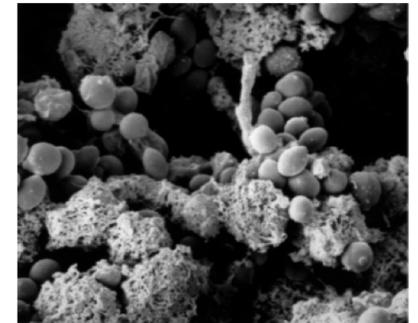


BIOFILM (BF)
ET
INFECTIONS OSTÉO-ARTICULAIRES COMPLEXES (IOAC)



26 OCTOBRE 2018



Pr Manuel ETIENNE

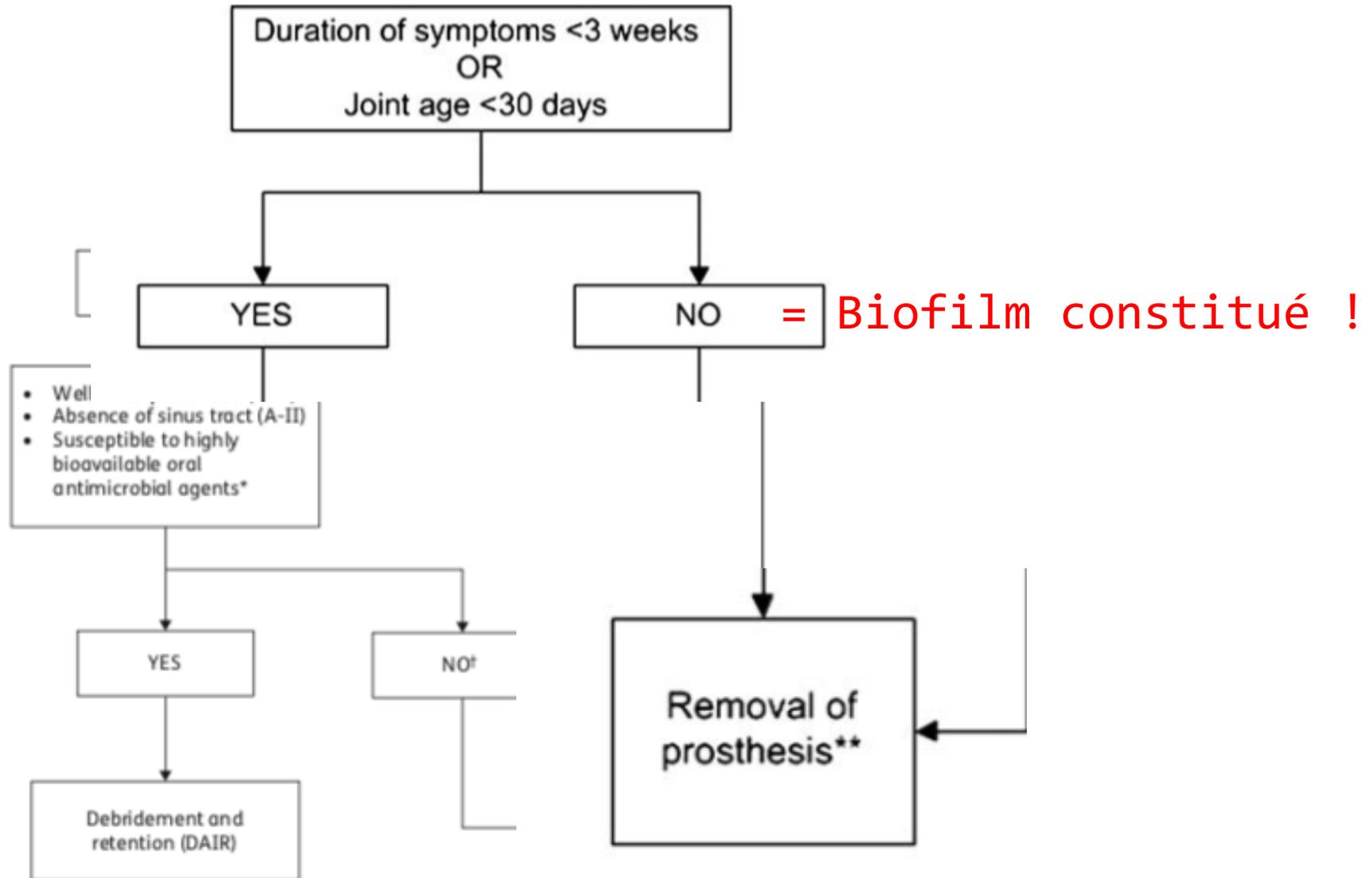
Maladies Infectieuses et Tropicales

Groupe de recherche sur l'adaptation microbienne (EA2656)

CHU Rouen

manuel.etienne@chu-rouen.fr

BF ET IOAC, VOUS SAVEZ TOUS QUE...



BF ET LITTÉRATURE IOAC...

Tous les articles commencent par « BF important »...

MAIS

Exemple d'une infection de prothèse articulaire :

- prélèvement de liquide articulaire
- isolement de la bactérie sur gélose
- détermination à partir d'une colonie de la sensibilité aux antibiotiques
- +/-détermination de la concentration minimale inhibitrice en gélose (E test)

- Infection <3 semaines : efficacité *in vitro* prédit ± succès *in vivo*
 - Lavage/débridement... + antibiotiques x 3 mois

≠

- Infection > 3-4 semaines : efficacité *in vitro* ne prédit PAS succès *in vivo*
 - Changement de prothèse + antibiotiques 6 à 12 semaines

Pronostic : mortalité ≈ 20%, chirurgie ≈ 60%

LE BF EST DÉCRIT DEPUIS...1720 ?



Antonie Van Leeuwenhoek (1720) :
animalicules (plaque dentaire)



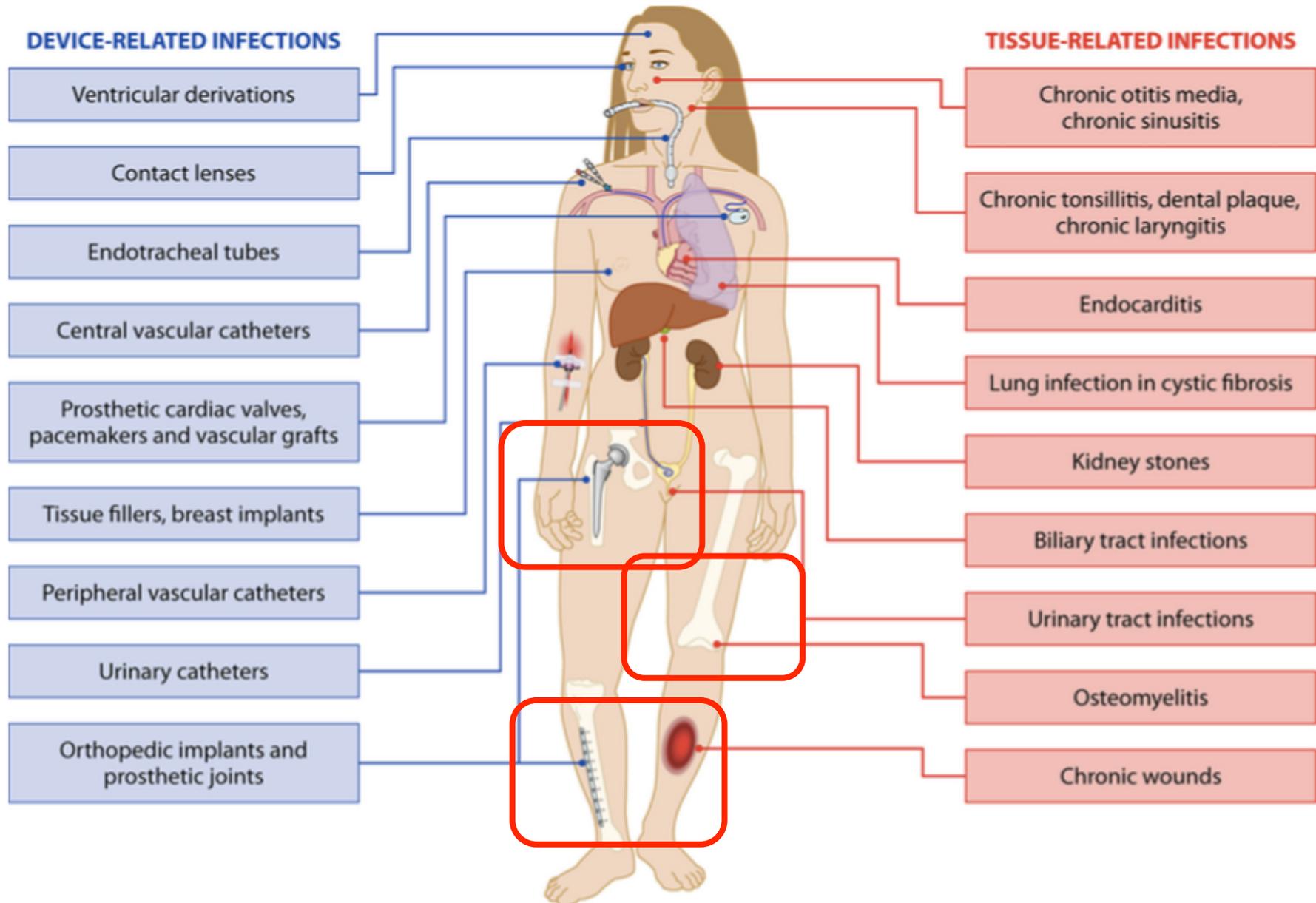
1971: systèmes microbiens dans conduites d'eau « tenaces et résistants aux désinfectants



1973: théorie des biofilms (plaque dentaire, torrents de montagne)

Costerton, Science, 1973

LE BIOFILM EST PARTOUT...



ANCIEN, PARTOUT... DONC... RECOS DE TRAITEMENT ?

ESCMID GUIDELINES

ESCMID* guideline for the diagnosis and treatment of biofilm infections 2014



ESCMID* guideline for the diagnosis and treatment of biofilm infections 2014

ET POURTANT...



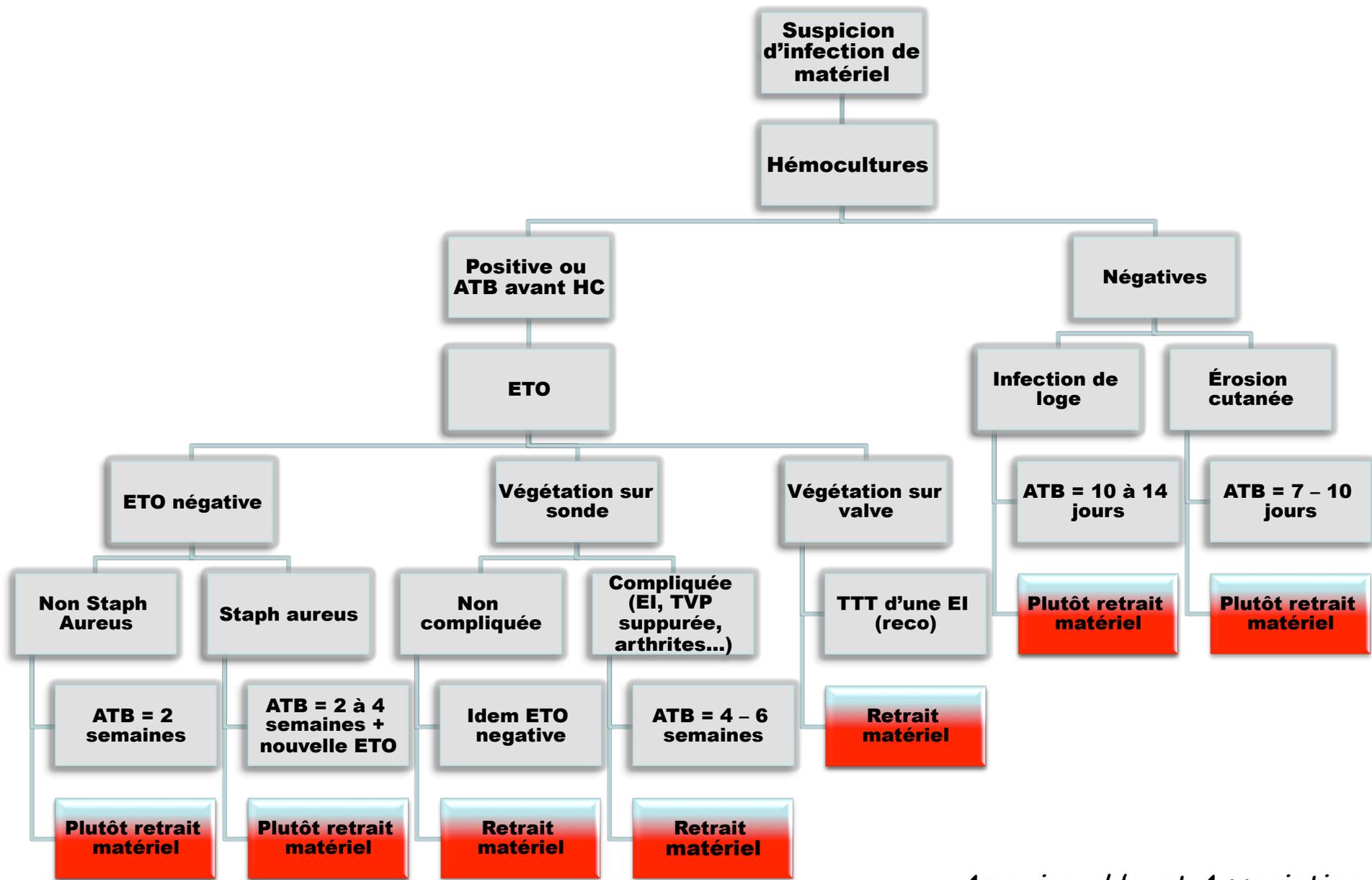
Pas de marqueur de stérilisation du biofilm (BF)

Peu de méthodes de prévention

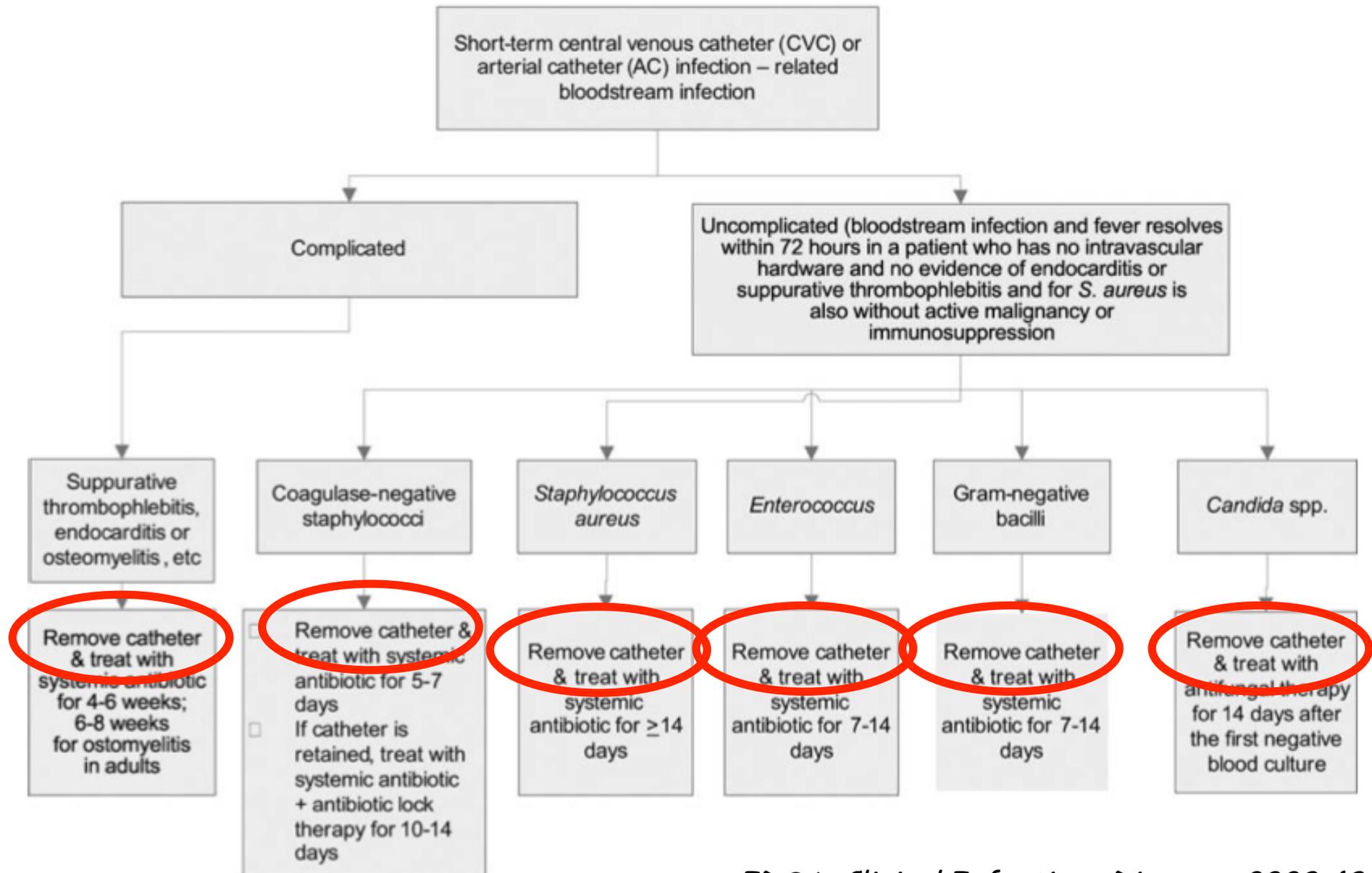
Pas d'évaluation de l'efficacité des ATB en BF

Principale mesure thérapeutique : retrait du BF...

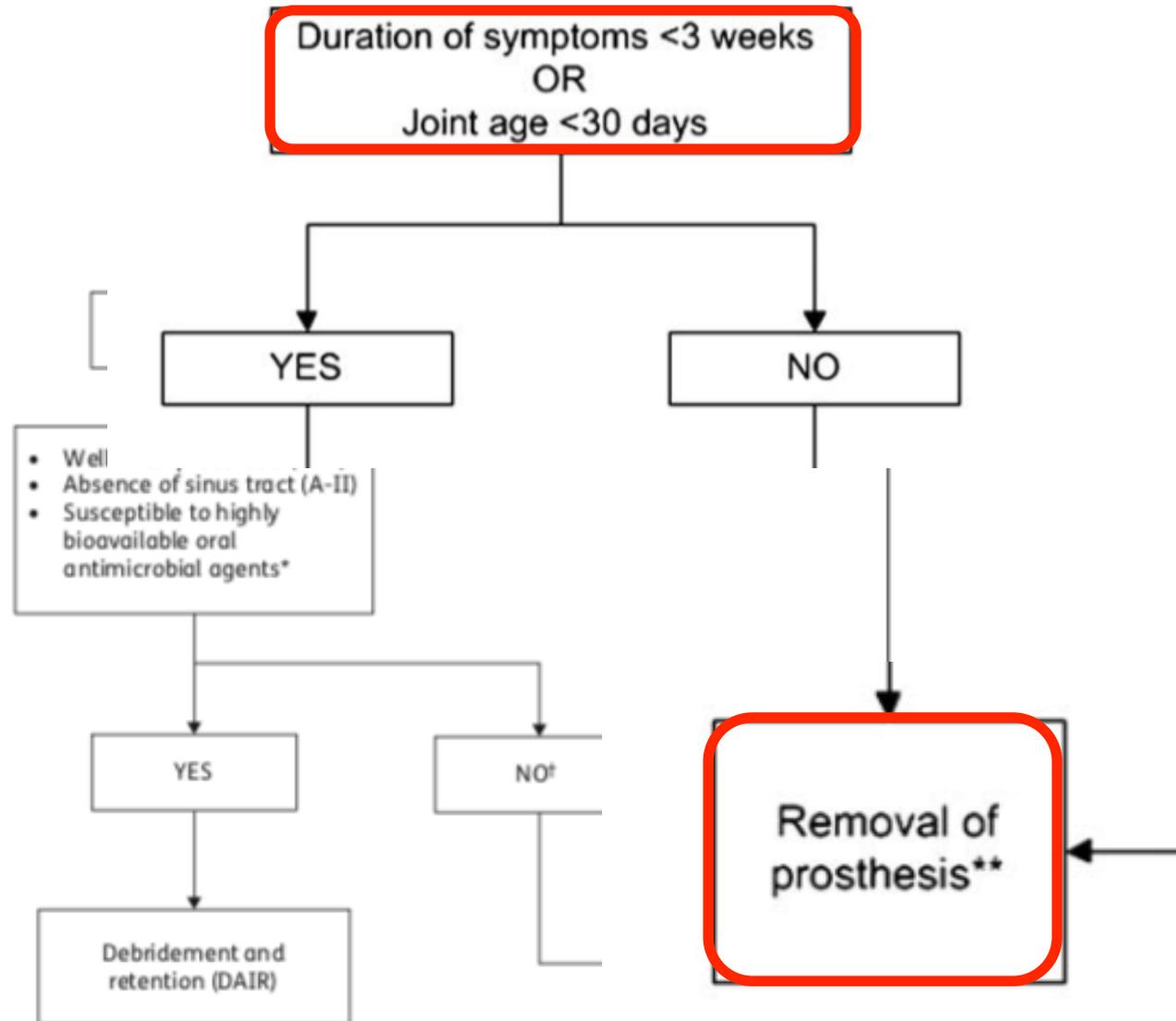
BIOFILM ET FRUSTRATION...PACE MAKER



BIOFILM ET FRUSTRATION...CATHÉTER CENTRAL



BF ET FRUSTRATION : INFECTION DE PROTHÈSE ARTICULAIRE



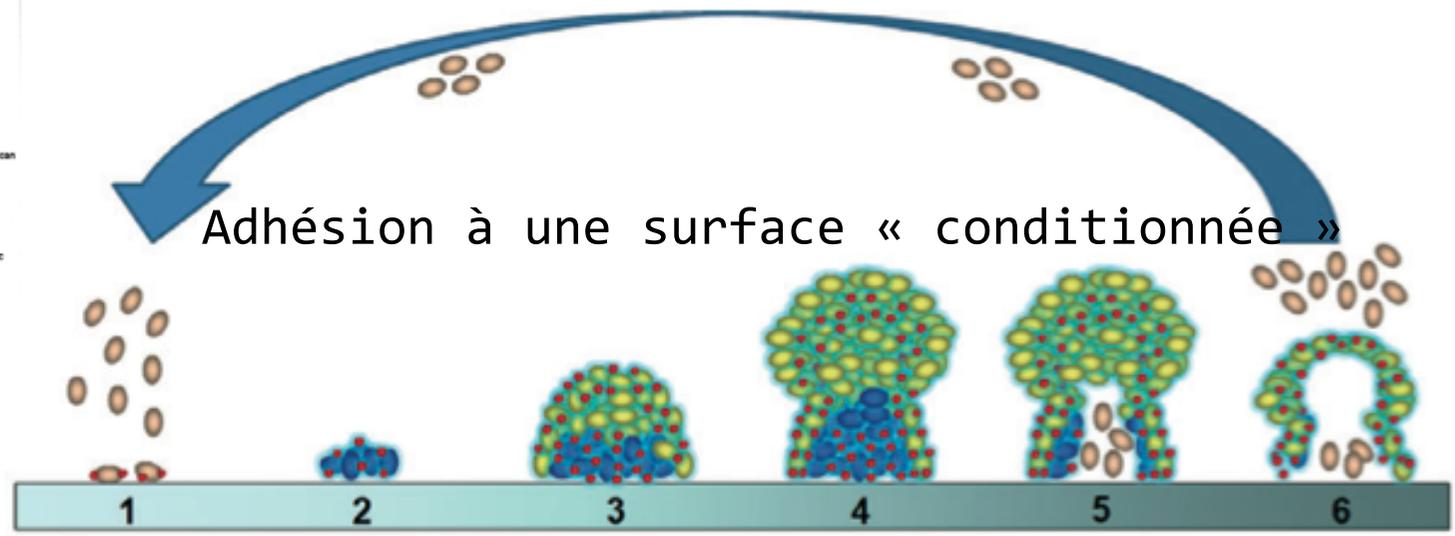
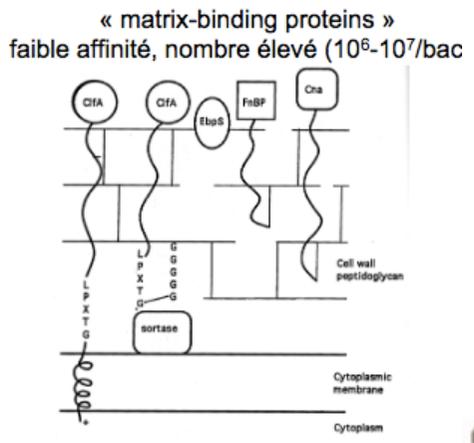
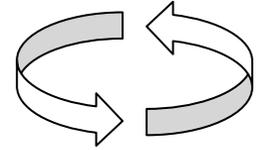
INSIDE THE BIOFILM



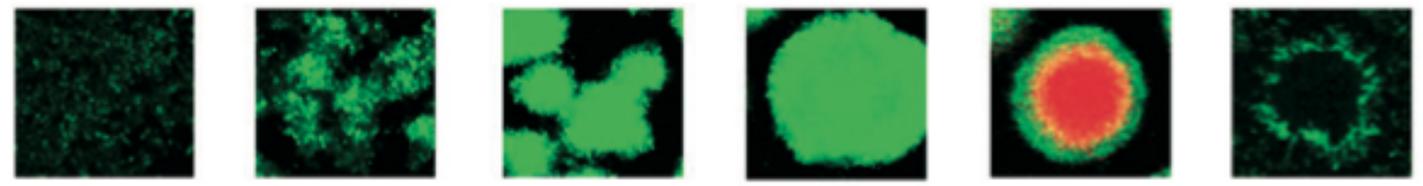
BIOFILM : « THE RACE FOR THE SURFACE »

Biofilm : processus progressif

Bactéries planctoniques (libres)



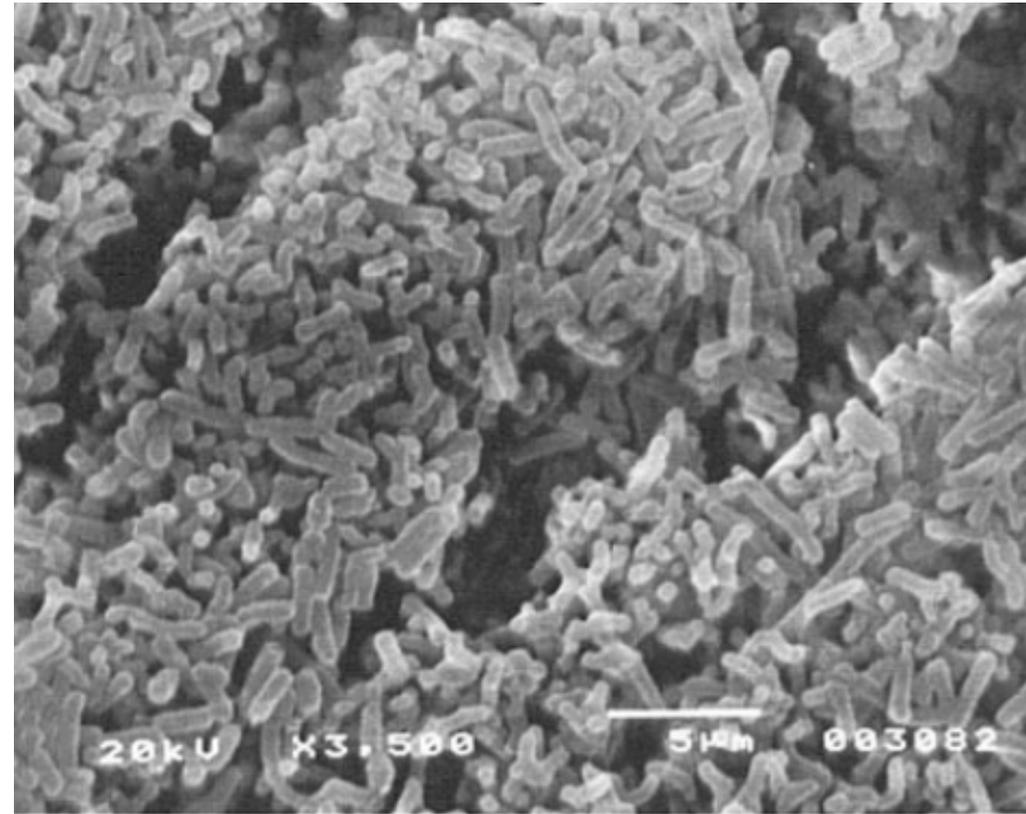
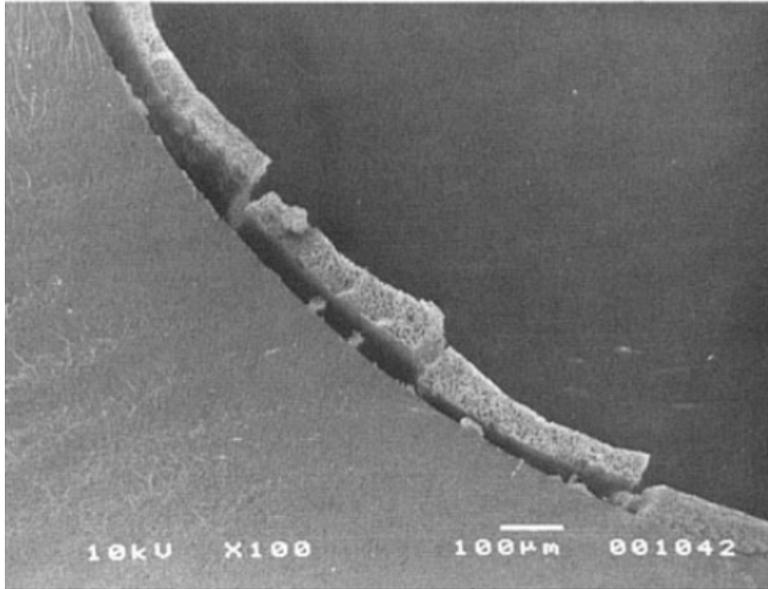
Biofilm development stages



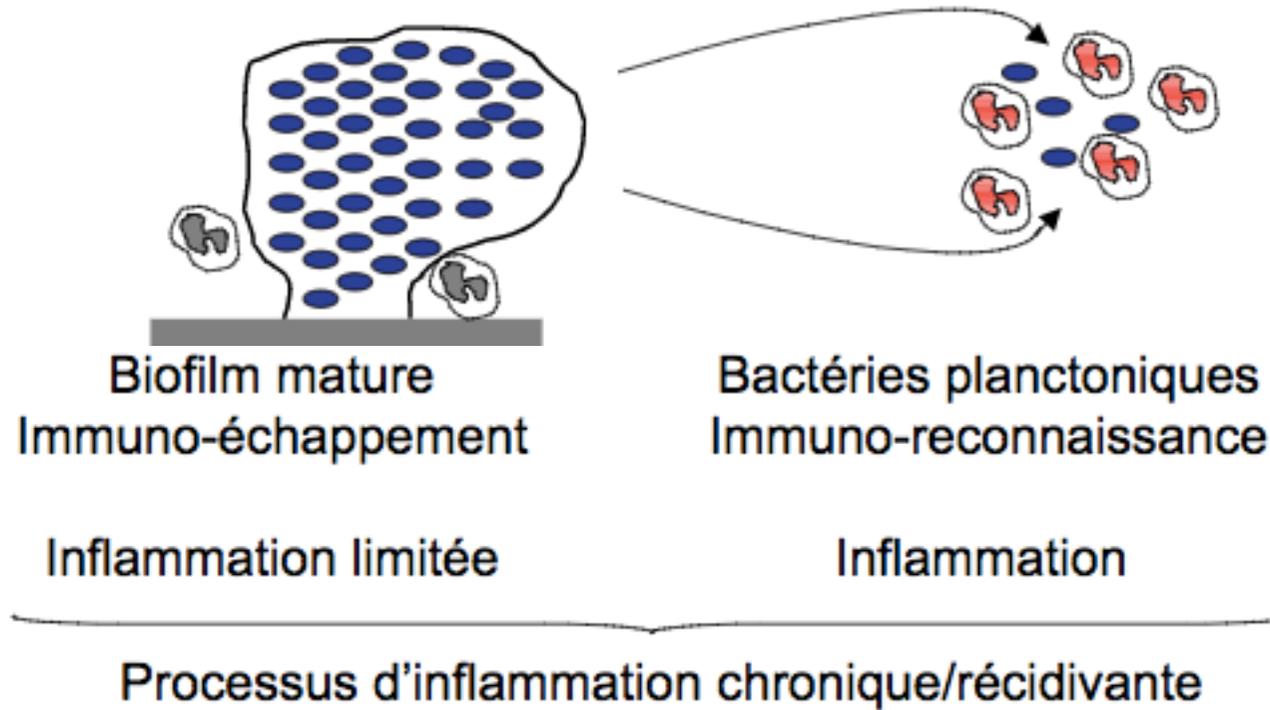
Attachment Microcolony Subpopulation interactions Macrocolony Activation Dispersion

Secondes Minutes Heures Jours

BIOFILM ET INFECTION...



BIOFILM ET IMMUNITÉ



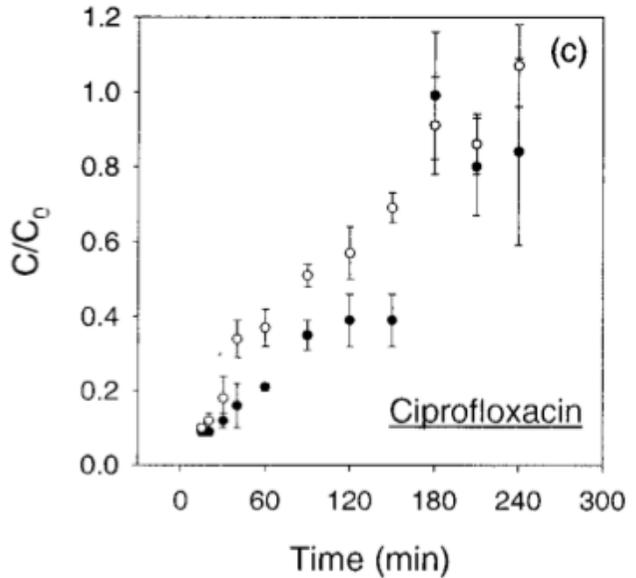
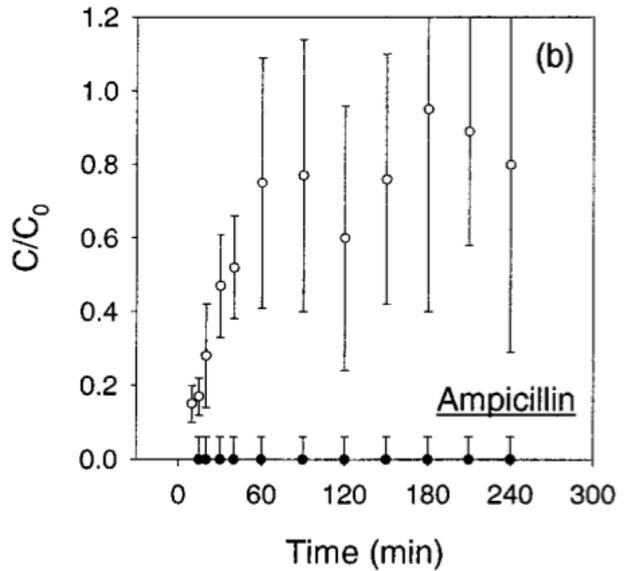
Taille du BF : obstacle à la phagocytose... enzymes toxiques pour les tissus

Matrice extracellulaire: obstacle à la reconnaissance des Ag par les Ac

DIFFUSION ATB

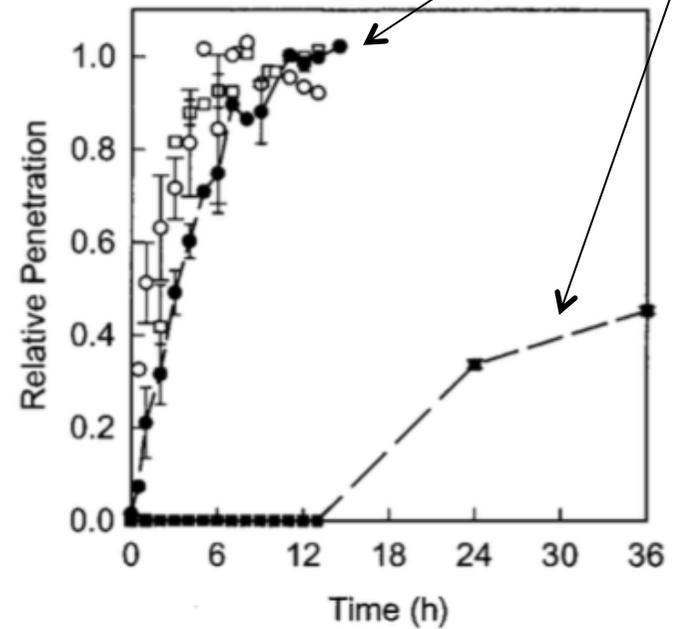
- Barrière exo-polysaccharidique + ADN dégradé

K. pneumoniae



Anderl, *Antimicrob. Agents Chemother.* 2000;44(7):1818-24

Pseudomonas (cipro vs tobra)

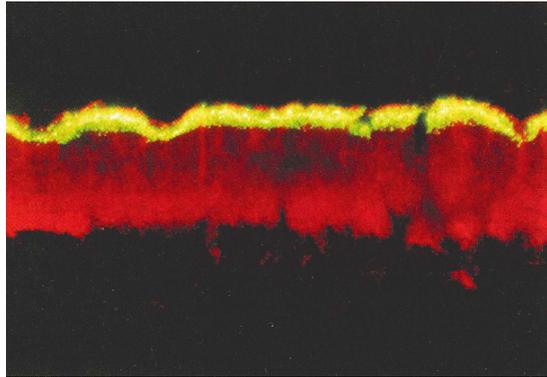


Walters. *Antimicrob Agents Chemother.* 2003 Jan;47(1):317-23.

➔ barrière perméable / retarde la diffusion / gradients f(structure BF/ATB)

ALTERATION DU MICRO-ENVIRONNEMENT

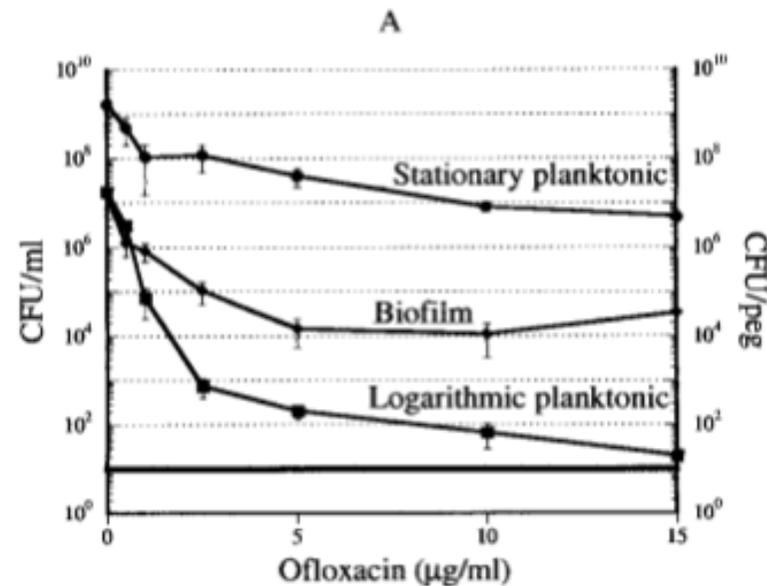
Surface → profondeur : gradient nutriments, O_2 ... ↘ division bactérienne



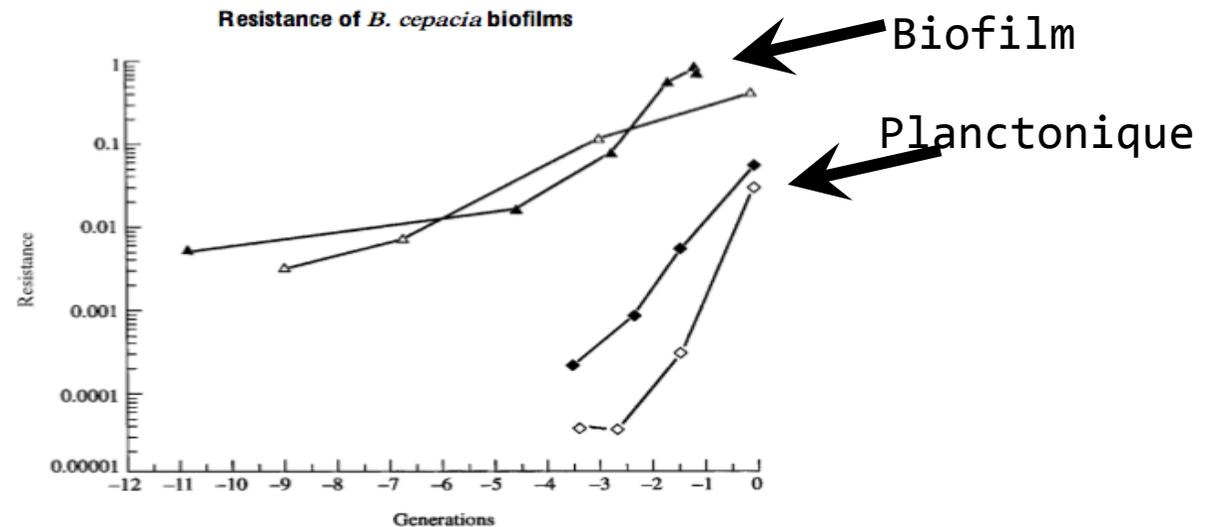
Synthèse protéique dans une biofilm de *P. aeruginosa*

Activité métabolique forte (jaune) ou faible (rouge)

Xu, FEMS Microbiol lett, 2001;199:167-71



Spoering, J Bact 2001(183)23:6746-51



Desai, J of Antimicrob Chemother, 1998(42):153-60

COMMUNICATION INTER-BACTÉRIENNE ?



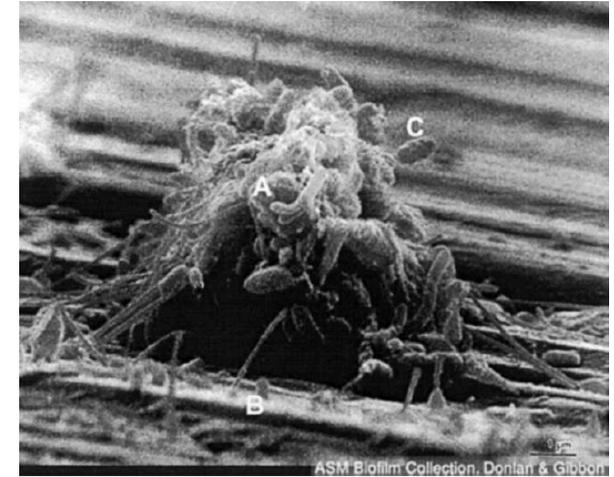
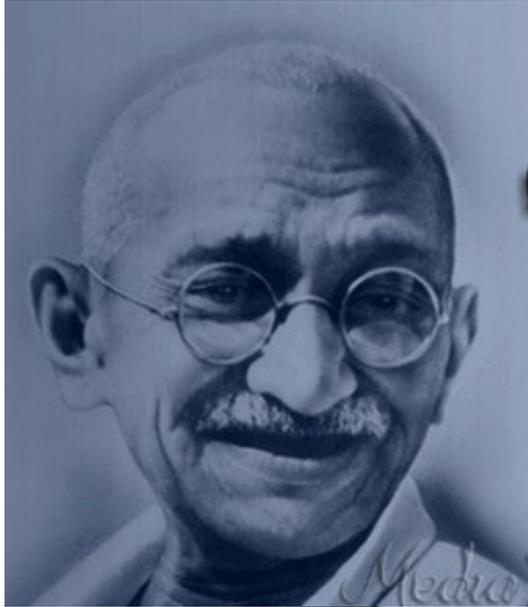
Bactéries à droite :

- PAS de production de ce signal moléculaire
- captent
- s'allument

Bactéries à gauche :

- capables de produire un signal moléculaire
- capables de détecter ce signal
- s'allument quand signal atteint un certain niveau

SOCIO-MICROBIOLOGIE

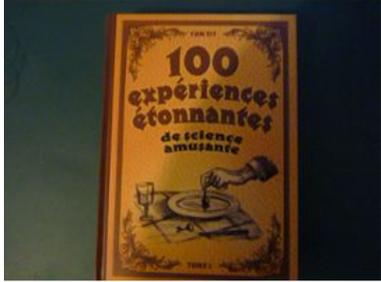


Effet stade ?
Effet internat ?

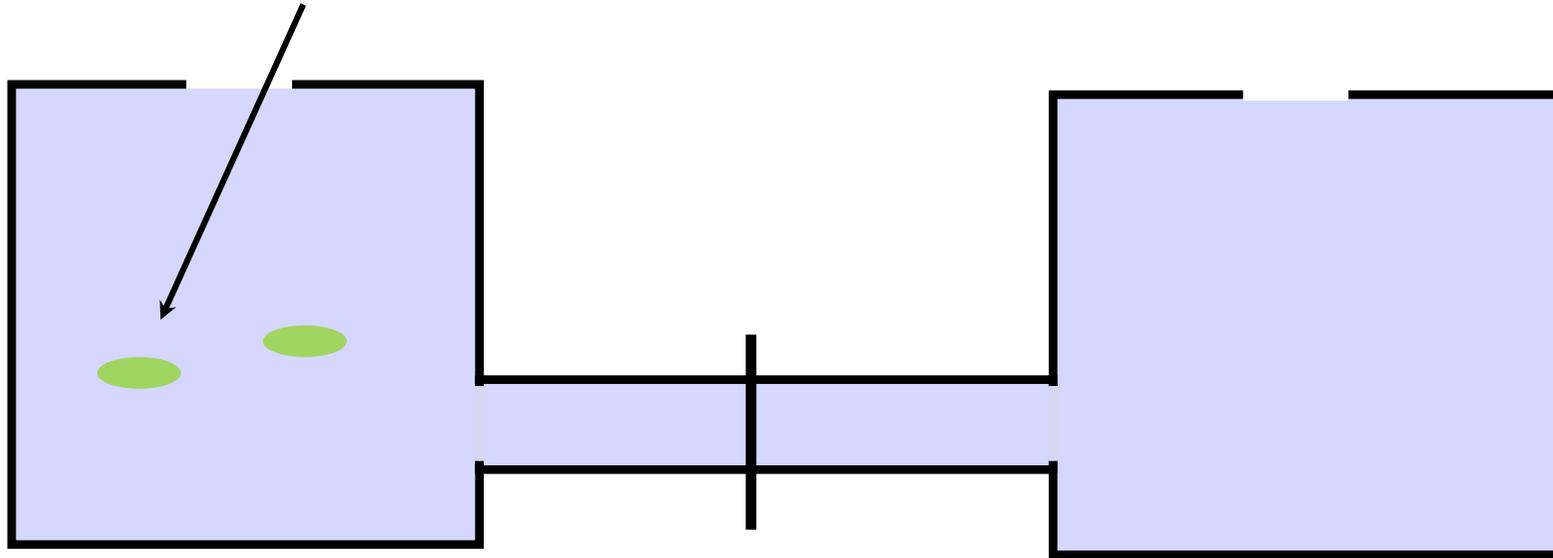
Quorum Sensing



QUORUM SENSING : « GOSSIP IN SLIME CITY »



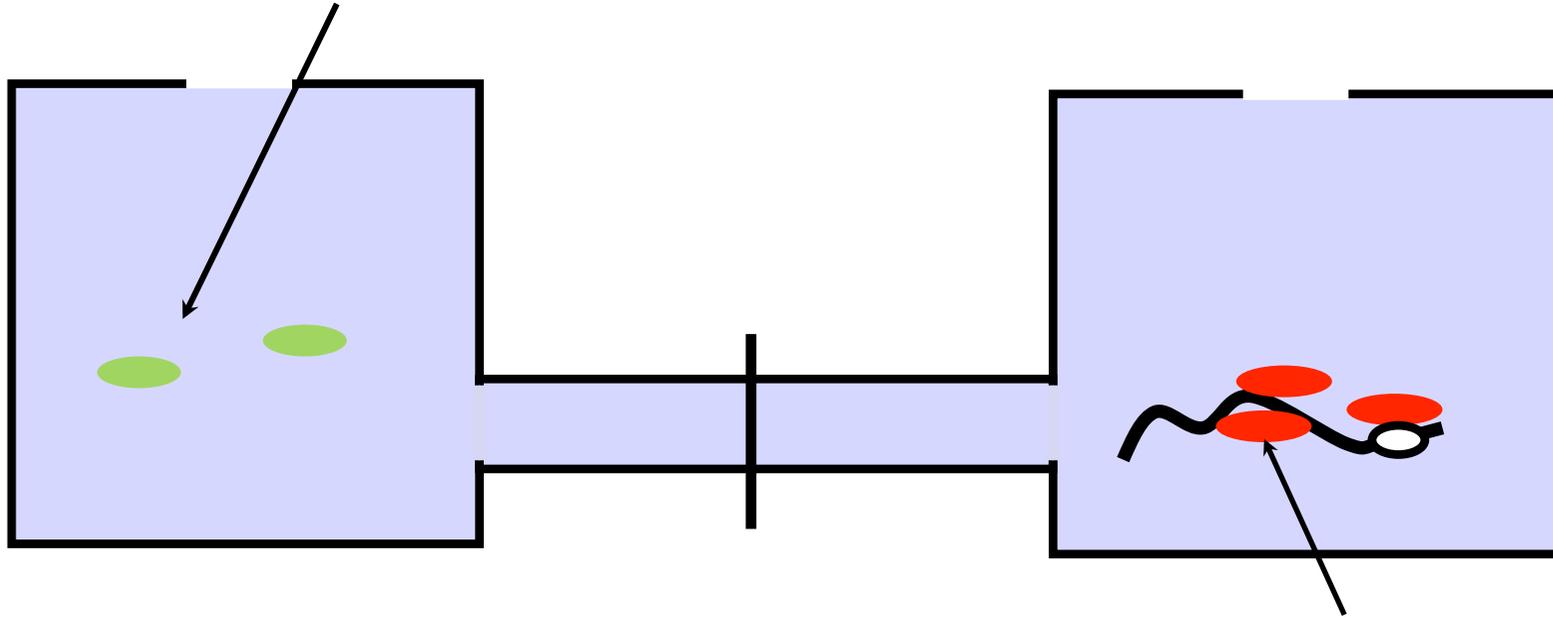
Concentration bactéricide = 1 mg/L



 Cellules libres : Concentrations minimales inhibitrice et bactéricide

QUORUM SENSING : « GOSSIP IN SLIME CITY »

Concentration bactéricide = 1 mg/L

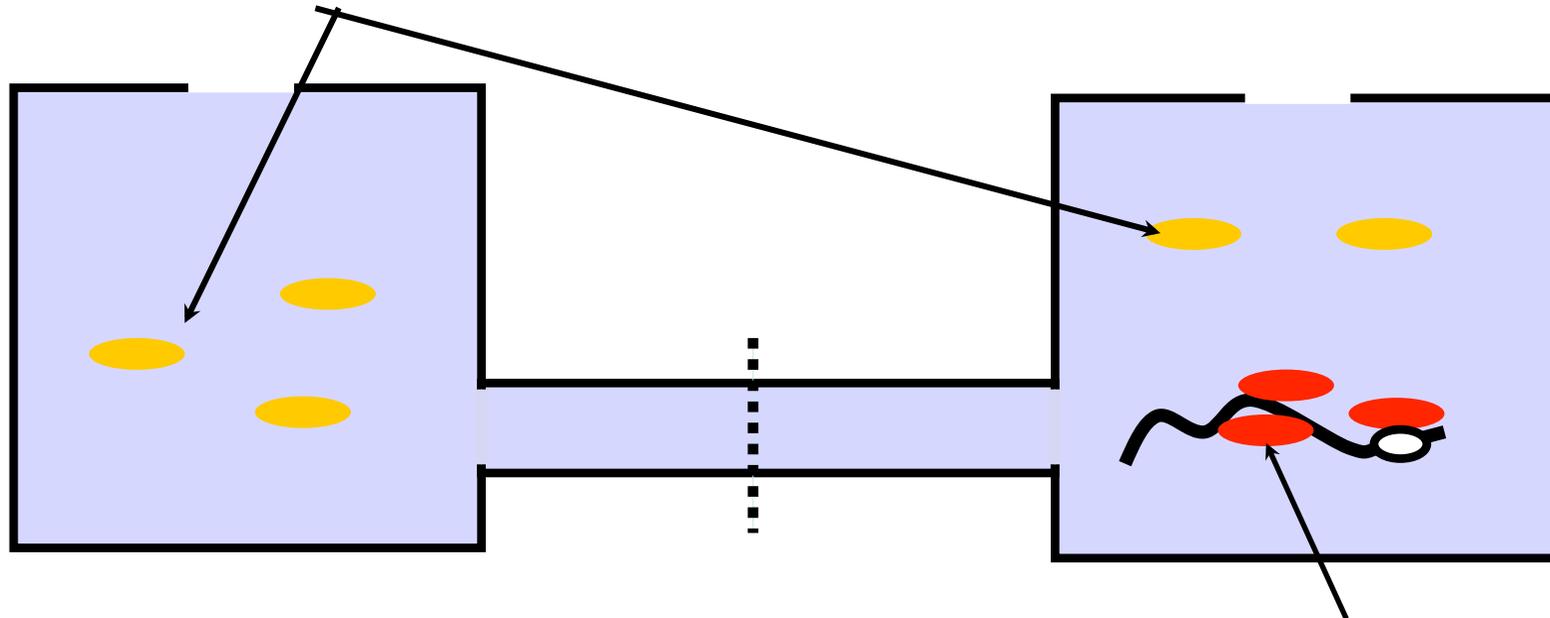


Concentration bactéricide ≈ 100 à 1000 mg/L

-  Cellules libres : Concentrations minimales inhibitrice et bactéricide
-  Cellules en biofilm : Concentration minimale d'éradication du biofilm (CMEB)

QUORUM SENSING : « GOSSIP IN SLIME CITY »

Concentration bactéricide = 10 mg/L



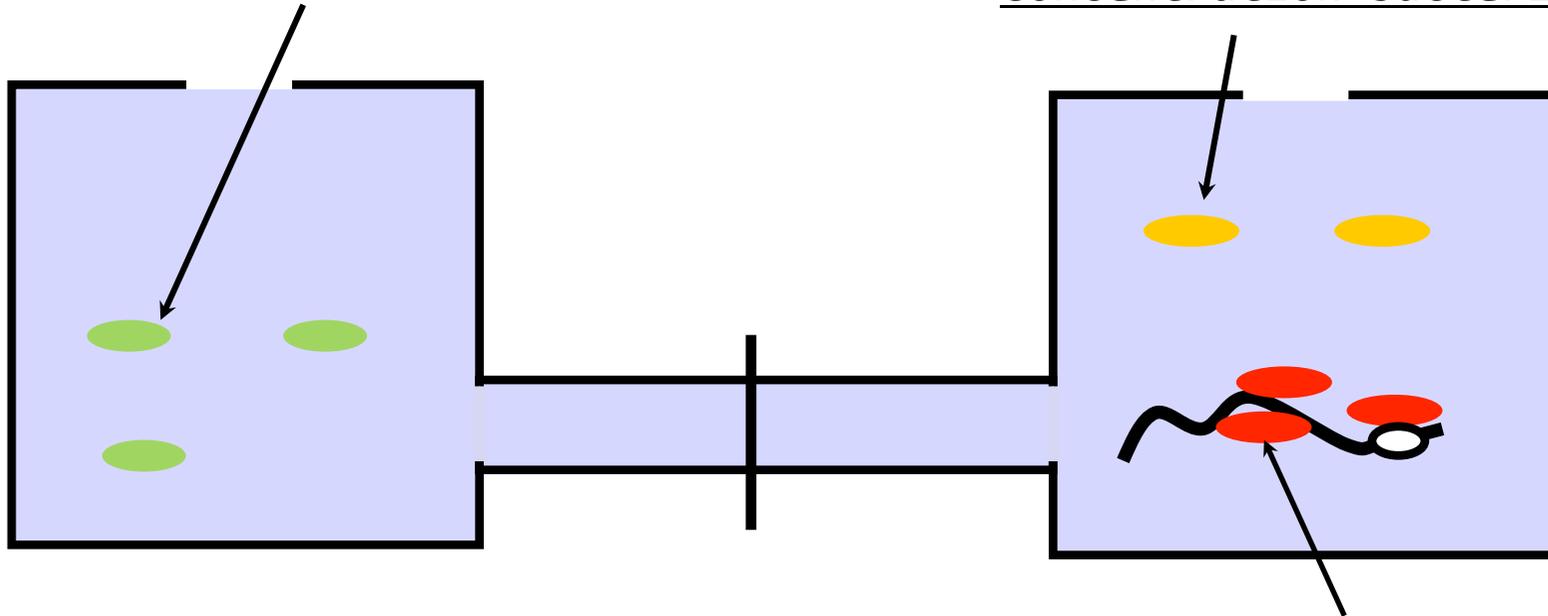
Concentration inhibitrice $\approx n \times 100$ à 1000 mg/L

-  Cellules libres : Concentrations minimales inhibitrice et bactéricide
-  Cellules planctoniques : Concentrations minimales inhibitrice et bactéricides des cellules planctoniques
-  Cellules en biofilm : Concentration minimale d'éradication du biofilm (CMEB)

QUORUM SENSING : « GOSSIP IN SLIME CITY »

Concentration bactéricide = 1 mg/L

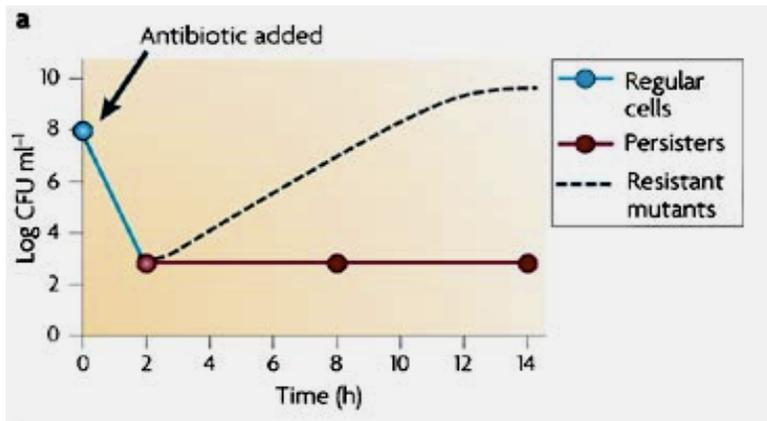
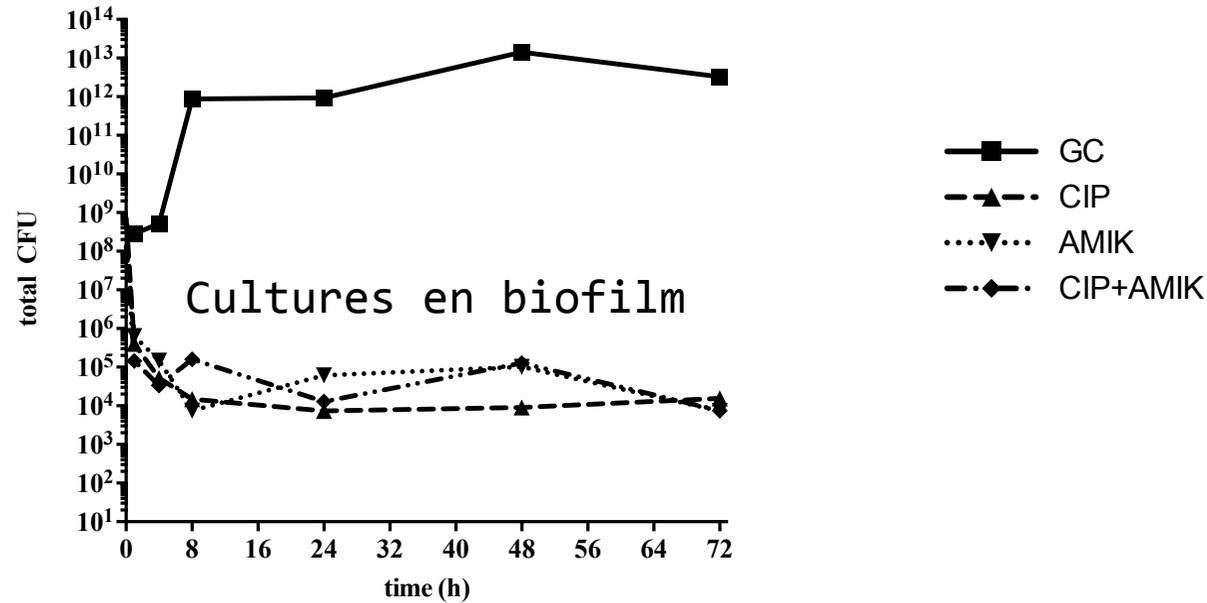
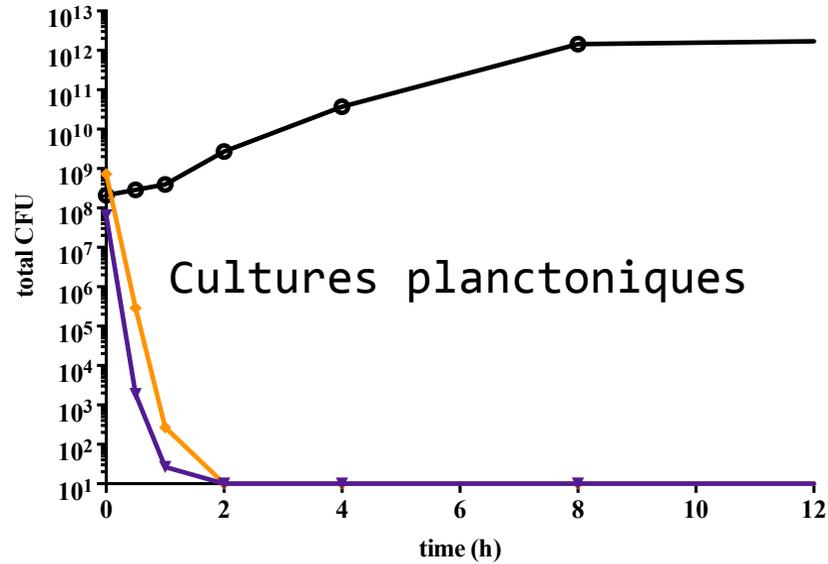
Concentration bactéricide = 10 mg/L



Concentration inhibitrice $\approx n \times 100$ à 1000 mg/L

-  Cellules libres : Concentrations minimales inhibitrice et bactéricide
-  Cellules planctoniques : Concentrations minimales inhibitrice et bactéricides des cellules planctoniques
-  Cellules en biofilm : Concentration minimale d'éradication du biofilm (CMEB)

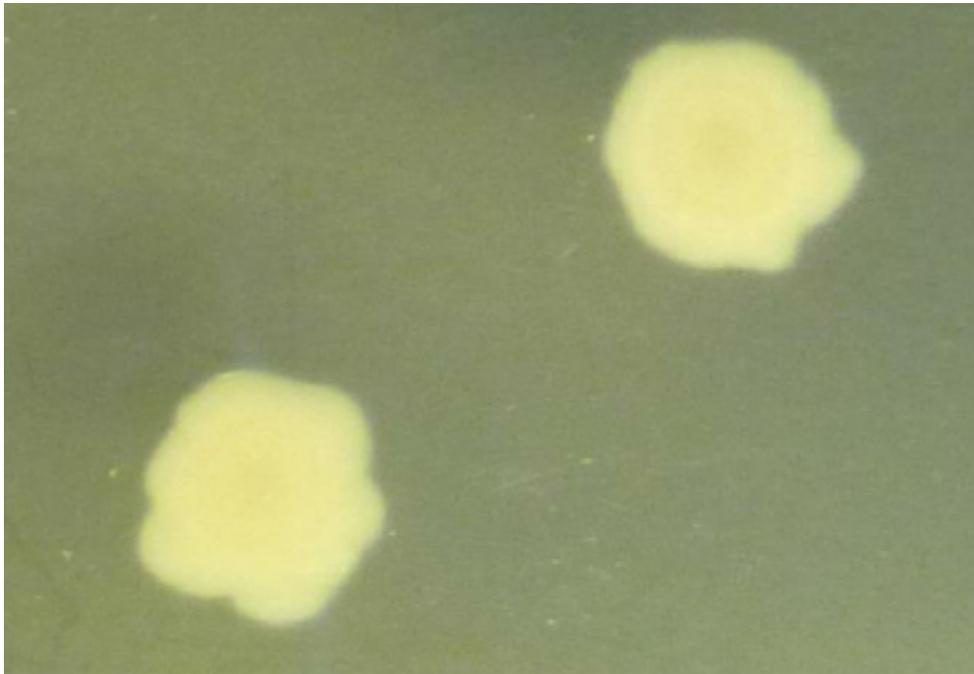
QUORUM SENSING : « GOSSIP IN SLIME CITY »



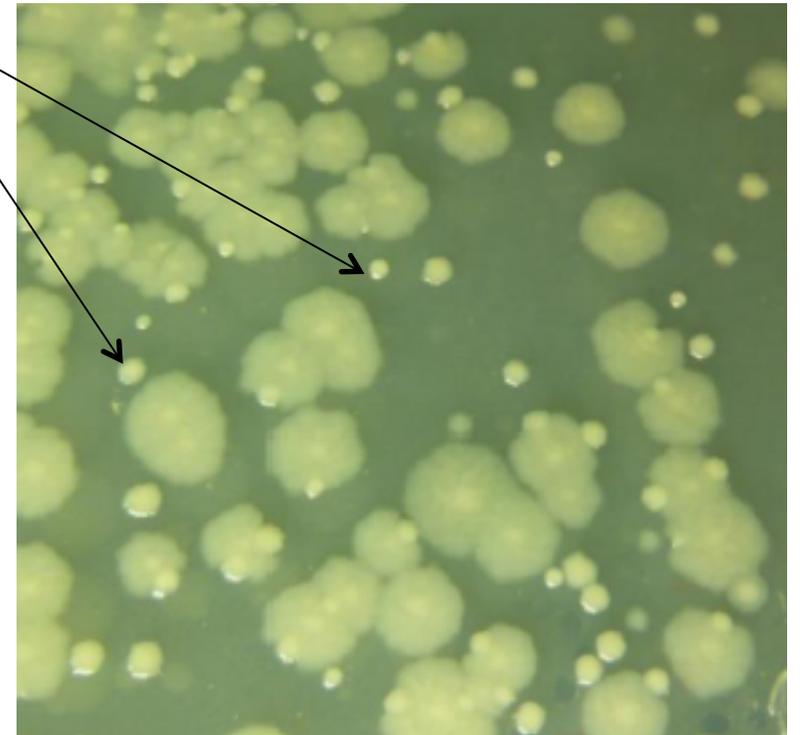
Bactéries tolérantes aux antibiotiques
 Transitoirement
 Du fait de leur environnement
 Mais qui ne sont pas résistantes

ALTÉRATIONS MORPHOLOGIQUES

Variants à petites colonies de la même bactérie



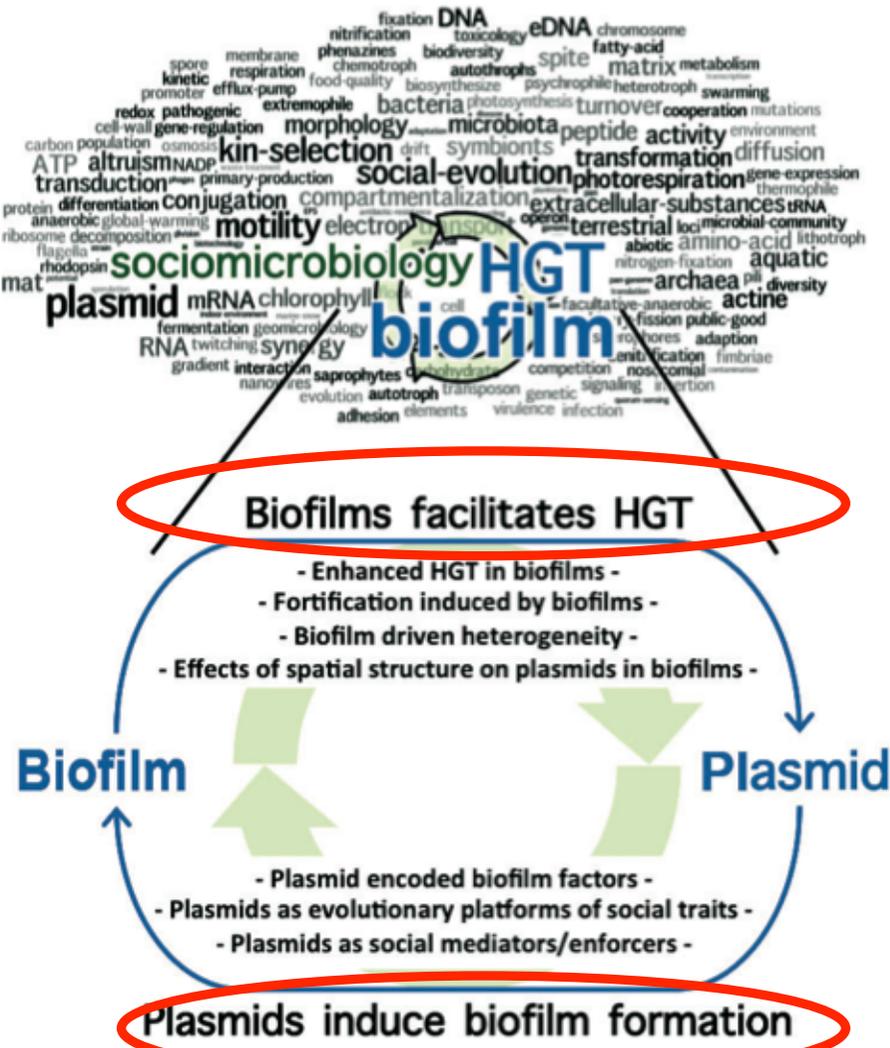
Culture « planctonique »



Culture en biofilm

TRANSFERT GÉNÉTIQUE HORIZONTAL

Biofilm : facilitateur de transfert de matériel génétique



		Effect on recipient	
		+	-
Effect on actor	+	Mutualism + \ +	Selfishness + \ -
	-	Altruism - \ +	Spite - \ -
		Cooperation	Competition

« Mafia methods ensuring stability » :

« addiction systems that give the bacterial hosts an offer they cannot refuse »

BF = MESURER L'IMPACT IN VITRO

In vitro

and proteomics-based studies on biofilms that have necessitated growth of larger quantities of biofilms for DNA, RNA, or protein extraction. To this end, several investigators have used growth strategies involving substrata with large surface areas (Oosthuizen et al., 2001; Whiteley et al., 2001a). Biofilms have also been cultured under batch as well as continuous culture conditions (Whiteley et al., 1997). For several reasons (cost, ease of experimental

manipulation, special logistical requirements, etc.) there are many variations on these concepts. While at first the plethora of techniques can be quite confusing to many people there are several basic concepts and strategies most commonly involved in biofilm studies. It is our intention in this section to focus on some of the most commonly used techniques for routine biofilm growth (Table 5), imaging (Table 6), and broadly based growth and

TABLE 5 Common laboratory biofilm techniques*

Technique	Organism and study	Reference(s)
Batch culture		
Cover slip on culture	<i>S. aureus</i> <i>hacS</i>	Meece et al., 2003
Flow cell (once-through)*	<i>E. coli</i> gene array	Schembra et al., 2003
Microtiter assay*	<i>P. aeruginosa</i> chemotaxis	Davey et al., 2003
Modified microtiter assay (additional substrate added)	<i>P. aeruginosa</i> <i>rel</i> gene	O'Toole and Kolter, 1998
Polystyrene-petal dishes	<i>C. albicans</i> biofilm formation	Chaplin et al., 2001
Polycarbonate membrane on culture	Dispersion of oral bacteria	Kaplan and Ficek, 2002
Robbins' device (original)*	<i>K. pneumoniae</i> antibiotic resistance	Anderl et al., 2003
Robbins device—modified (MRD)*	<i>P. aeruginosa</i> antibiotic resistance	Walters et al., 2005
Rotating-disk bioreactor*	Industrial biofouling	McCoy et al., 1981
	<i>P. aeruginosa</i> biofilms and antibiotic resistance	Nidetz et al., 1985
	<i>P. aeruginosa</i> heavy metal resistance	Taitel and Parash, 2003
	MBed culture production	Lawrence et al., 2003
Continuous culture systems		
Chemostat wall	Competition in mixed culture biofilms	Christensen et al., 2002
Chemostat with once-through tubing	<i>P. putida</i> biofilm production	Sauer and Cooper, 2001
Chemostat with suspended surfaces	<i>S. aureus</i> biofilm dispersal susceptibility	Lappens et al., 2003
Chemostat with flow cell*	<i>S. aureus</i> biofilm transformation	Li et al., 2001
Chemostat with recirculating culture*	<i>P. aeruginosa</i> cell signaling	Purosoj et al., 2002
	Growth rate control of biofilms in MRD	Whiteley et al., 1997
	Mixed culture biofilms	Whiteley et al., 2001b
	<i>E. coli</i> <i>gcs</i> and stringer response genes	Adams and McLean, 1999; Balcer and McLean, 2002
Utip flow system	Phosphate-limited biofilms	Huang et al., 1998
Nutrient-limiting membrane	Growth rate control of biofilm populations	Gilbert et al., 1989
Minimizing biofilm formation*		
Agar-embedded cells (simulated biofilm)	Fiber optic mapping of biofilms	Thar et al., 2004
Aquarium gravel	Drug resistance of model biofilms	Jouenne et al., 1994
Bioreactor (pulsed bed)	Gene array study of <i>P. aeruginosa</i> biofilm	Whiteley et al., 2001a
Glass wool in liquid culture	Mercury removal from wastewater	von Gunstein et al., 2002
Sand suspension in bioreactor	Proteomic analysis of <i>B. subtilis</i>	Steyn et al., 2001
	Hexadecane biotransformation	Holten et al., 2002
	σG regulation of <i>E. coli</i> biofilms	Prigent-Combaret et al., 2001

* * * addressed in detail in the text.

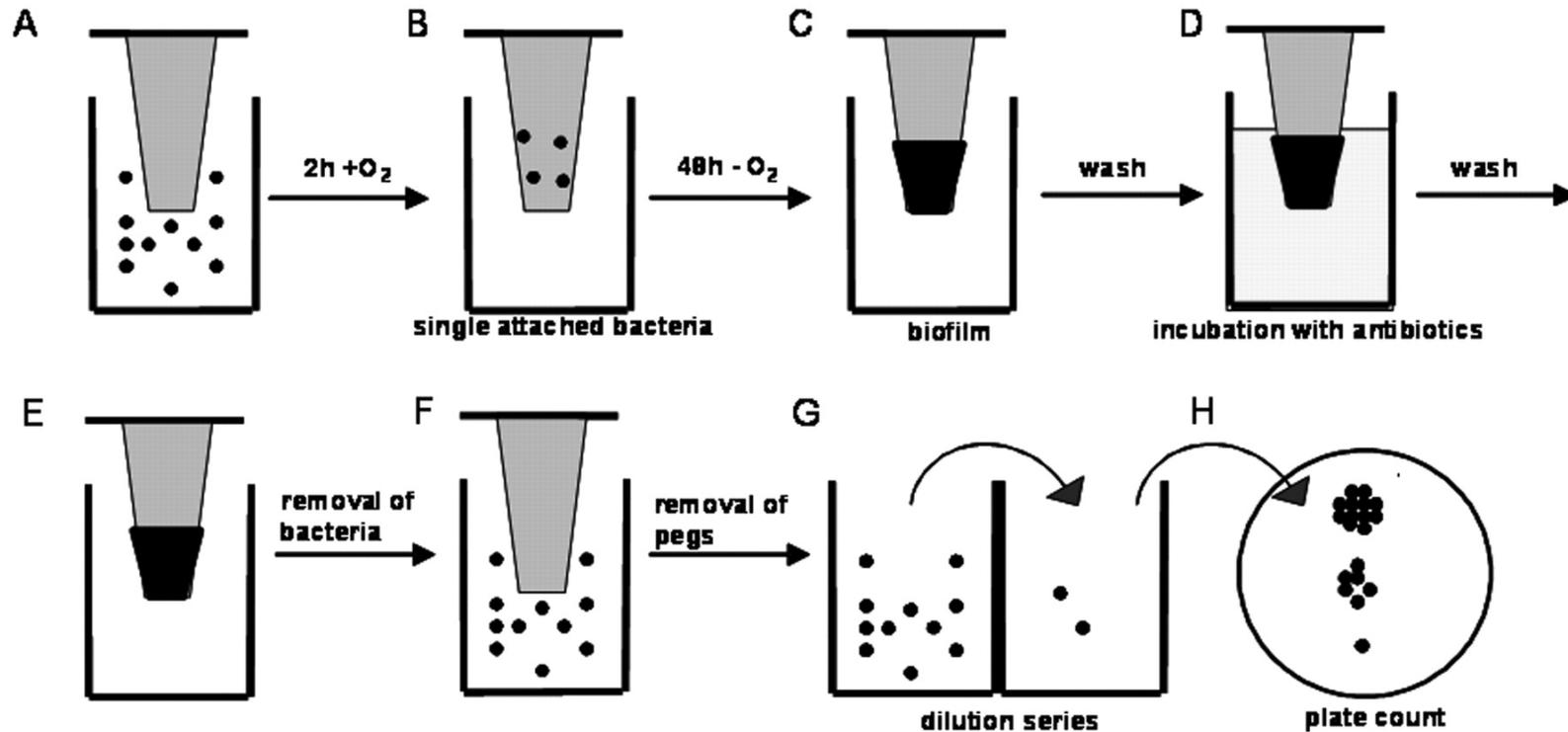
TABLE 6 Biofilm imaging, image analysis, and infrared spectroscopy*

Technique	Organism and study	Reference(s)
SCM and related techniques		
Original SCM technique*	Complex biofilm architecture	Lawrence et al., 1991
Deconvolution microscopy	Development of technique for biofilms analysis	Phipps et al., 1990
Two-photon microscopy	Development of technique for biofilm analysis	Vincent et al., 1999
Flow cell (once-through)	<i>E. coli</i> gene array	Schembra et al., 2003
Fluorescence in situ hybridization	<i>P. aeruginosa</i> chemotaxis	Davey et al., 2003
Growth rate determination	Identification of occurrence numbers	Amann et al., 1992
	Acridine orange correlation with growth rate	Muller et al., 1996; Stenzberg et al., 1999; Wenfand et al., 1996
	Differential respiration stain	Huang et al., 1995
Reporter gene	Unstable <i>gfp</i> and other reporter genes for in situ gene expression	Anderson et al., 1998, 2001; Korber et al., 1999; Muller et al., 1998
Reporter stains and other chemicals	Dyes and particles to measure biofilm diffusion	de Beer et al., 1997; Stoodley et al., 1994
	Biofilm chemical microenvironment	de Beer and Schramm, 1999
	Protection of acid labile crystal	Molven et al., 1991
Time-lapse microscopy	Biofilm flow and surface detachment phenomena	Stoodley et al., 1999a, 2002
Electron microscopy techniques		
Antibody stabilization for electron microscopy	Identification of biofilm matrix polymers and coagulating preparation for SEM and TEM	Mackie et al., 1979
Elemental identification	Mineral precipitation and identification in aquatic biofilms, SEM with X-ray analysis	Kochauer et al., 1994
Environmental SEM	Examination of partially hydrated corrosion biofilms	Litke and Wagner, 1996
Image analysis		
COMSTAT	Quantitative image analysis	Heylorn et al., 2000b
Image structure analyzer	Quantitative image analysis	Purosoj et al., 2002
Ultra-Lapse imaging	Biofilm migration on surfaces	Stoodley et al., 1999b
Other imaging techniques		
Microelectrodes	Oxygen diffusion in biofilms	Stewart et al., 2000
Nuclear magnetic resonance	Imaging biofilms in opaque environments	Hodkins et al., 1999; Kowandowski et al., 1993
Glass slides (sliding stage)	Biofilms conjugation	Ghigo, 2001; Haugler and Wiersma, 1999
Infrared spectroscopy		
ATR spectroscopy	Antibiotic penetration into biofilms	Nichols et al., 1985; Sued et al., 1994
ATR flow cell	Algalite structure in biofilms	Nevens et al., 2001
ATR and Raman spectroscopy	Antibiotic effect against <i>C. albicans</i>	Choo-Smith et al., 2003; Sued and Tyler, 2002
Mix spectroscopy and surface analysis techniques		
Auger and X-ray spectroscopy	Biofilm copper corrosion	Jolly et al., 1989
X-ray photoelectron spectroscopy, secondary ion mass spectroscopy	Study of conditioning films on surfaces	Tyler, 1997
Mass spectrometry	Evolution of biofilm growth rates	Freeman and Lock, 1995

* ATR spectroscopy, attenuated total internal reflectance infrared spectroscopy.

BF = MESURER L'IMPACT IN VITRO

In vitro : Calgary Biofilm Device



Herrmann G et al. J Infect Dis. 2010;202:1585-1592

Ceri, J Clin Microbiol 1999;37(6): 1771-6

AUCUNE CORRÉLATION IMPACT IN VITRO / IN VIVO

Pseudomonas aeruginosa : CMI versus CMEB (Conc. Min. d'Eradication du Biofilm)

Antibiotic	MIC ($\mu\text{g/ml}$)		MBEC ($\mu\text{g/ml}$)		
	NCCLS assay ^a	Assay with CBD ^a	A_{650} ^a	0 CFU/peg ^b	
Amikacin	2	4	16	16	
Aztreonam	2	4	>1,024	>1,024	x 520
Ceftazidime	1	2	>1,024	>1,024	
Ciprofloxacin	0.25	0.25	4	4	x 16
Gentamicin	2	4	128	128	
Imipenem	1	4	>1,024	>1,024	
Piperacillin	2	16	>1,024	>1,024	
Tobramycin	0.5	1	2	2	

exposed to various concentrations of antibiotics to establish their 'biofilm eradication concentrations'. However, such testing has not yet resulted in reliable prediction of therapeutic success [34,113–115] (DII).

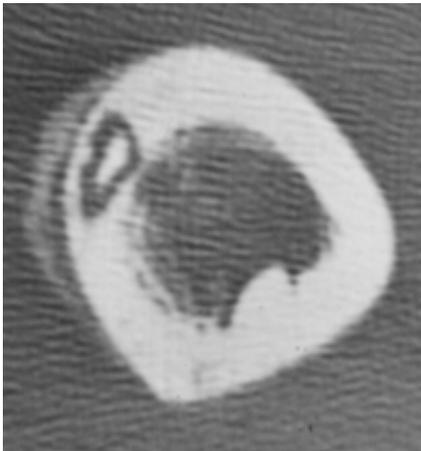


BIOFILM = IMPACT SUR IOAC, EXEMPLES

RECHUTES TARDIVES



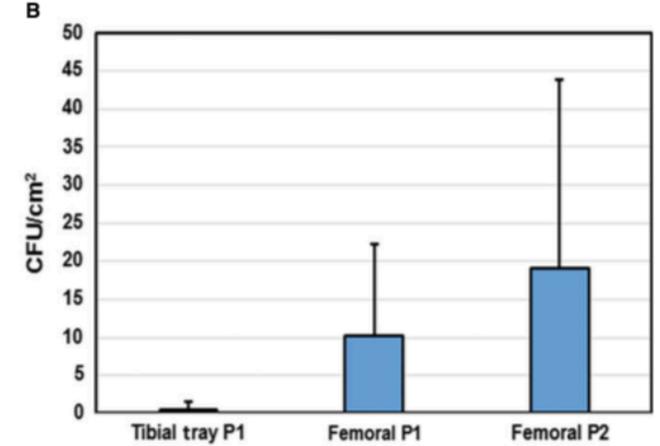
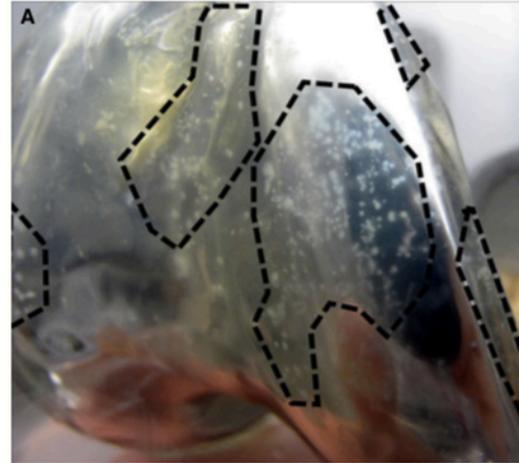
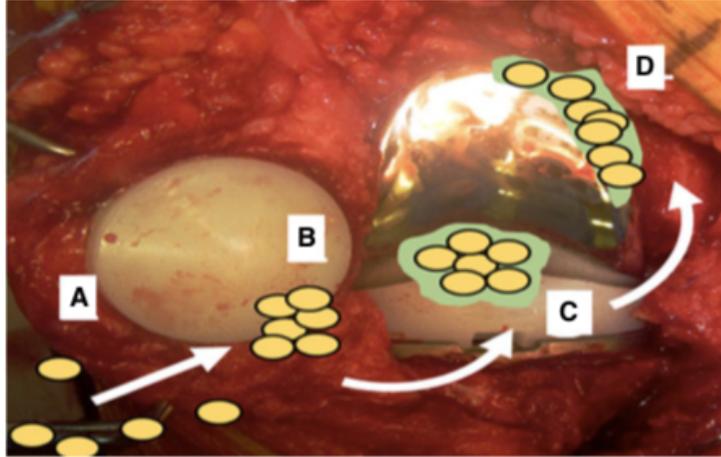
Rechute tardive :
+ 26 ans (staphylocoque)



Zimmerli, APMIS 2017

Moser, J Radiol Diag Interv (2012)

BF ET DIFFICULTÉS DIAGNOSTIQUES



Répartition hétérogène, aux différentes interfaces

- quantitativement
- ET
- qualitativement

BF ET DIFFICULTÉS DIAGNOSTIQUES



Variants à petites colonies :

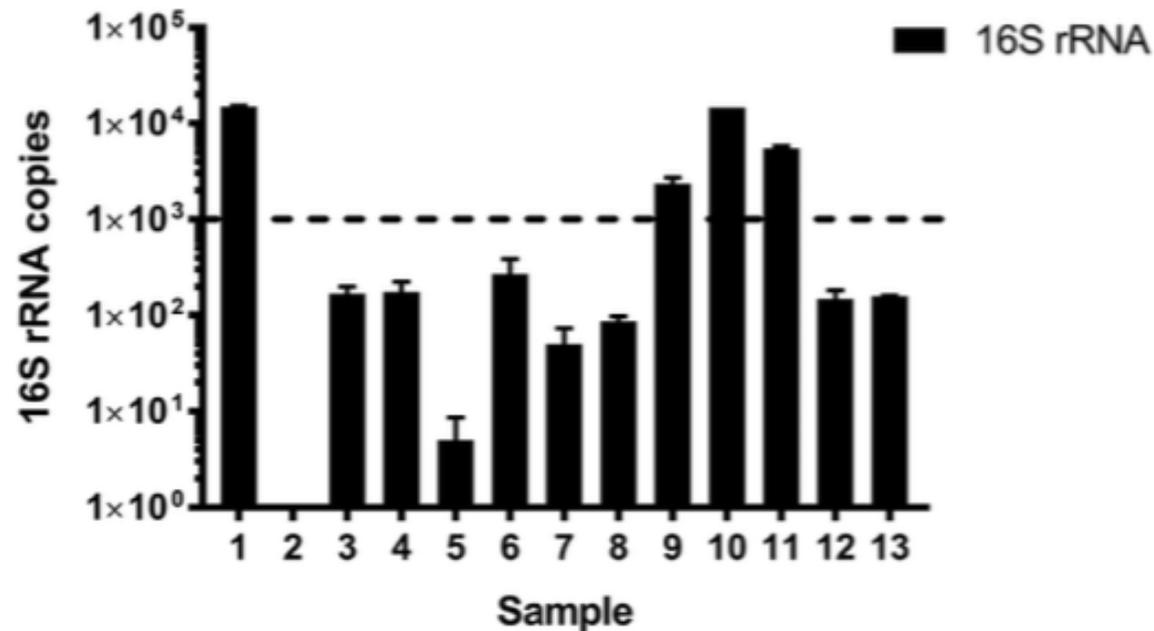
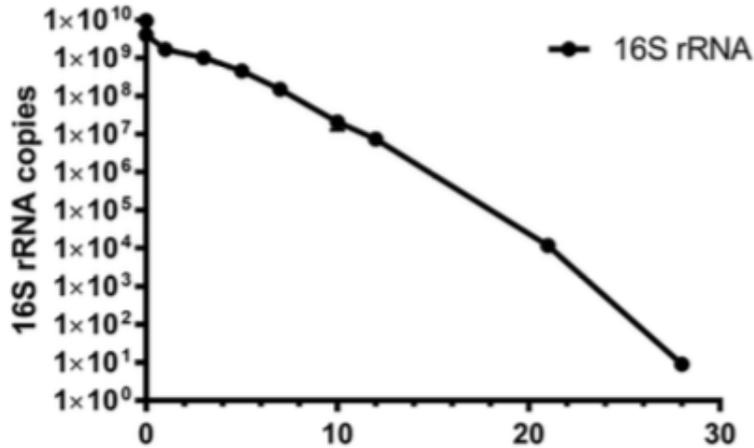
- forme différente d'un pathogène
- croissance lente
- morphologie différente
- localisation intracellulaire (échappement immun)
- faible réponse aux antibiotiques

Piffaut C, et al. BMJ Case Rep 2013

Neut, Acta Orthopaedica 2007

BF ET IMPACT DIAGNOSTIQUE/THERAPEUTIQUE

Cinétique naturelle de dégradation des ARN



Nombre de copies d'ARN présentes sur chaque spacer au moment de la repose

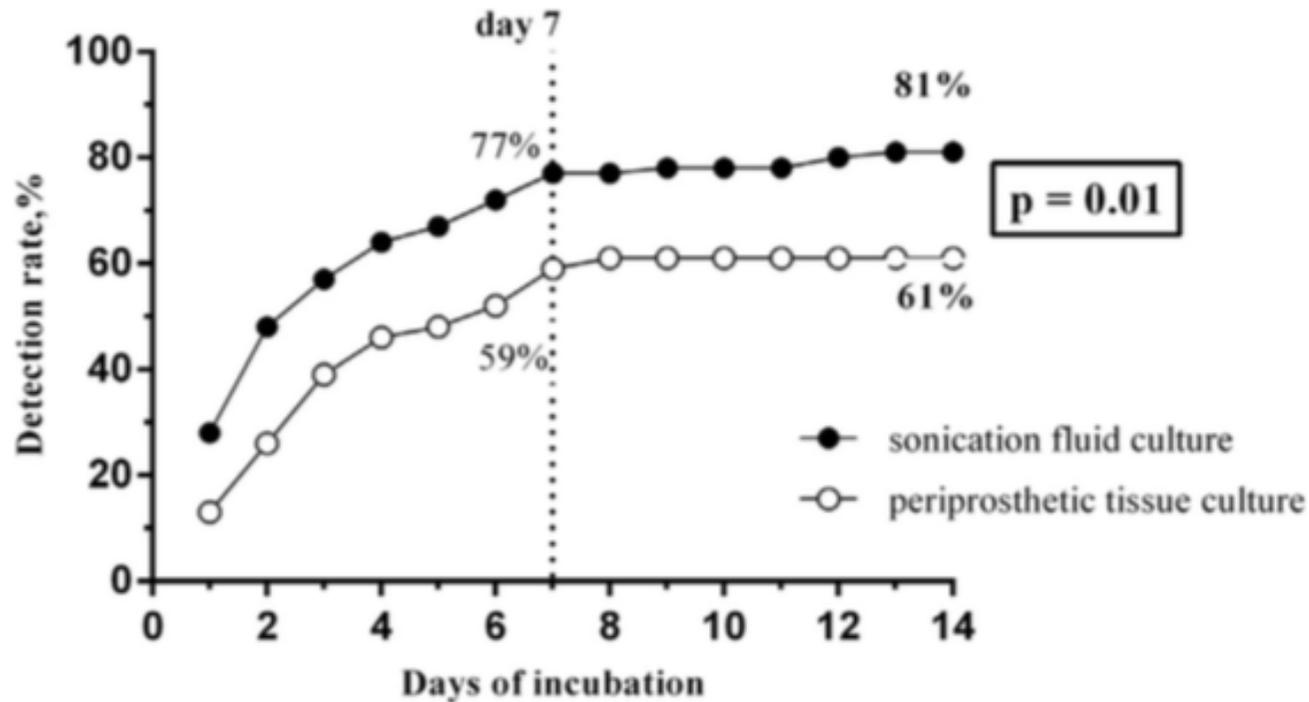
DISRUPTEZ LE BF !

Nécessité de méthodes diagnostiques spécifiques :
ex fragmentation par sonication

	Sensitivity of sonication fluid culture (%)	Sensitivity of periprosthetic tissue culture (%)	<i>p</i> value
Overall	77.04	55.73	0.01
Type of infection			
Early	87.5	62.5	0.48
Delayed	64.7	41.1	0.13
Late	80.6	61.1	0.14
Site of infection			
Hip	84.6	58.9	0.02
Knee	63.6	50.0	0.45

DISRUPTEZ LE BF !

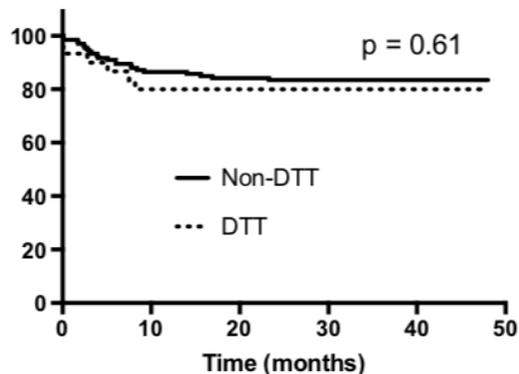
Nécessité de méthodes diagnostiques spécifiques :
ex. fragmentation par sonication



BF ET IMPACT PRONOSTIQUE

Pathogènes résistants aux traitements présumés efficaces sur les bactéries en BF (Difficult to treat = DTT)

Variable	All patients (n = 163)	DTT PJI group (n = 30)	Non-DTT PJI group (n = 133)	p value
Treatment				
Time until reimplantation (days)	63.9 ± 34.5	89.4 ± 50.5	58.1 ± 26.9	<0.001
CRP prior to reimplantation (mg/l)	11.9 ± 13.1	10 ± 11.8	12.3 ± 13.4	0.164
Total duration of antimicrobial therapy (days)	123.3 ± 57.7	150.8 ± 74.7	117.4 ± 51.7	0.003
Duration of i.v. antimicrobial therapy (days) ^a	32.8 ± 20.2	50.4 ± 31	29 ± 14.7	<0.001
Duration of oral antimicrobial therapy (days) ^a	84.1 ± 32.2	90.1 ± 47.3	82.8 ± 28.2	0.553
No. of revisions during interval	1.7 ± 1.3	2 ± 1.6	1.5 ± 1	0.324
Duration of hospital stay (days)	31.2 ± 15.9	44.5 ± 26.5	28.2 ± 10.4	<0.001



Survie =
 Délai ré-implantation >
 Durée de traitement >
 Durée d'hospitalisation >

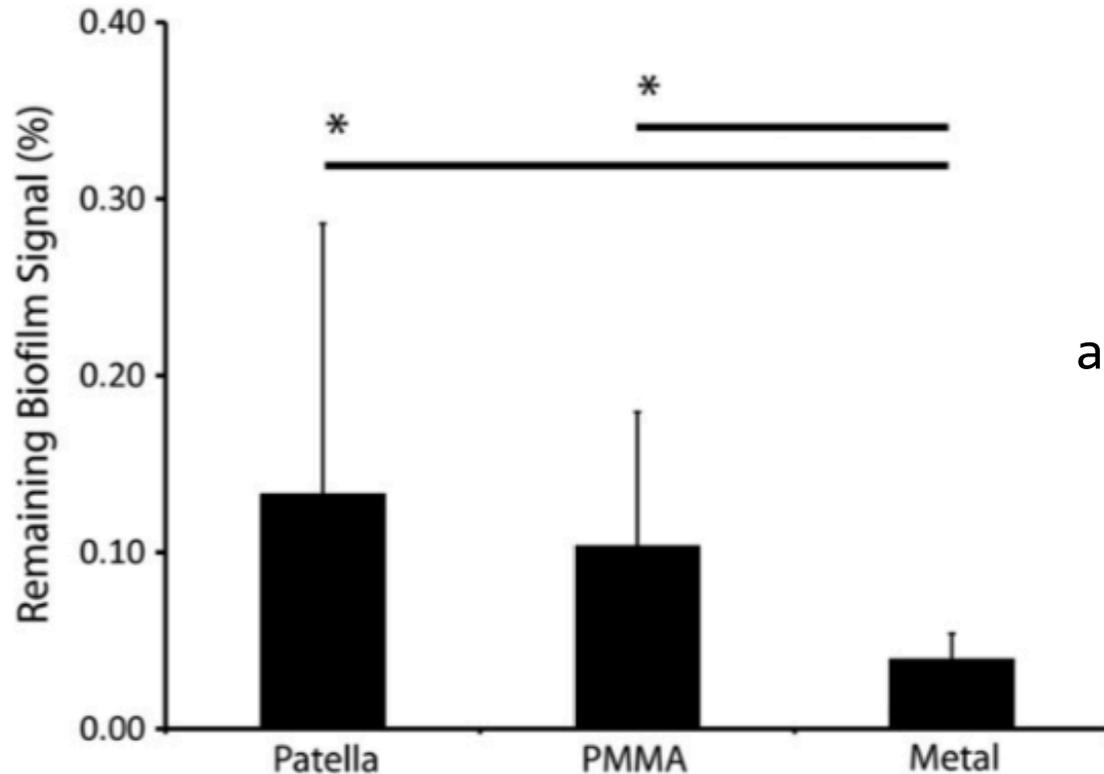
BF ET IMPACT PRONOSTIQUE

	Adjusted Hazard Ratio (95% Confidence Interval)	P-Value
Performing DR in the presence of a joint prosthesis implanted for more than 3 months in absence of hematogenous infection	3.6 (1.3; 10.2)	.02
Performing DR in the presence of symptoms for more than 3 weeks	0.7 (0.2; 2.9)	.6
Performing DR in the presence of an abscess or sinus tract	2.2 (0.8; 6.2)	.1
Performing DR in the presence of unstable implant	1.1 (0.1; 9.8)	.9
Performing DR in the presence of multidrug-resistant organism, <i>Enterococcus</i> sp., quinolone-resistant <i>Pseudomonas aeruginosa</i> or a fungus	0.6 (0.2; 2.4)	.5
Performing DR in the presence of an aerobic GNB PJI	5.2 (1.4; 19.8)	.02

Legend: DR: retention and debridement; GNB: Gram-negative bacilli; PJI: prosthetic joint infection.

BF ET IMPACT THÉRAPEUTIQUE

Méthodes chirurgicales de disruption du Biofilm



Biomasse persistante en BF
après pulse-lavage-irrigation (%)

Etc.

BF : ETAT DES CONNAISSANCES ?

Prévention :

- limiter le recours aux matériels étrangers / ATB prophylaxie / hygiène...
- pas de matériau prévenant efficacement et durablement les infections en BF
- pas de molécule permettant l'inhibition *in vivo* de la constitution d'un BF

Diagnostic :

- Des prélèvements issus de milieux liquides peuvent ne pas détecter une infection en BF
 - Pas de méthode pour déterminer l'ancienneté/stabilité/stérilisation du BF
 - L'isolement des pathogènes requiert une dispersion mécanique du BF
 - la séparation des bactéries de leur BF entraîne une réversion de leur phénotype
- ➔ Les tests de sensibilité planctoniques peuvent ne pas refléter l'efficacité des ATB en BF / pas de test fiable en BF

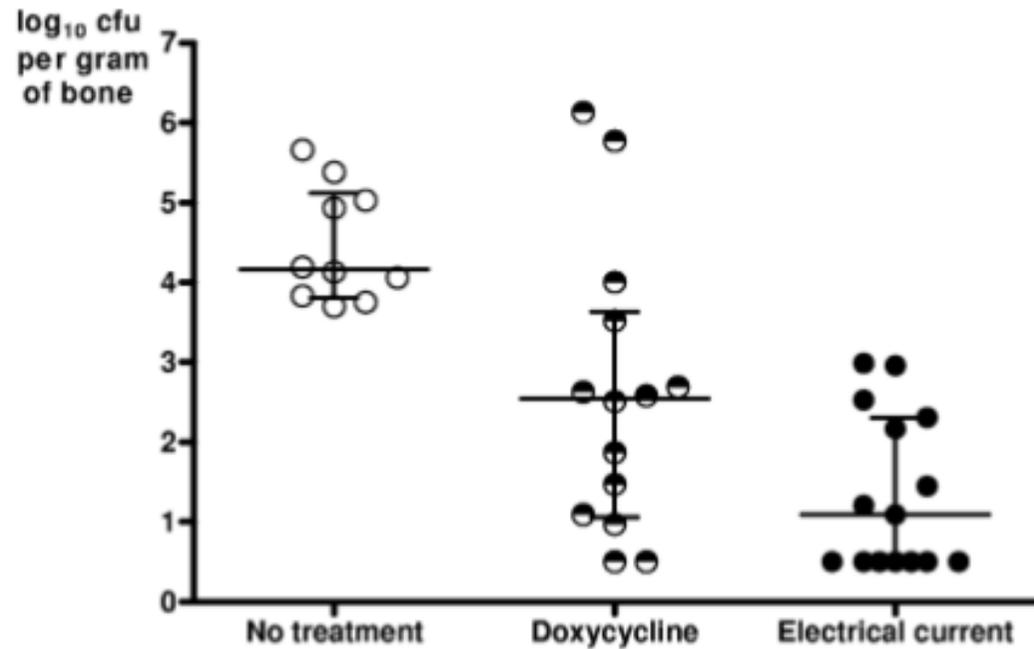
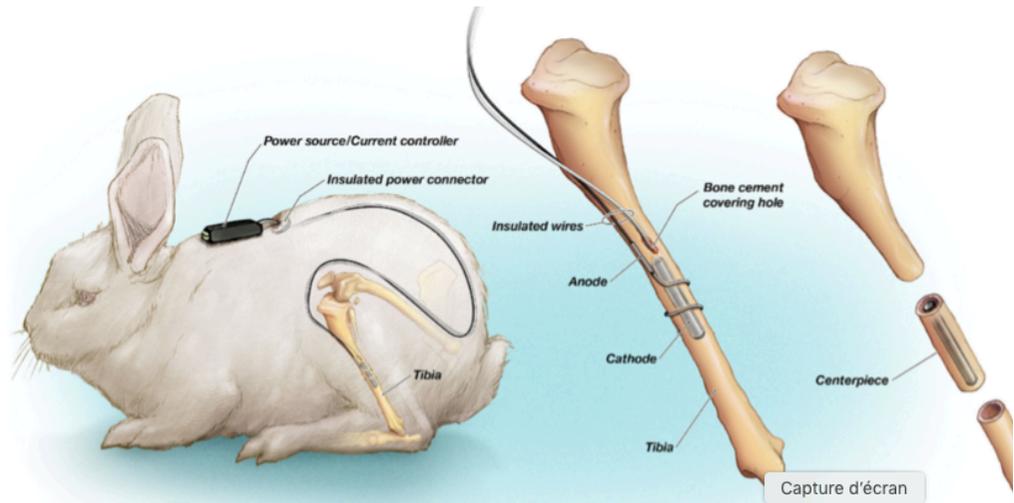
ETAT DES CONNAISSANCES/RECOMMANDATIONS ?

Traitement :

- Le traitement antibiotique seul échoue
- La détersion chirurgicale est le plus souvent nécessaire
- Il n'existe pas à l'heure actuelle de molécules *in vivo* permettant la dispersion des BF ou la potentialisation de l'effet des ATB

BF ET PERSPECTIVES THÉRAPEUTIQUES

Disruption du BF



INFECTIONS EN BF : RECOMMANDATIONS

Pistes de recherche :

Antibiotiques antibiofilm

Dégradeurs de matrice

Antibiotiques locaux

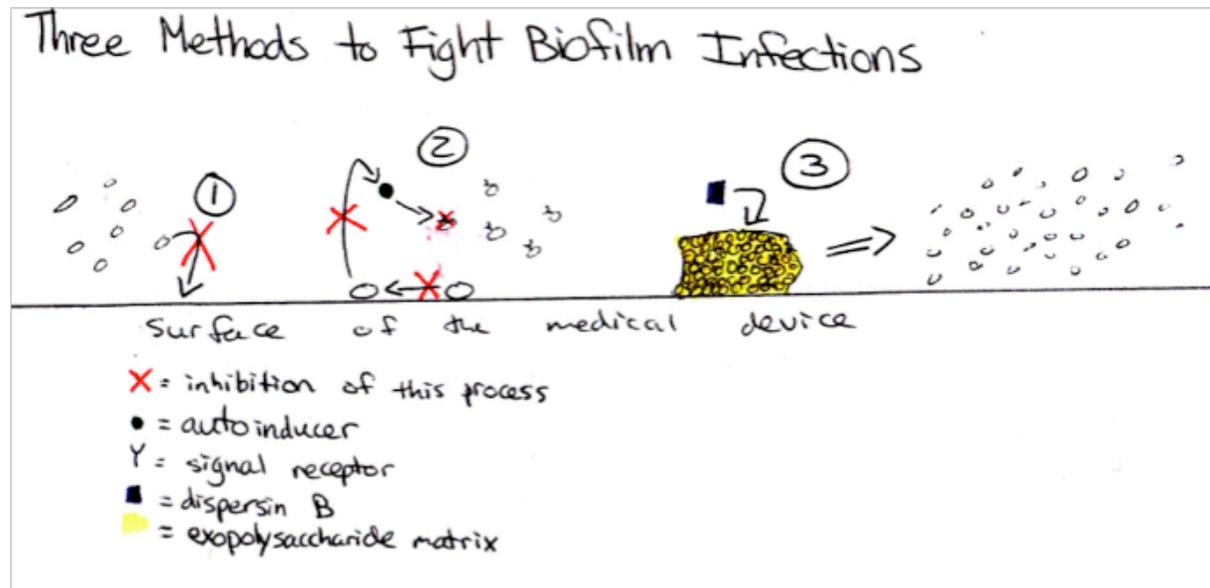
Associations antibiotiques / UV / électricité...

Leucopatch

Vaccination

Mucus shaver

Phages...



Merci de votre attention

