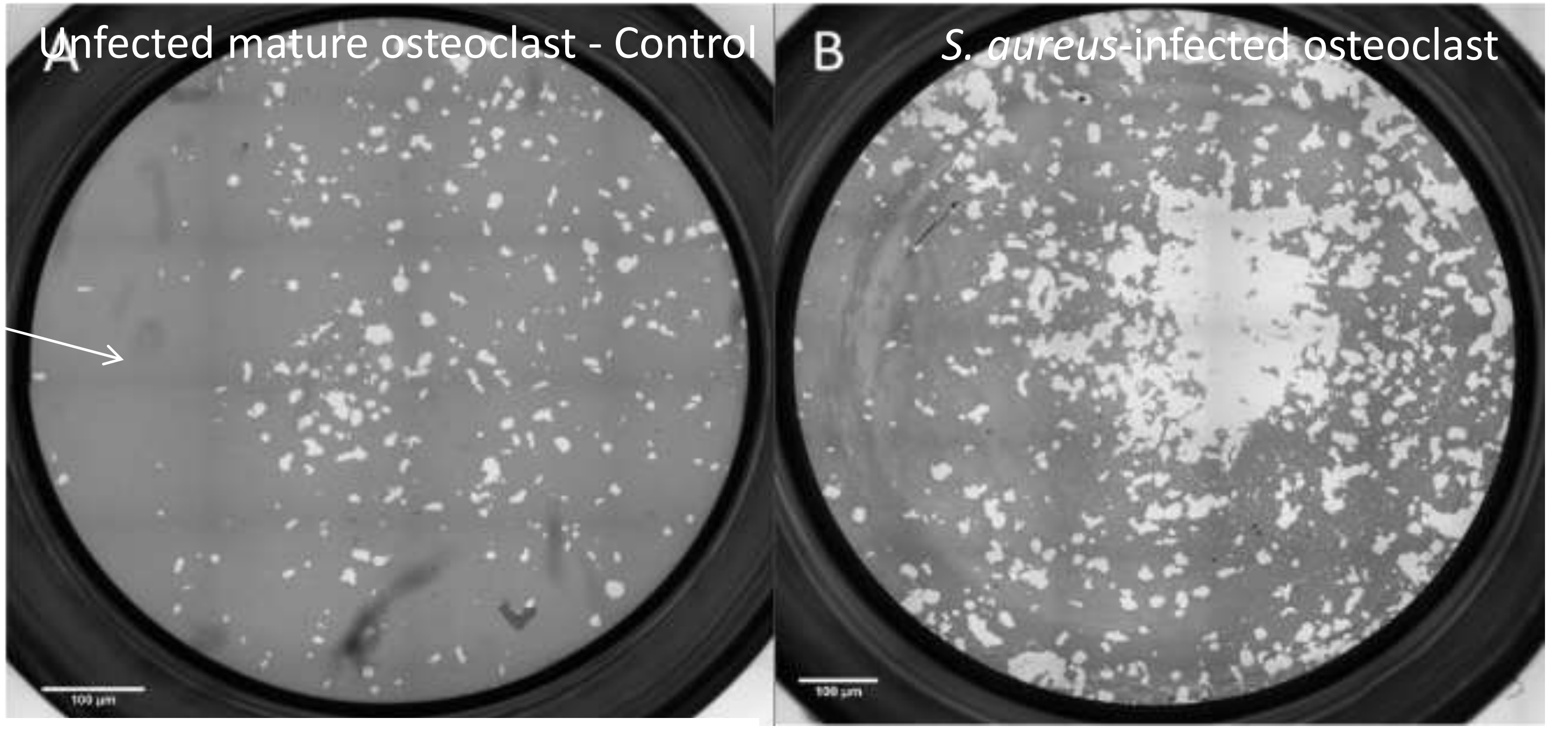
- Typage cytométrique = **macrophages**
- **Relargage de cytokines pro-inflammatoires**
- Surnageant = **boost de la résorption osseuse** par des ostéoclastes non infectés

Interactions avec les cellules osseuses : ostéoClastes

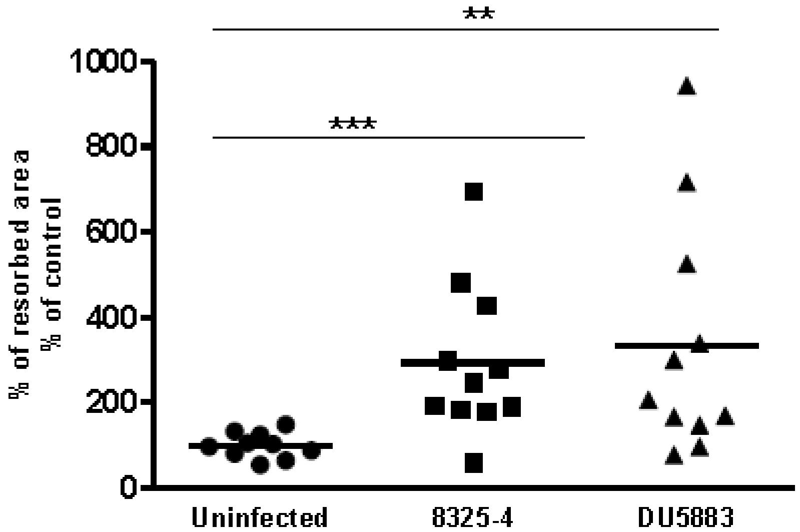

PU.1
M-CSF
RANKL
c-fos
NFκB
αvβ3
TRAF6
c-Src
Cathepsin K
Carbonic
Anhydrase II
H+-ATPase
Determination
Proliferation,
Survival
Differentiation
Polarization
Resorption



Interactions avec les cellules osseuses : ostéoClastes


A
Unfected mature osteoclast - Control
B
S. aureus-infected osteoclast
100 µm
100 µm

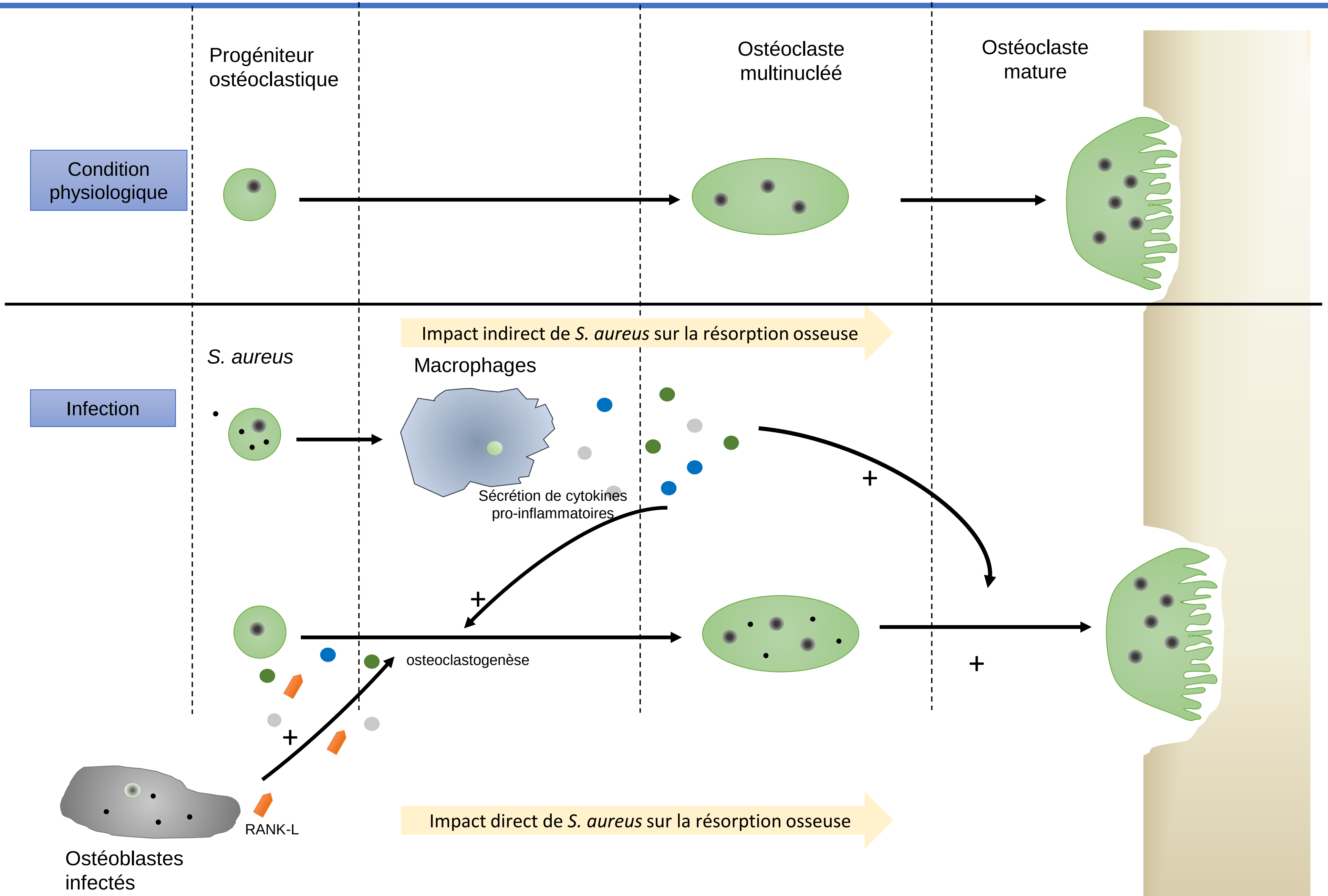
Matrice minérale mimant la matrice osseuse



Infection par *S. aureus*

Augmentation de la résorption osseuse par les OC matures

Interactions avec les cellules osseuses : ostéoClastes



Progéniteur ostéoclastique

Ostéoclaste multinucléé

Ostéoclaste mature

Condition physiologique

Impact indirect de *S. aureus* sur la résorption osseuse

S. aureus

Macrophages

Infection

Sécrétion de cytokines pro-inflammatoires

+

+

+

osteoclastogenèse

+

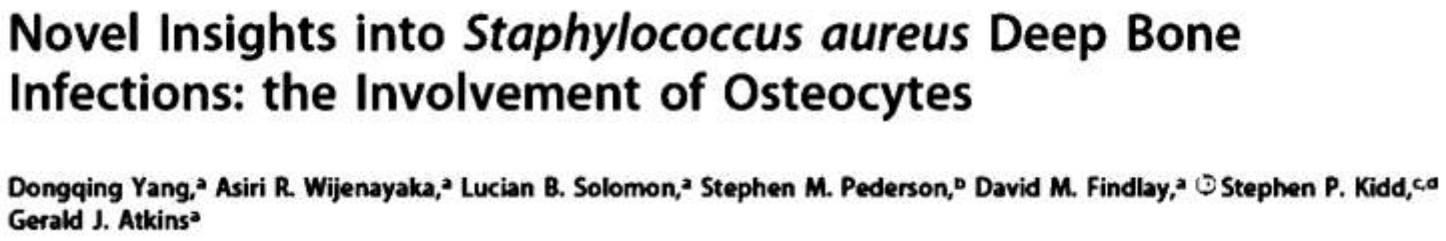
+

RANK-L

Impact direct de *S. aureus* sur la résorption osseuse

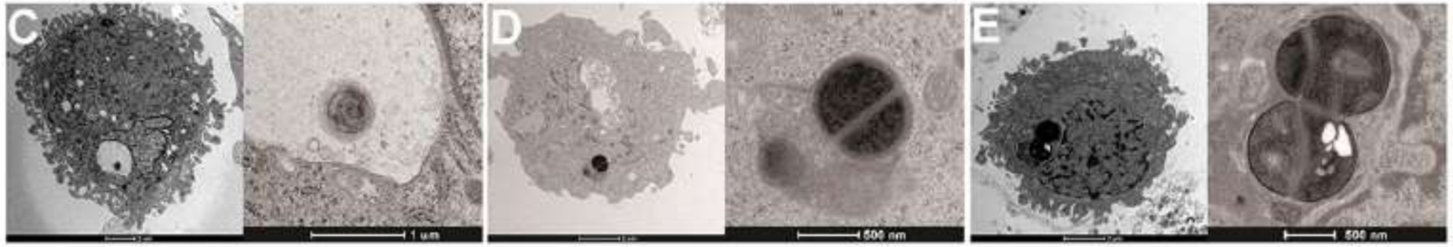
Ostéoblastes infectés

Interactions avec les cellules osseuses : ostéocytes



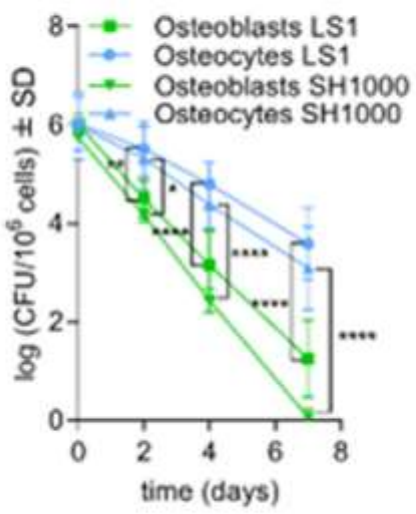
Novel Insights into *Staphylococcus aureus* Deep Bone Infections: the Involvement of Osteocytes

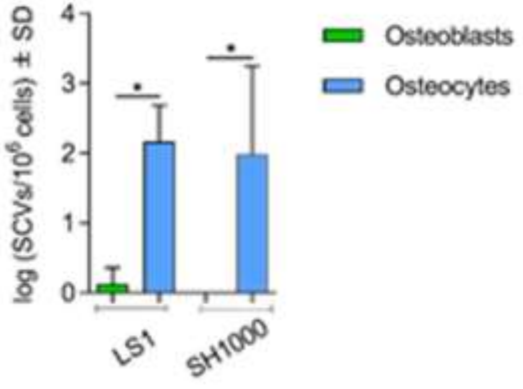
Dongqing Yang,[a] Asiri R. Wijenayaka,[a] Lucian B. Solomon,[a] Stephen M. Pederson,[b] David M. Findlay,[a] Stephen P. Kidd,[c,d] Gerald J. Atkins[a]

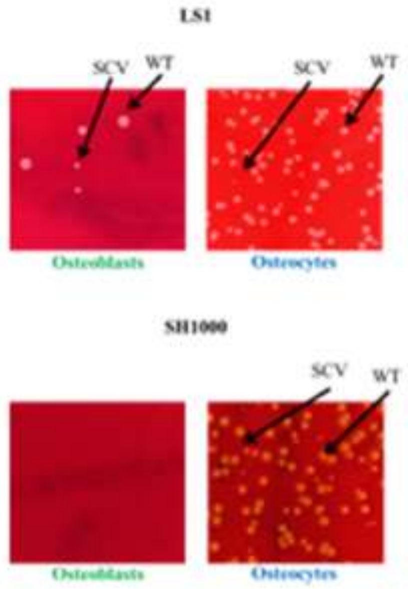

C
D
E
1 µm
500 nm
500 nm

Osteocytes Serve as a Reservoir for Intracellular Persisting *Staphylococcus aureus* Due to the Lack of Defense Mechanisms

Marina Garcia-Moreno[1], Paul M. Jordan[2], Kerstin Günther[2], Theresa Dau[3], Christian Fritzsch[1], Monika Vermes[1], Astrid Schoppa[4], Anita Ignatius[4], Britt Wildemann[3], Oliver Werz[2], Bettina Löffler[1] and Lorena Tuchscherr[1]*






LS1
SCV
WT
SCV
WT
Osteoblasts
Osteocytes
SH1000
SCV
WT
Osteoblasts
Osteocytes

Modèles *in vitro* + prélèvements cliniques osseux (fracture fémorale (contrôles) et sepsis / PTH)

En comparaison avec les ostéoblastes :

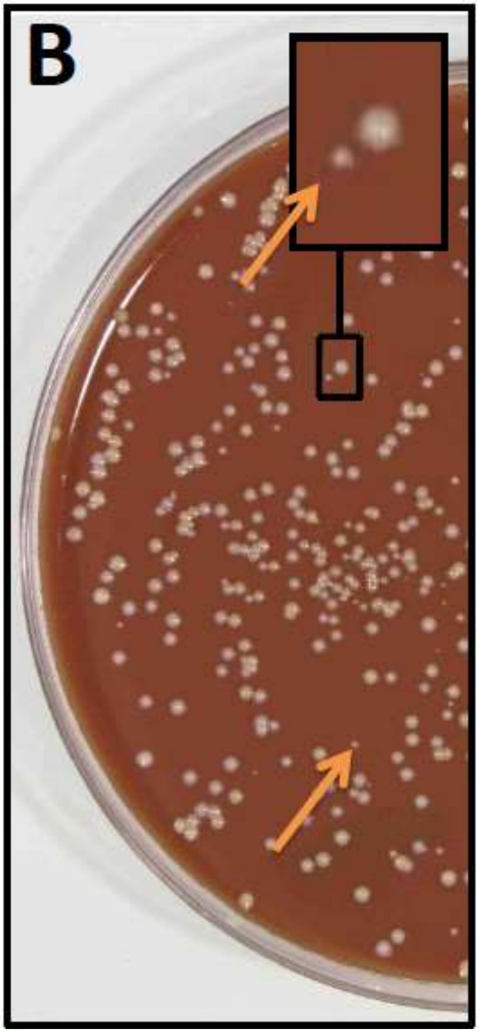
- Internalisation et **persistance +++**
- Recrutement et **inflammation**, mais faible clairance
- Émergence de **SCV** +++

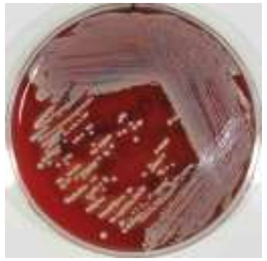
Small colony variants et persisters

Formation de Small Colony Variant (SCV)

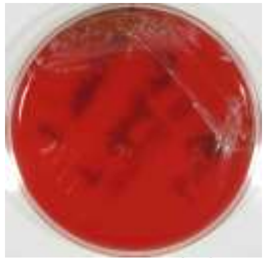
Difficulté de détection des SCV

Détection de Small Colony Variant





S. aureus « sauvage »

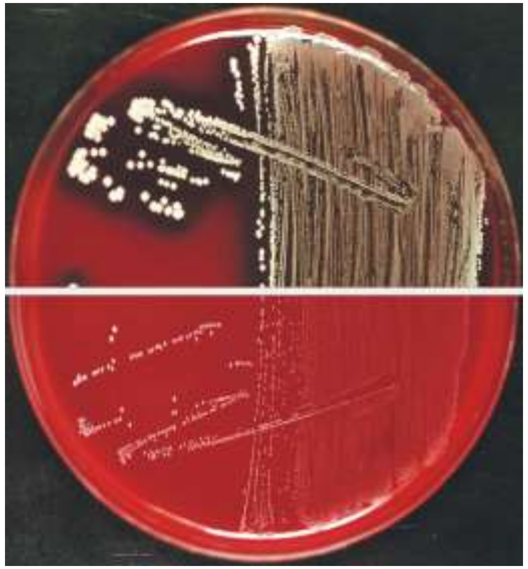


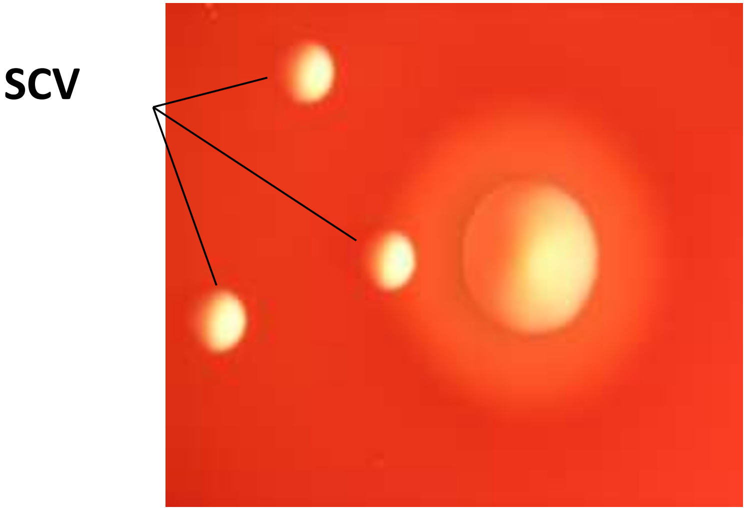
S. aureus SCV

Formation de Small Colony Variant (SCV)

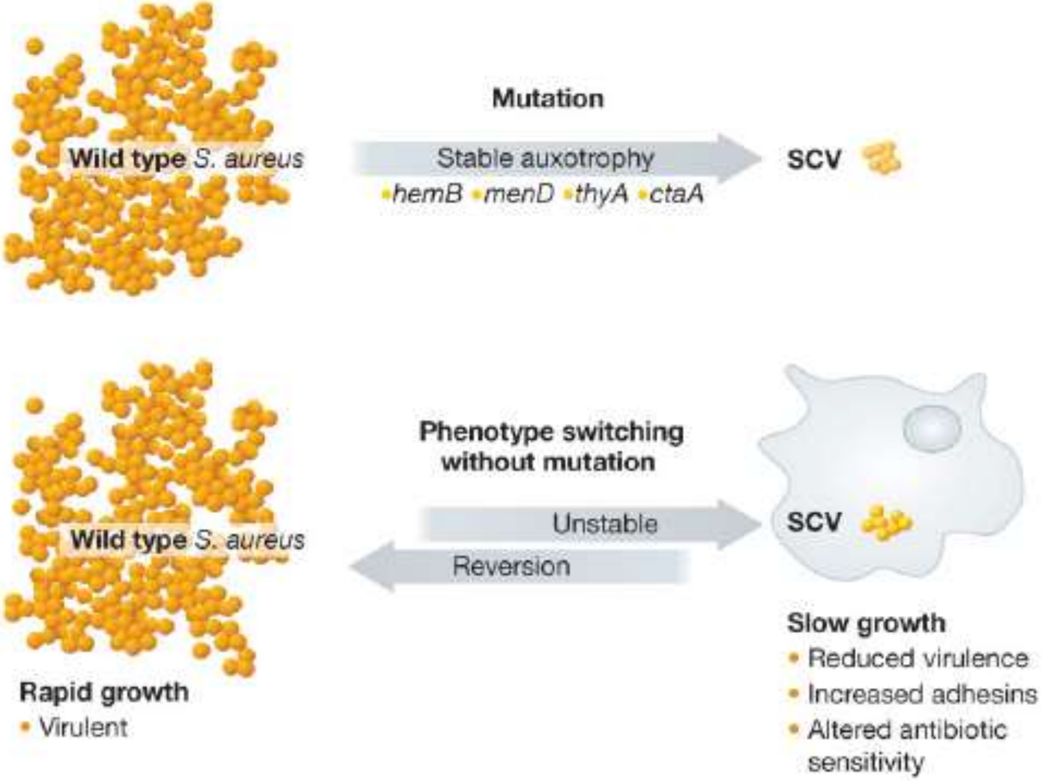
Modifications phénotypiques des SCV
Adaptation à un environnement hostile : os, biofilm, intracellulaire

- Croissance lente (temps de doublement x 10)
- Petites colonies atypiques
- Résistance accrue aux antibiotiques
- Adhérence +++
- Survie intracellulaire +++
- Révertant





Proctor, Nat Rev Microbiol 2006 – Proctor, CID 1995 – Tuchscherr, JID 2010 – Tuchscherr, *JAC 2016*


Wild type S. aureus
Mutation
Stable auxotrophy
•hemB •menD •thyA •ctaA
SCV
Wild type S. aureus
Phenotype switching without mutation
Unstable
Reversion
SCV
Rapid growth
• Virulent
Slow growth
• Reduced virulence
• Increased adhesins
• Altered antibiotic sensitivity

Garzoni *et al.* EMBO Mol Med 2011

Formation de Small Colony Variant (SCV)

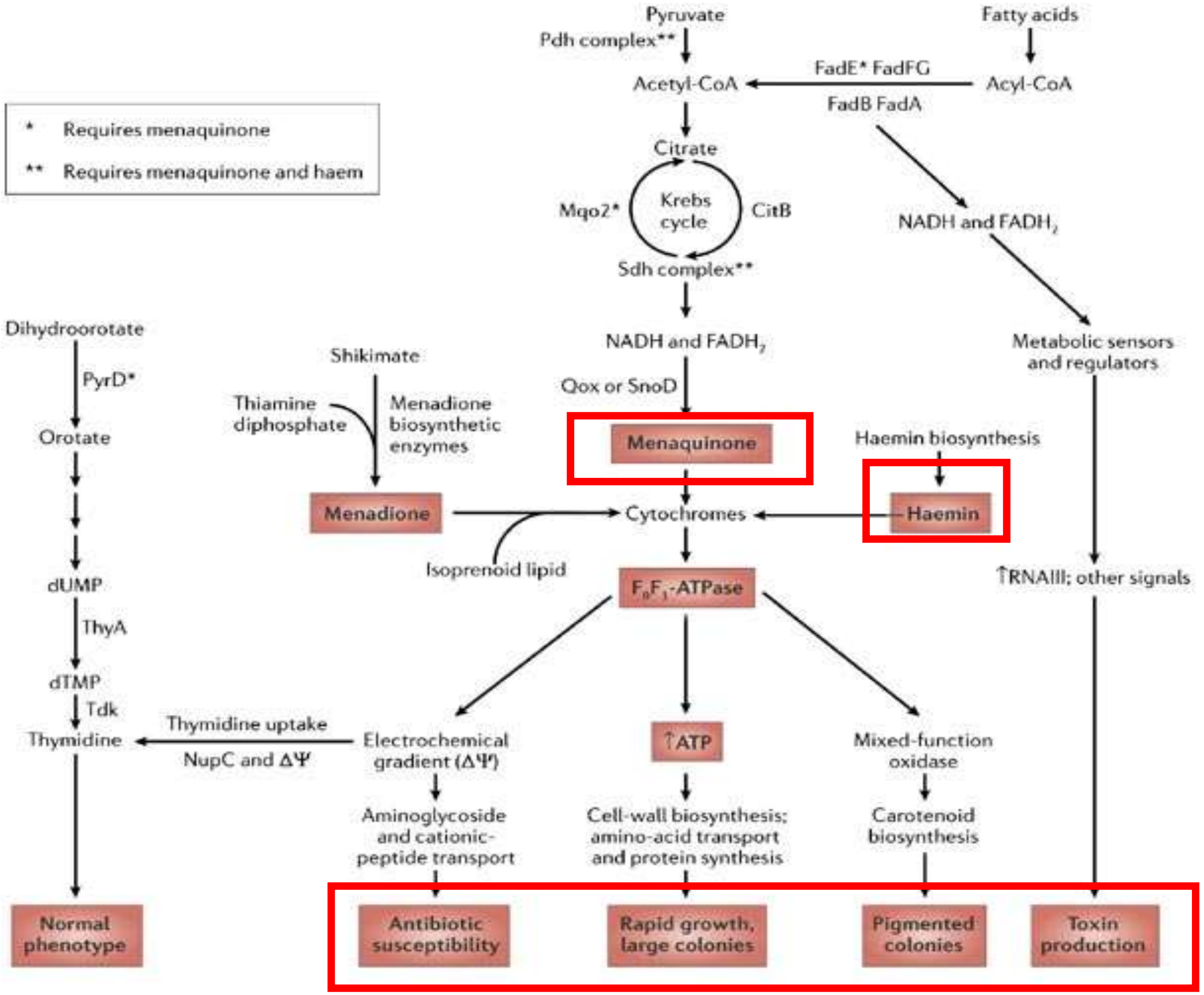
Apparition de SCV chez les staphylocoques :

Anomalies dans transport d'électron/perte de certaines voies métaboliques

Mécanismes ?

- Altérations dans la chaîne de transport d'électrons (ménadione, hémine) ou dans la synthèse de thymidine

- Dysrégulations de l'expression ou mutations

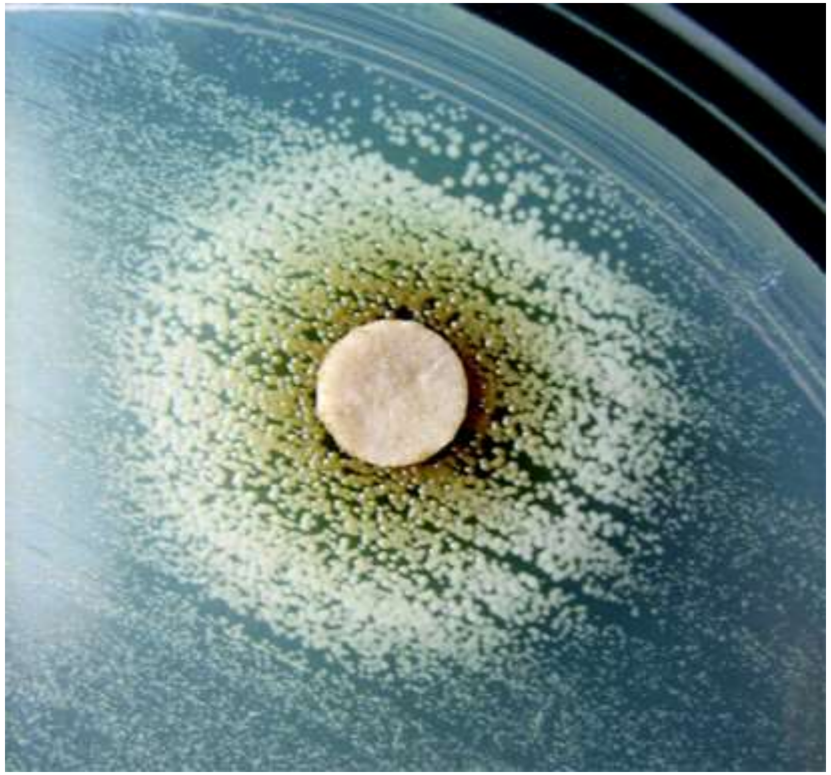

* Requires menaquinone
** Requires menaquinone and haem
Pyruvate
Pdh complex**
Acetyl-CoA
Fatty acids
Acyl-CoA
FadE* FadFG
FadB FadA
Citrate
Krebs cycle
Mqo2*
CitB
Sdh complex**
NADH and $FADH_2$
NADH and $FADH_2$
Metabolic sensors and regulators
Qox or SnoD
Menaquinone
Haemin biosynthesis
Haemin
Cytochromes
Dihydroorotate
PyrD*
Orotate
dUMP
ThyA
dTMP
Tdk
Thymidine
Shikimate
Thiamine diphosphate
Menadione biosynthetic enzymes
Menadione
Isoprenoid lipid
F_0F_1-ATPase
↑RNAIII; other signals
Thymidine uptake
NupC and ΔΨ
Electrochemical gradient (ΔΨ)
↑ATP
Mixed-function oxidase
Aminoglycoside and cationic-peptide transport
Cell-wall biosynthesis; amino-acid transport and protein synthesis
Carotenoid biosynthesis
Normal phenotype
Antibiotic susceptibility
Rapid growth, large colonies
Pigmented colonies
Toxin production

Proctor RA *et al. Nature Reviews Microbiology 2006*

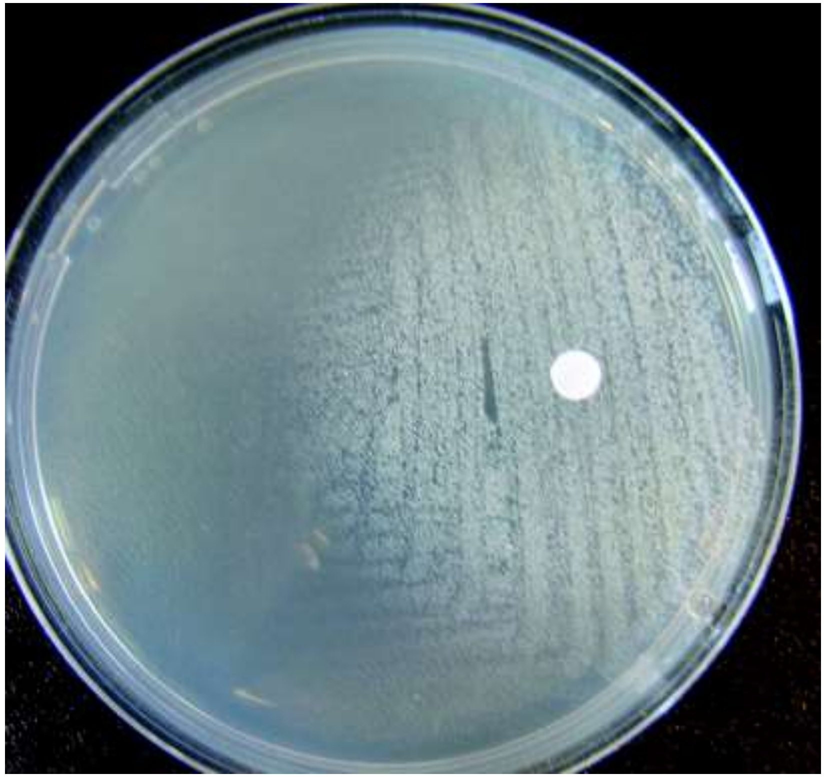
Formation de Small Colony Variant (SCV)

Apparition de SCV chez les staphylocoques :
Anomalies dans transport d'électron/perte de certaines voies métaboliques

Complementation of hemin auxotrophy



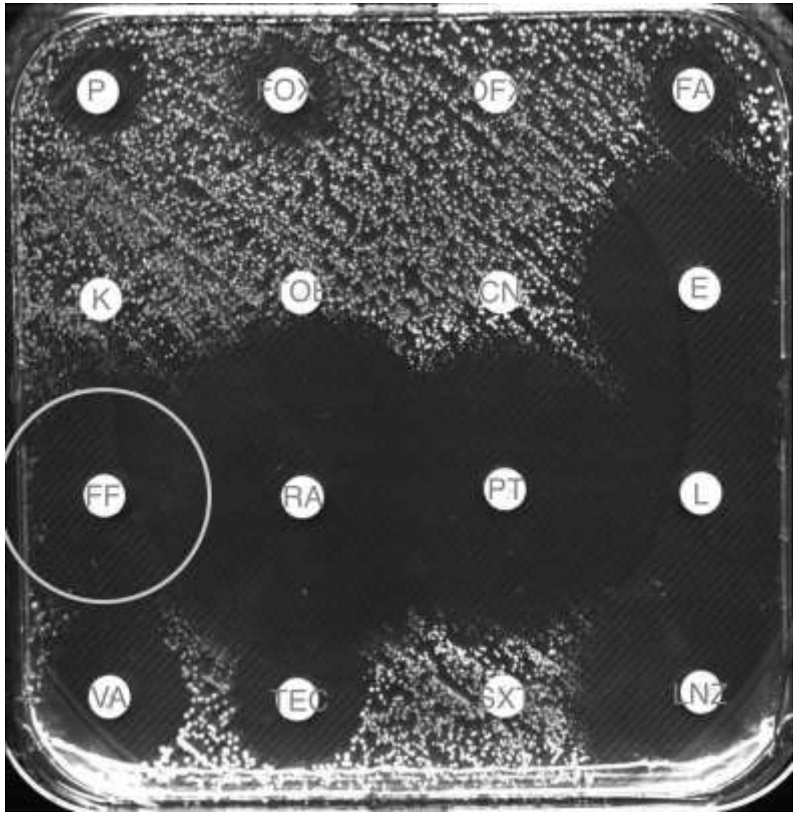
Complementation of menadione auxotrophy



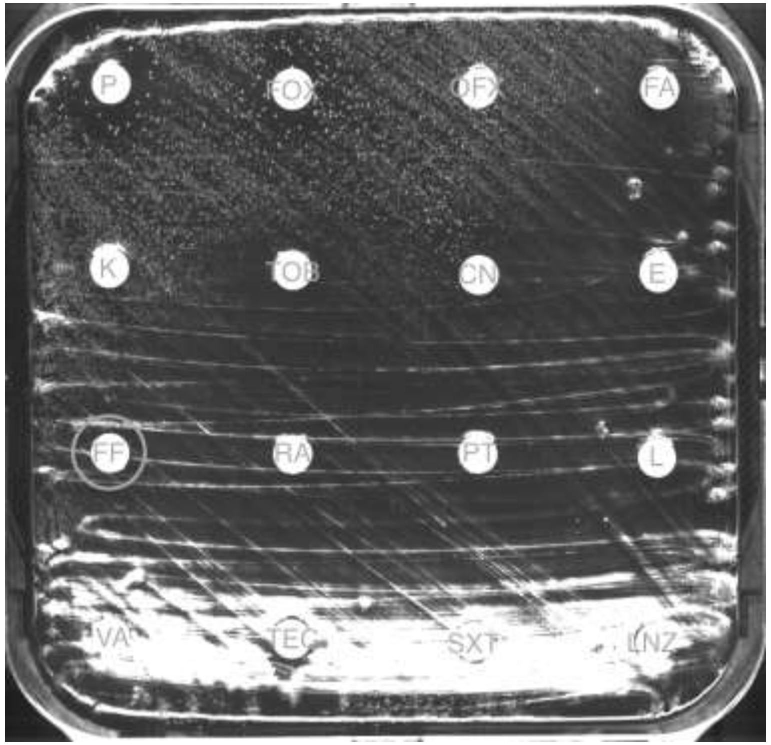
Norström T *et al.* Antimicrob Agents Chemother 2007

Formation de Small Colony Variant (SCV)

Apparition de SCV chez les staphylocoques :
Anomalies dans transport d'électron/perte de certaines voies métaboliques


P
FOX
OFX
FA
K
TOB
CN
E
FF
RA
PT
L
VA
TEC
SXT
LNZ

S. epidermidis

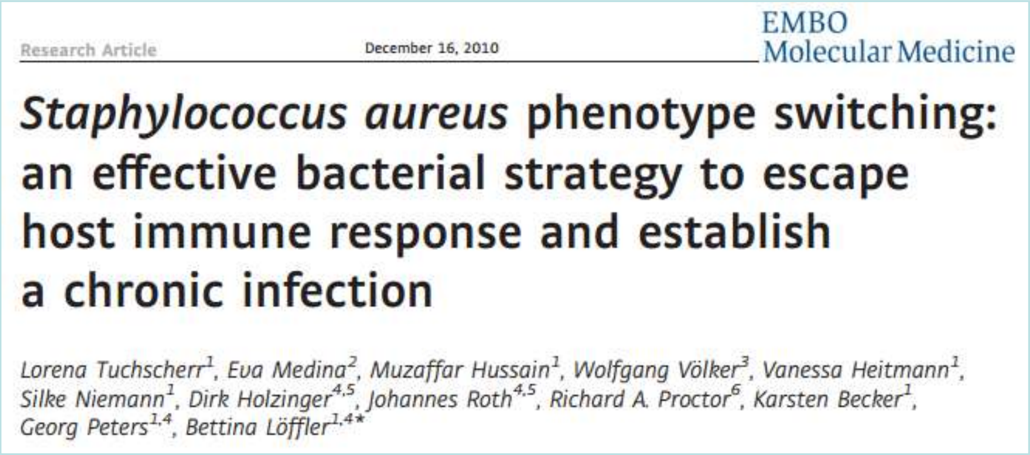

P
FOX
OFX
FA
K
TOB
CN
E
FF
RA
PT
L
VA
TEC
SXT
LNZ

S. epidermidis de phénotype SCV

Formation de Small Colony Variant (SCV)

Décrits dans de nombreuses situations cliniques
(IOA, infections / matériel, mucoviscidose …)

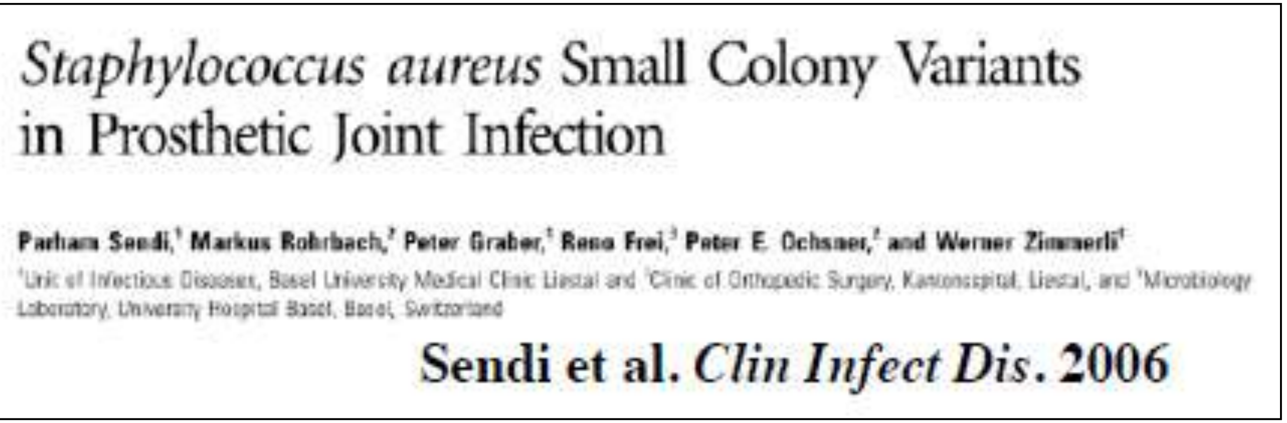
… et chez de nombreuses espèces
(staphylocoques, *Pseudomonas*, *E. coli*, *P. acnes* …)



Research Article December 16, 2010 EMBO Molecular Medicine

Staphylococcus aureus **phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection**

*Lorena Tuchscherr[1], Eva Medina[2], Muzaffar Hussain[1], Wolfgang Völker[3], Vanessa Heitmann[1], Silke Niemann[1], Dirk Holzinger[4,5], Johannes Roth[4,5], Richard A. Proctor[6], Karsten Becker[1], Georg Peters[1,4], Bettina Löffler[1,4]**

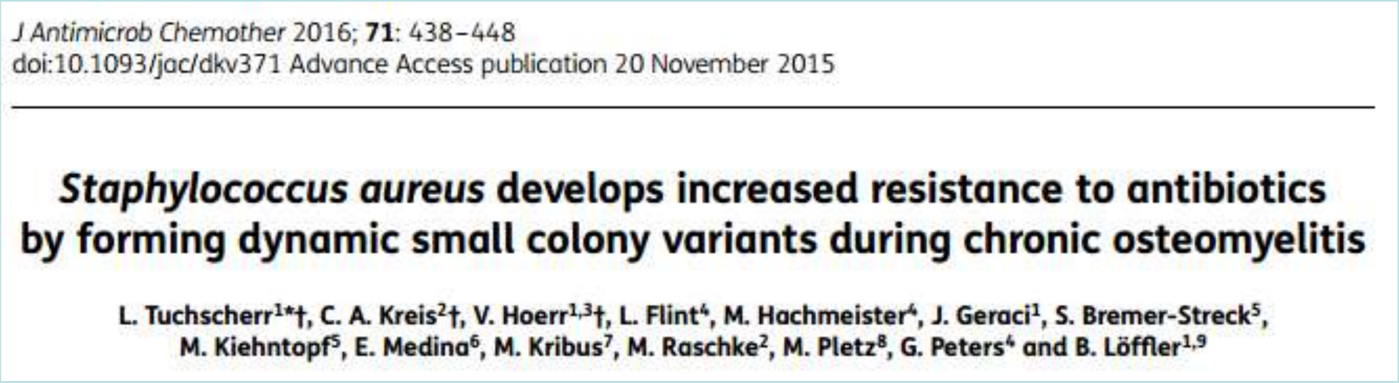


Staphylococcus aureus Small Colony Variants in Prosthetic Joint Infection

Parham Sendi,[1] Markus Rohrbach,[2] Peter Graber,[1] Reno Frei,[3] Peter E. Ochsner,[2] and Werner Zimmerli[1]

[1]Unit of Infectious Diseases, Basel University Medical Clinic Liestal and [2]Clinic of Orthopedic Surgery, Kantonsspital, Liestal, and [3]Microbiology Laboratory, University Hospital Basel, Basel, Switzerland

Sendi et al. *Clin Infect Dis*. 2006



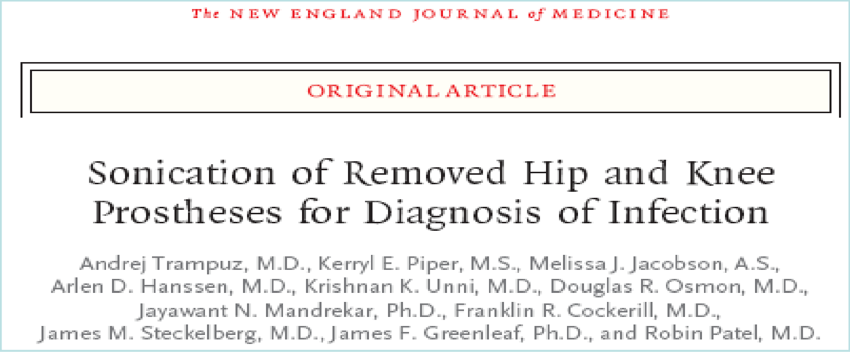
J Antimicrob Chemother 2016; **71**: 438–448
doi:10.1093/jac/dkv371 Advance Access publication 20 November 2015

Staphylococcus aureus **develops increased resistance to antibiotics by forming dynamic small colony variants during chronic osteomyelitis**

L. Tuchscherr[1]*†, C. A. Kreis[2]†, V. Hoerr[1,3]†, L. Flint[4], M. Hachmeister[4], J. Geraci[1], S. Bremer-Streck[5], M. Kiehntopf[5], E. Medina[6], M. Kribus[7], M. Raschke[2], M. Pletz[8], G. Peters[4] and B. Löffler[1,9]

Formation de Small Colony Variant (SCV)

Intérêt de la sonication

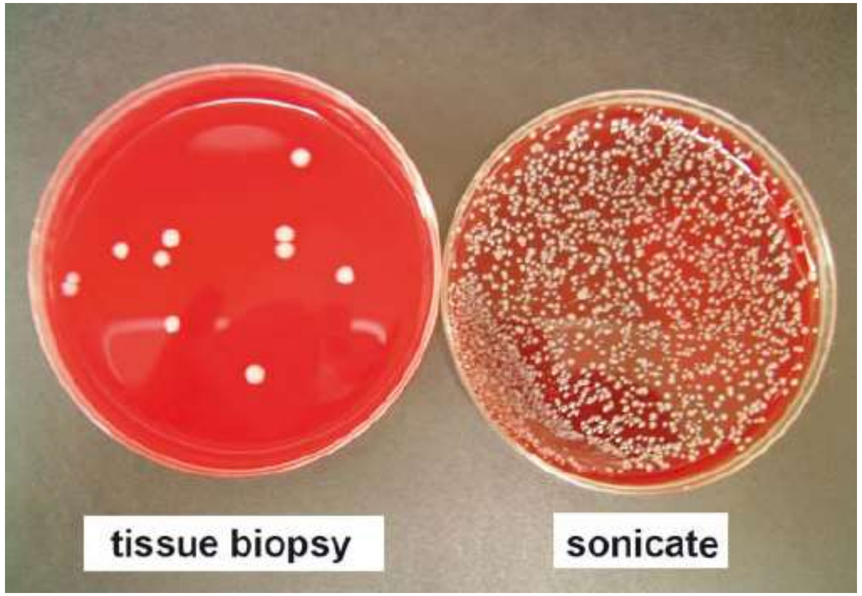


The NEW ENGLAND JOURNAL of MEDICINE

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.



Culture de sonicat

17 suspicion de sepsis, 9 cultures +
6 avec SCV

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

Formation de Small Colony Variant (SCV)

SCV et IOA à *S. aureus*

- SCV associés à des IOA persistantes, récurrentes et réfractaires aux traitements ATB

- Adaptés à la survie intracellulaire prolongée
  - **Réservoir** à l'abri du système immunitaire et des ATB
  - Relargués par mort cellulaire
  - Facilement internalisés par une nouvelle cellule-hôte car capacité d'invasion accrue

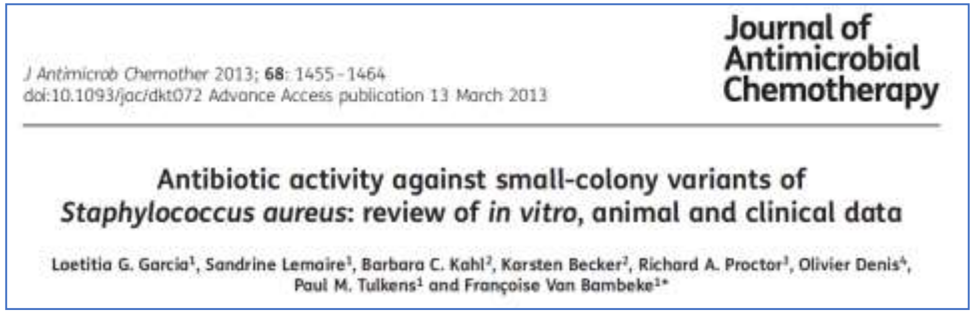
⇨ Source de **récidives**

- Résistance accrue à différents antibiotiques (aminosides, ATB agissant sur les bactéries en phase de multiplication)

⇨ Source d'**échecs thérapeutiques**

Von Eiff et al. Injury 2006 ; Proctor *et al.* Nat Rev Microbiol 2006 ; Tuchscherr et *al*. J Infec Dis 2011

Small colonie variants (SCVs)

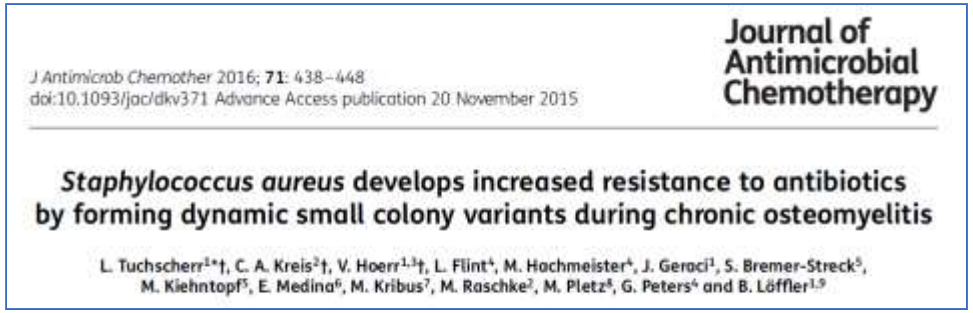


J Antimicrob Chemother 2013; **68**: 1455–1464
doi:10.1093/jac/dkt072 Advance Access publication 13 March 2013

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]*



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Journal of Antimicrobial Chemotherapy

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Données *in vitro*

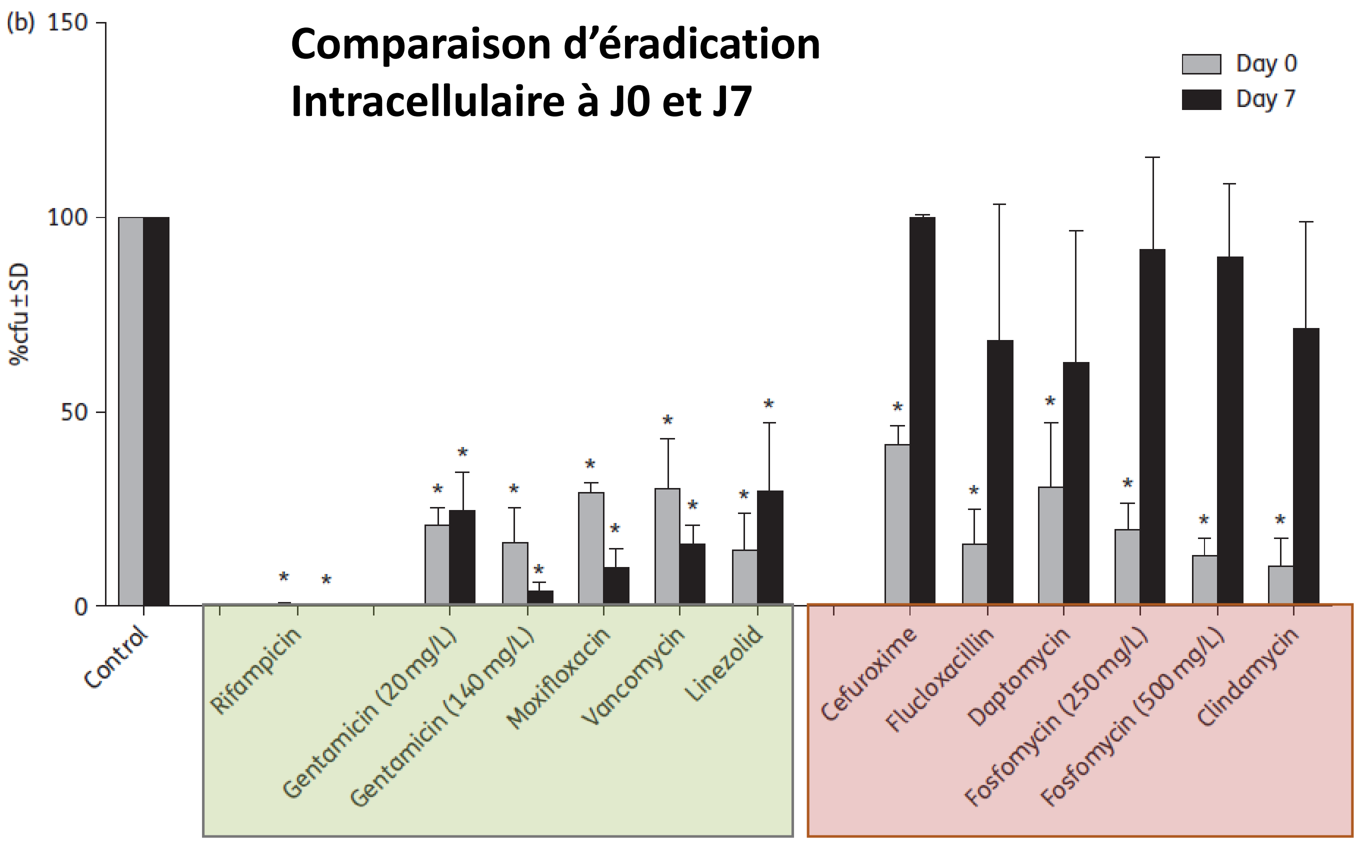
Limites - caractère révertant
- CMI développées pour bactéries à croissance rapide

Réduction d'activité
- aminoside +++
- cotrimoxazole +++
- ATB actifs sur la paroi : BL, GP, DAP

Données *in vivo* : conflictuelles +++

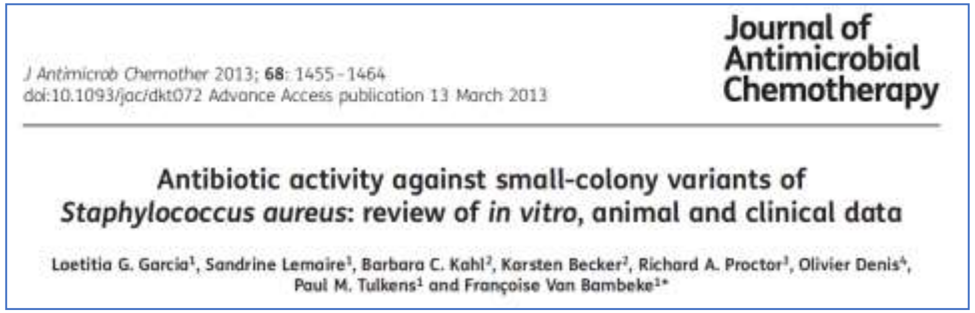
Très peu de données cliniques

Modèle cellulaire



Comparaison d'éradication Intracellulaire à J0 et J7

Small colonie variants (SCVs)



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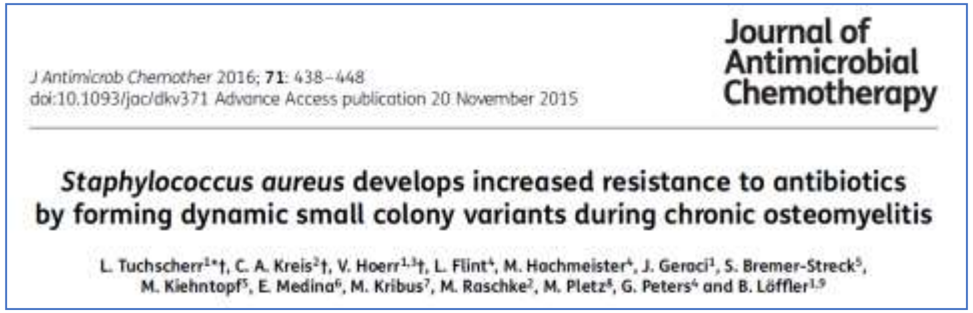
Données *in vivo* : conflictuelles +++

Très peu de données cliniques



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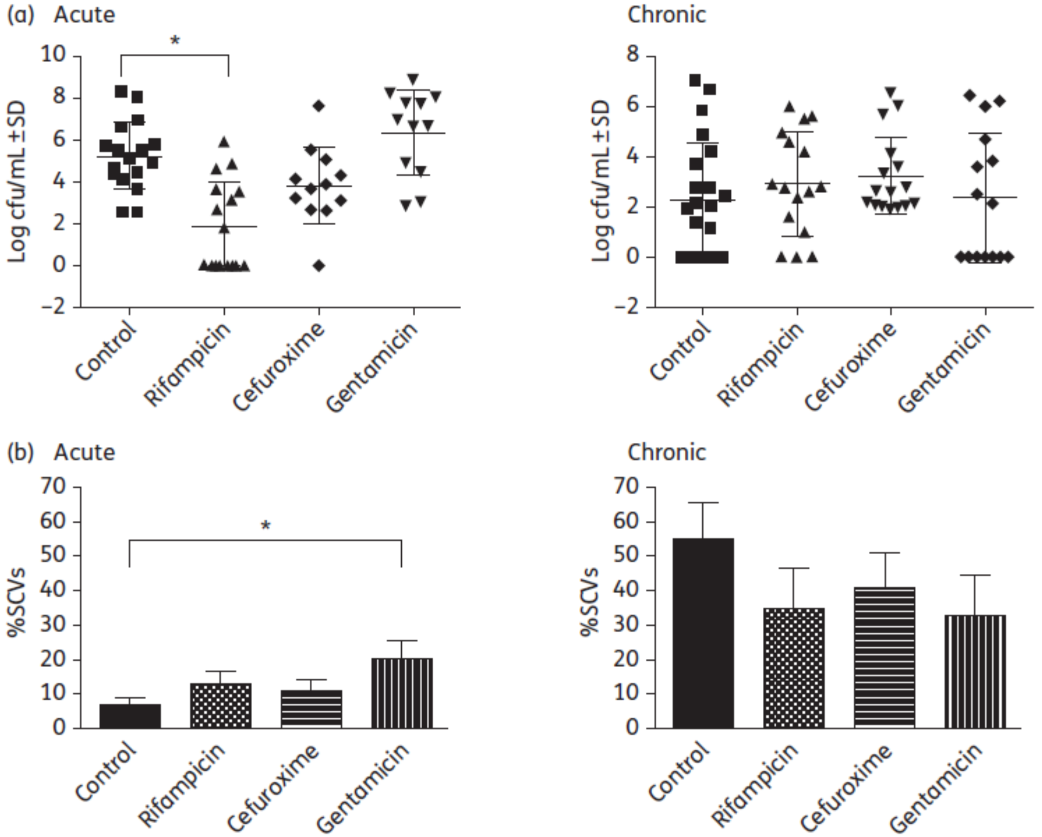
Journal of Antimicrobial Chemotherapy

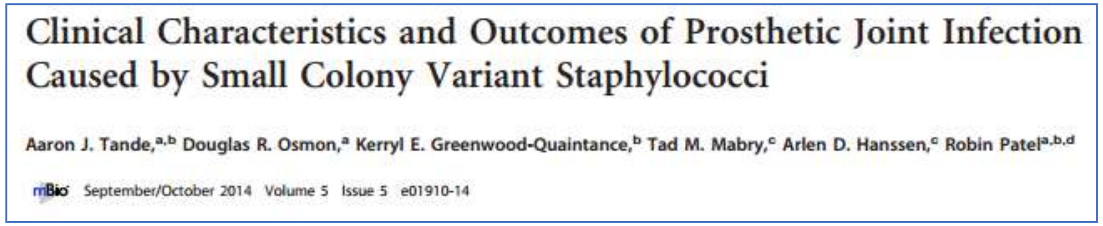
***Staphylococcus aureus* develops increased resistance to antibiotics by forming dynamic small colony variants during chronic osteomyelitis**

L. Tuchscherr[1*†], C. A. Kreis[2†], V. Hoerr[1,3†], L. Flint[4], M. Hachmeister[4], J. Geraci[1], S. Bremer-Streck[5], M. Kiehntopf[5], E. Medina[6], M. Kribus[7], M. Raschke[2], M. Pletz[8], G. Peters[4] and B. Löffler[1,9]

Modèle animal : ostéomyélite tibiale hématogène



Small colonie variants (SCVs)



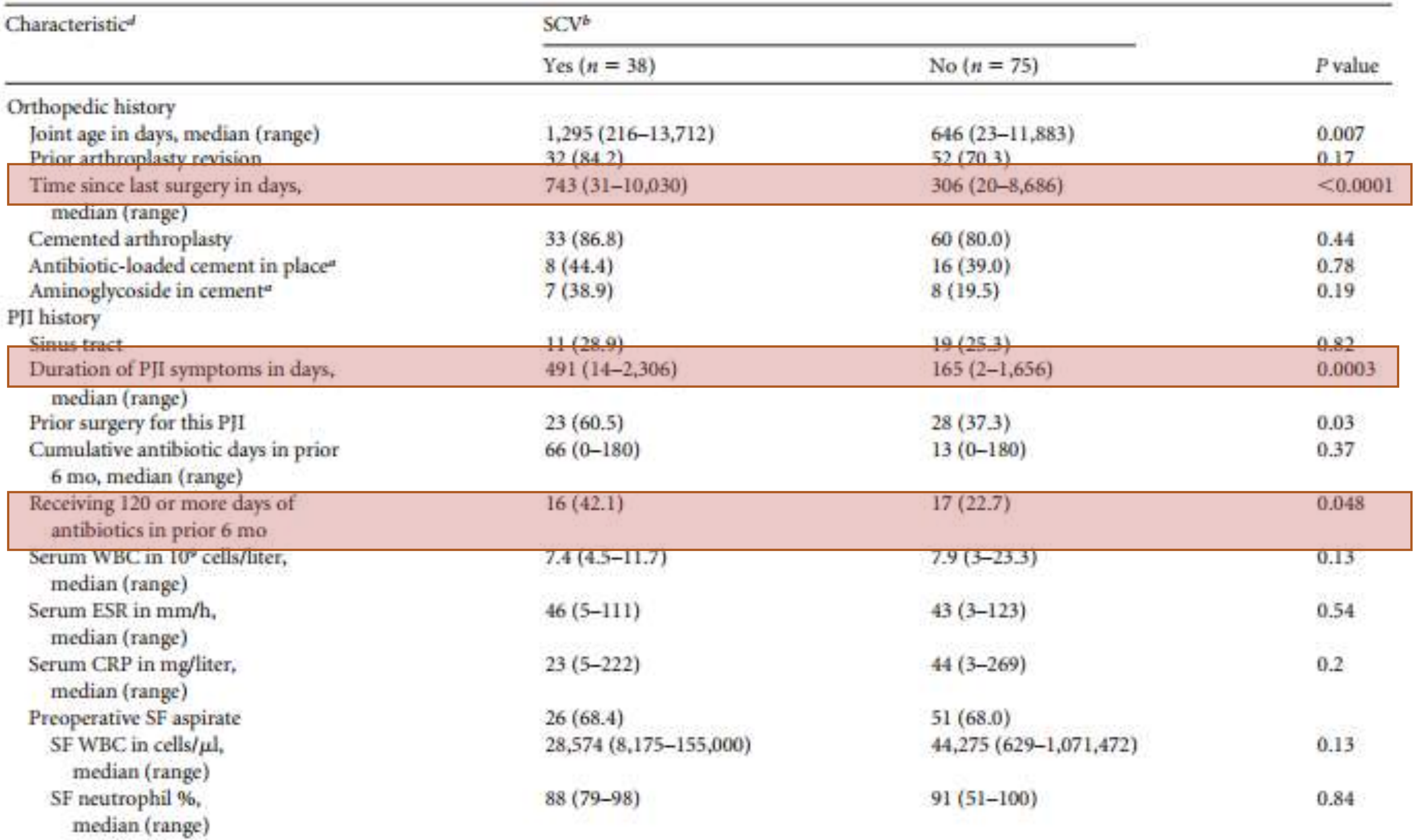
Clinical Characteristics and Outcomes of Prosthetic Joint Infection Caused by Small Colony Variant Staphylococci

Aaron J. Tande,[a,b] Douglas R. Osmon,[a] Kerryl E. Greenwood-Quaintance,[b] Tad M. Mabry,[c] Arlen D. Hanssen,[c] Robin Patel[a,b,d]

mBio September/October 2014 Volume 5 Issue 5 e01910-14

Retrospective series of 113 patients with staphylococcal PJI, with prospective testing of archived sonicate fluid samples

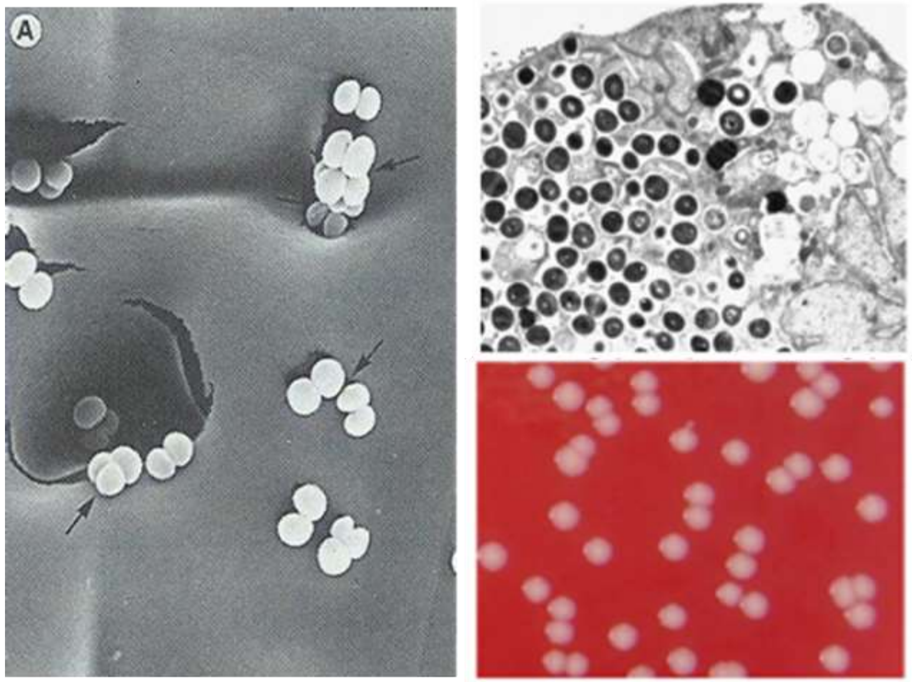
38 subjects (34%) with SCVs and 75 (66%) with only normal-phenotype (NP) bacteria



Characteristic[d]	SCV[b]		
	Yes ($n = 38$)	No ($n = 75$)	P value
Orthopedic history			
Joint age in days, median (range)	1,295 (216–13,712)	646 (23–11,883)	0.007
Prior arthroplasty revision	32 (84.2)	52 (70.3)	0.17
Time since last surgery in days, median (range)	743 (31–10,030)	306 (20–8,686)	<0.0001
Cemented arthroplasty	33 (86.8)	60 (80.0)	0.44
Antibiotic-loaded cement in place[a]	8 (44.4)	16 (39.0)	0.78
Aminoglycoside in cement[a]	7 (38.9)	8 (19.5)	0.19
PJI history			
Sinus tract	11 (28.9)	19 (25.3)	0.82
Duration of PJI symptoms in days, median (range)	491 (14–2,306)	165 (2–1,656)	0.0003
Prior surgery for this PJI	23 (60.5)	28 (37.3)	0.03
Cumulative antibiotic days in prior 6 mo, median (range)	66 (0–180)	13 (0–180)	0.37
Receiving 120 or more days of antibiotics in prior 6 mo	16 (42.1)	17 (22.7)	0.048
Serum WBC in 10^9 cells/liter, median (range)	7.4 (4.5–11.7)	7.9 (3–23.3)	0.13
Serum ESR in mm/h, median (range)	46 (5–111)	43 (3–123)	0.54
Serum CRP in mg/liter, median (range)	23 (5–222)	44 (3–269)	0.2
Preoperative SF aspirate	26 (68.4)	51 (68.0)	
SF WBC in cells/µl, median (range)	28,574 (8,175–155,000)	44,275 (629–1,071,472)	0.13
SF neutrophil %, median (range)	88 (79–98)	91 (51–100)	0.84

Adaptation bactérienne

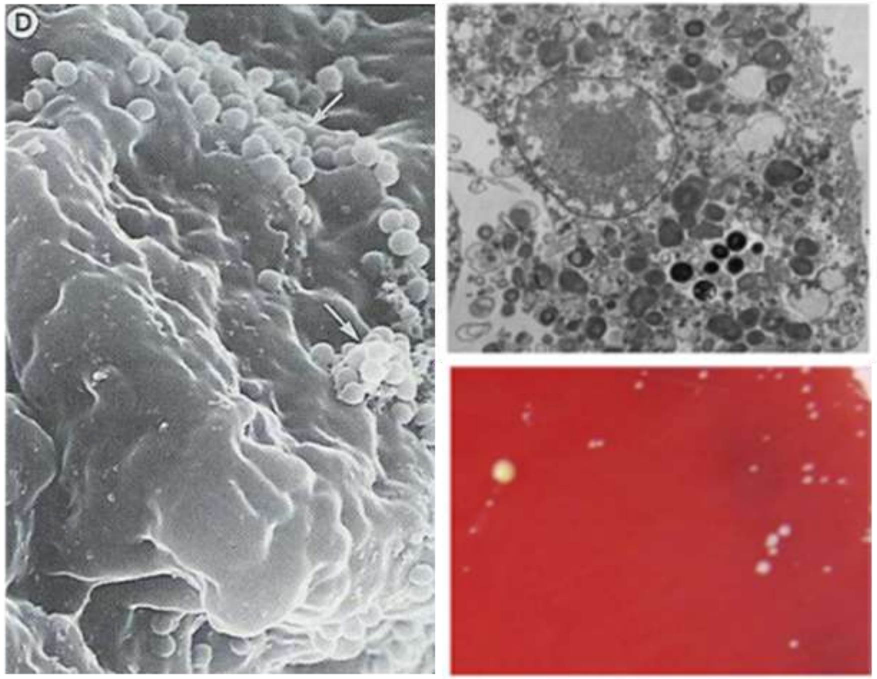
IOA aiguë → IOA chronique



Biofilm

Persistance intracellulaire

SCVs



↑ Toxines ↓ Adhésines

Agression tissulaire

Réponse immunitaire / inflammatoire

- Destruction cellulaire
- Invasion et destruction tissulaire

↓ Toxines ↑ Adhésines

↓ Inflammation, dommages tissulaires

- Échappement au système immunitaire
- « Tolérance » aux antibiotiques

⇓

DEBRIDEMENT, ABLATION MATERIEL

CHOIX DES ANTIBIOTIQUES : rmp, fq, dap

THERAPEUTIQUES CIBLEES ?

JACKPOT!!!