Infections associées aux biofilms, quoi de neuf sur le plan diagnostique et thérapeutique ?

Le point de vue du clinicien…

- Mardi 18 décembre 2018 -

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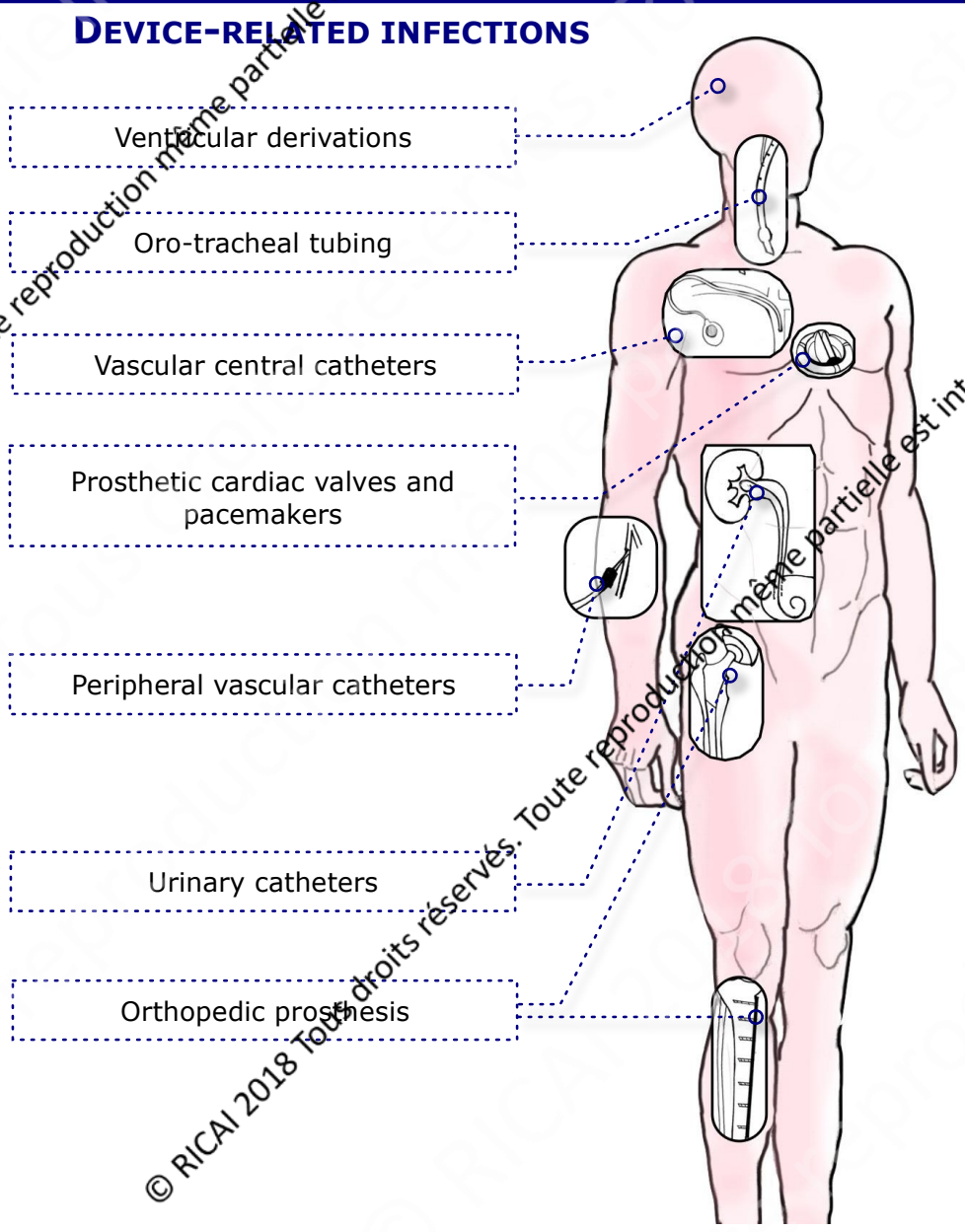
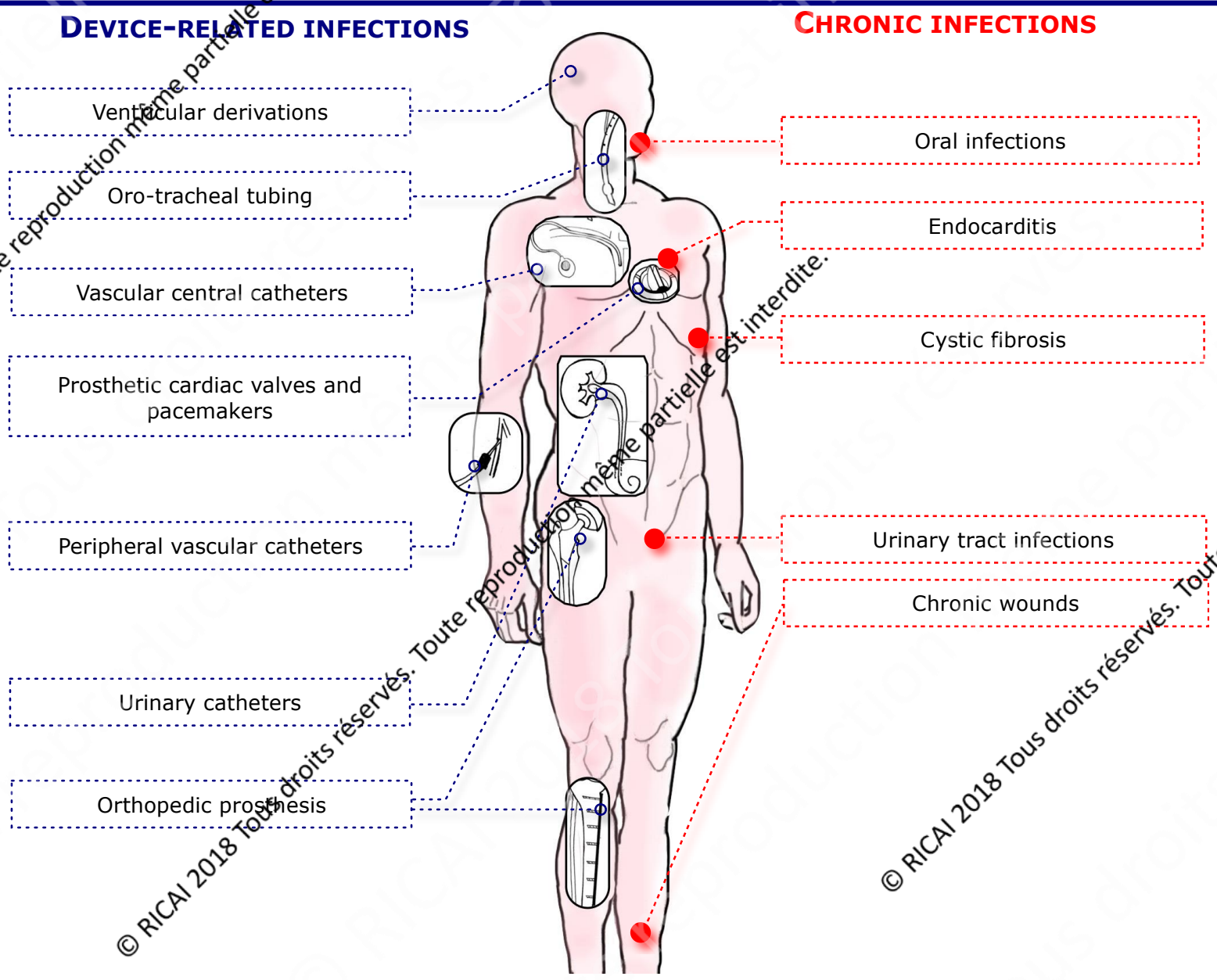
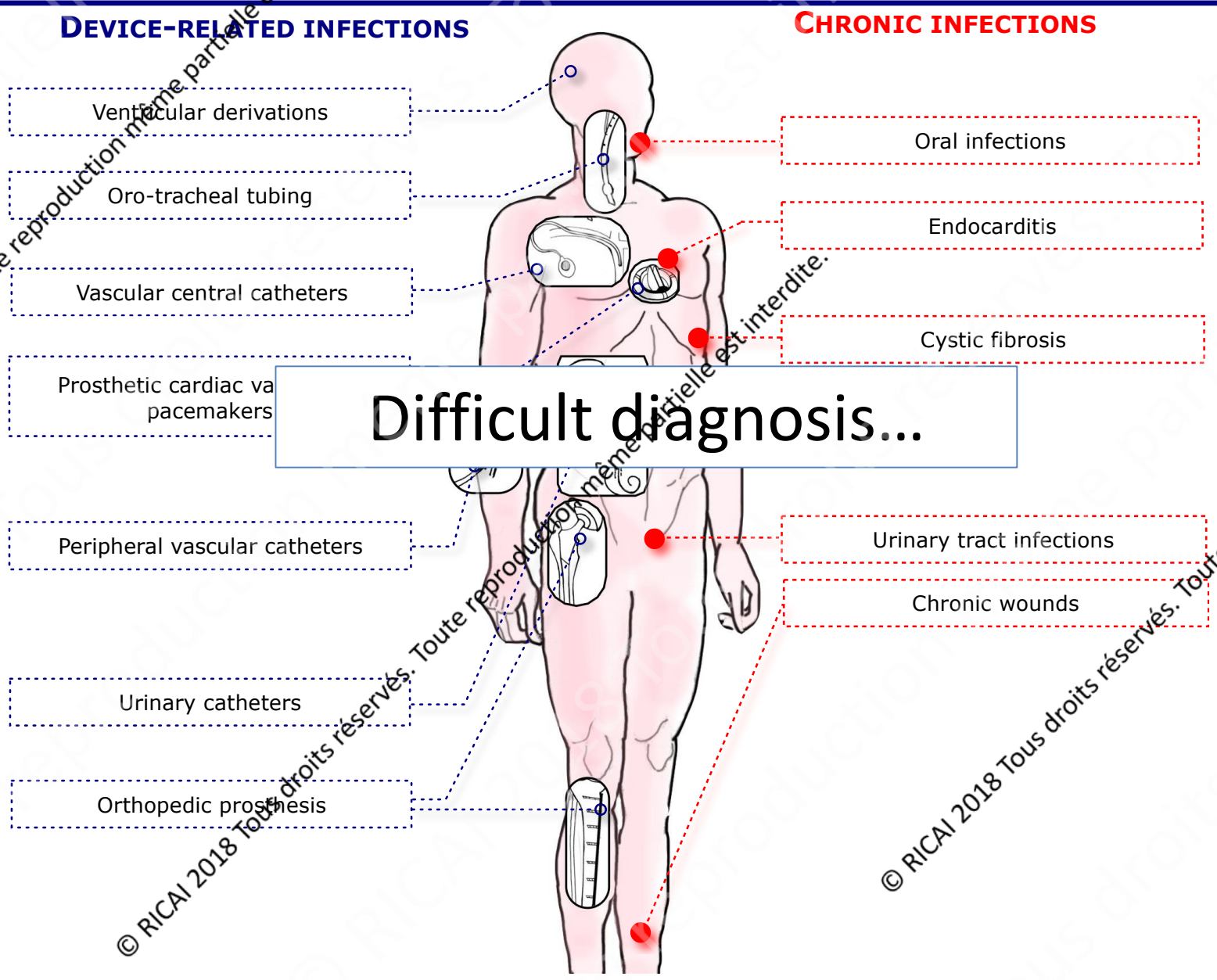
Medical complications of biofilm lifestyle



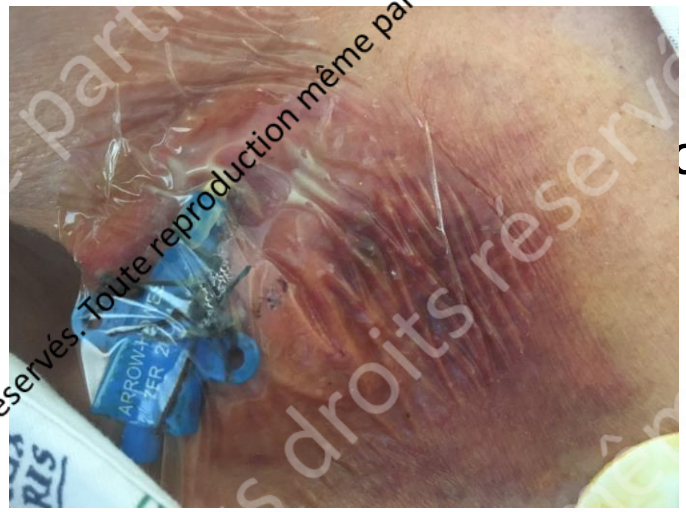
Medical complications of biofilm lifestyle

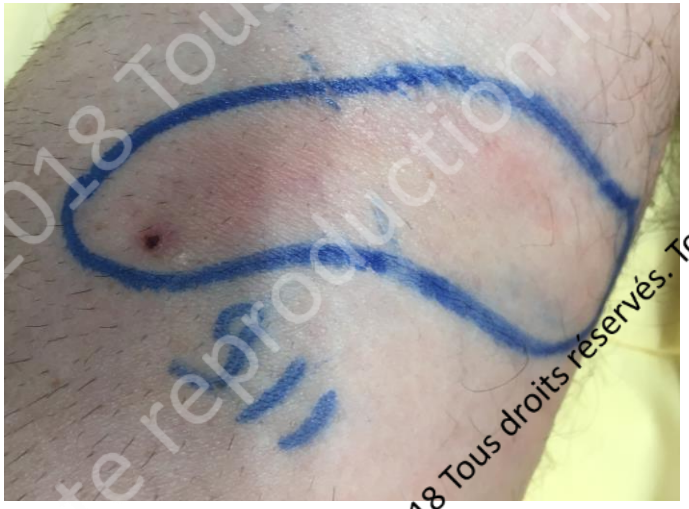


Medical complications of biofilm lifestyle

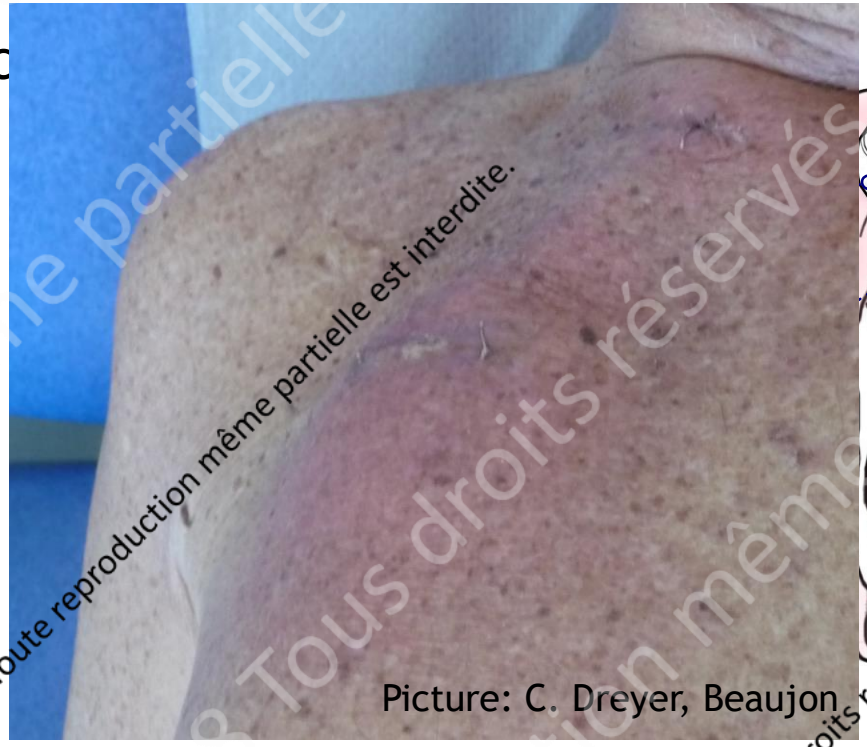


Diagnosis: clinical signs

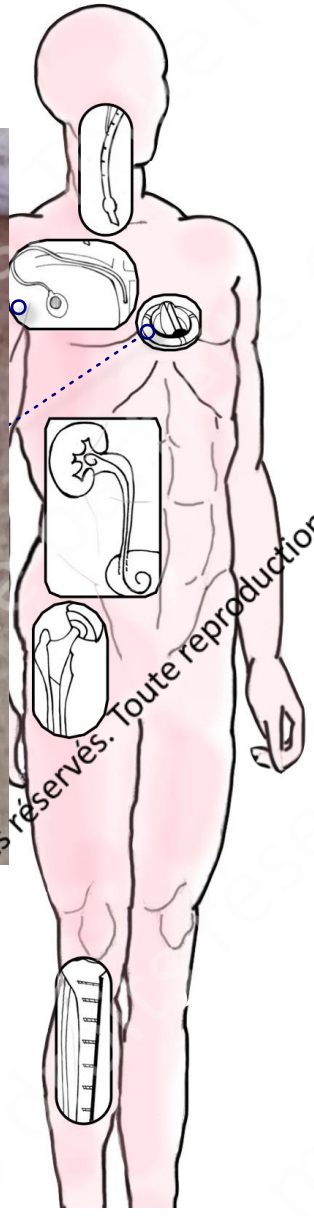




Only in 10-20% of the patients



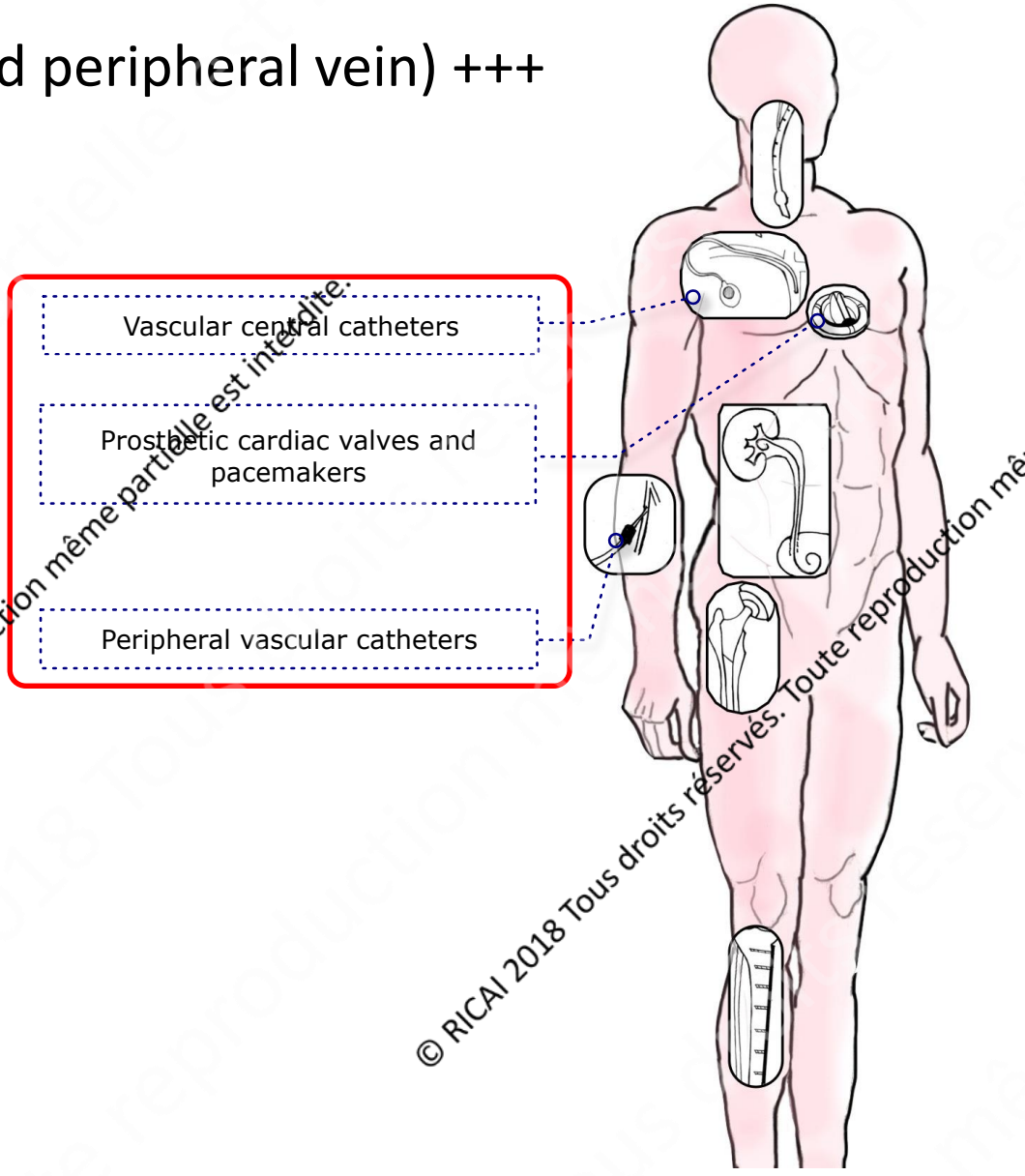
Picture: C. Dreyer, Beaujon



Pictures: Laure Delaval

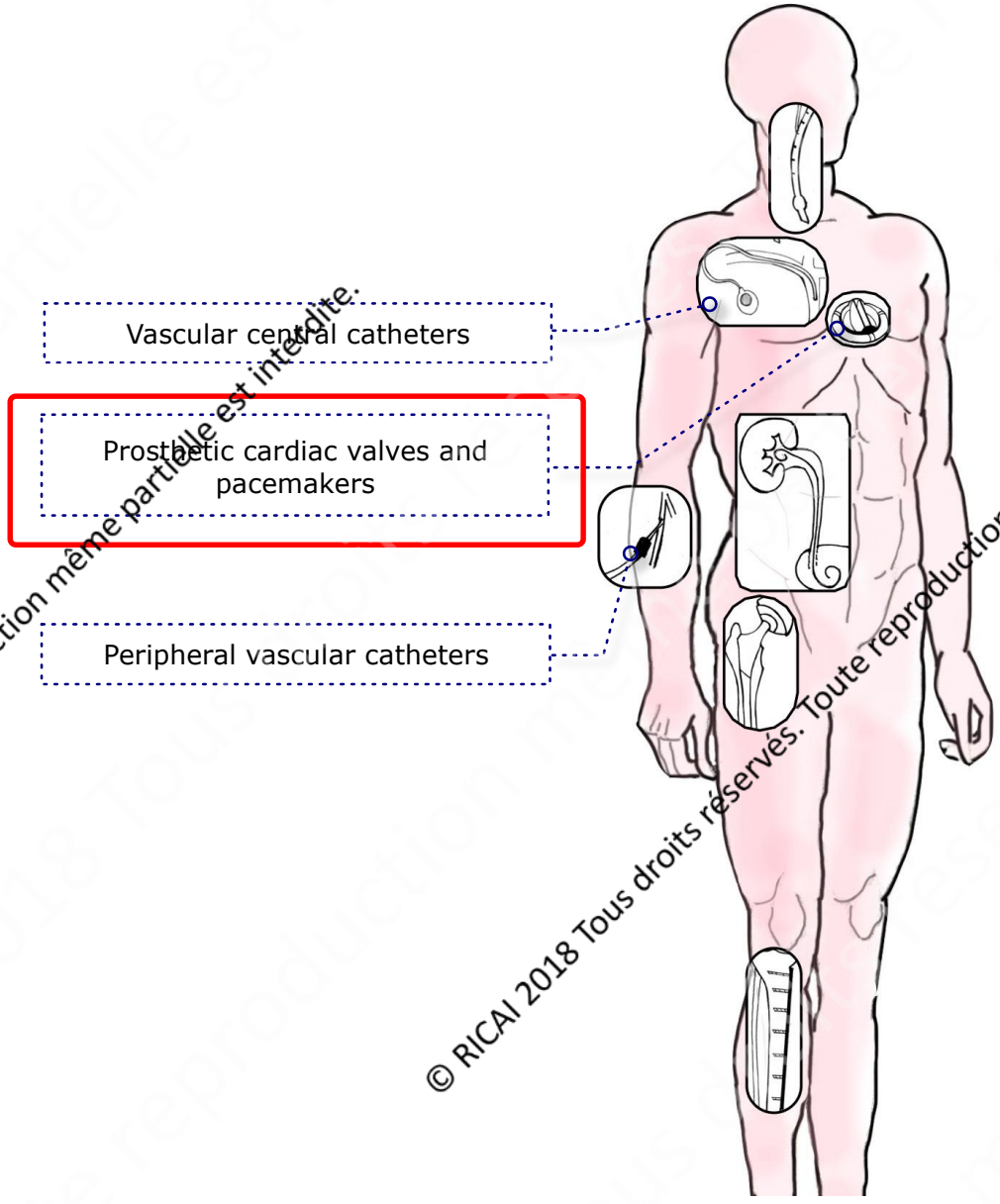
Diagnosis: microbiological tools

- Paired blood culture (CVC and peripheral vein) +++
- 14 days blood cultures (IE)
- Culture of removed device



Diagnosis: imaging

- Echocardiography +++
- (^{18}F-FDG) PET/CT
- ECG-gated cardiac CT
- leucocyte scintigraphy



Diagnosis: imaging

Definite IE

Pathological criteria

- Microorganisms demonstrated by culture or on histological examination of a vegetation, a vegetation that has embolized, or an intracardiac abscess specimen; or
- Pathological lesions: vegetation or intracardiac abscess confirmed by histological examination showing active endocarditis

Clinical criteria

- 2 major criteria; or
- 1 major criterion and 3 minor criteria; or
- 5 minor criteria

Possible IE

- 1 major criterion and 1 minor criterion; or
- 3 minor criteria

Rejected IE

- Firm alternate diagnosis; or
- Resolution of symptoms suggesting IE with antibiotic therapy for ≤4 days; or
- No pathological evidence of IE at surgery or autopsy, with antibiotic therapy for ≤4 days; or
- Does not meet criteria for possible IE, as above

2. Imaging positive for IE

a. Echocardiogram positive for IE:
- Vegetation;
- Abscess, pseudoaneurysm, intracardiac fistula;
- Valvular perforation or aneurysm;
- New partial dehiscence of prosthetic valve.

b. Abnormal activity around the site of prosthetic valve implantation detected by ^{18}F-FDG PET/CT (only if the prosthesis was implanted for >3 months) or radiolabelled leukocytes SPECT/CT.

c. Definite paravalvular lesions by cardiac CT.

Habib, G. *et al* 2015 Europ Heart J

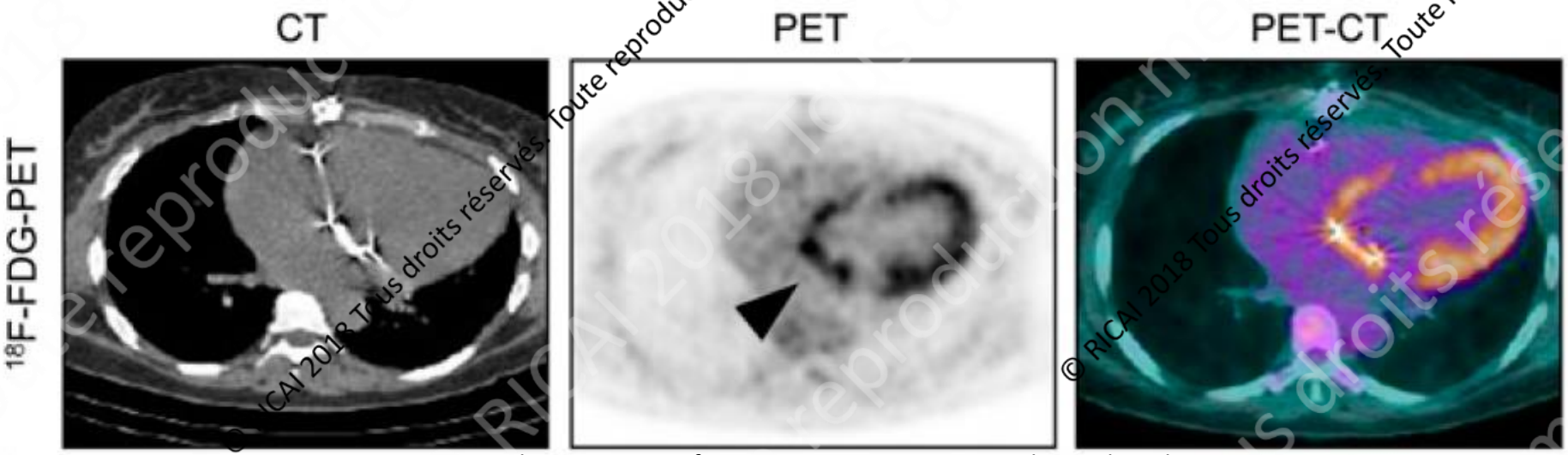
Diagnosis: imaging in infective endocarditis

- Cardiac CT (3 prospective studies, mostly **prosthetic**):
  - 30 patients/study. 93-100% Sensitivity; 83-88% Specificity
  - Before surgery: paravalvular lesions +++ / coro-TDM

Gomes, A. *et al* 2017 Lancet Infect Dis

Diagnosis: imaging in infective endocarditis

- Cardiac CT (3 prospective studies, mostly **prosthetic**):
  - 30 patients/study. 93-100% Sensitivity; 83-88% Specificity
  - Before surgery: paravalvular lesions +++ / coro-TDM
- (^{18}F-FDG) PET/CT (16/24 prospectives):
  - Prosthetic valves (8 studies) : 73-100% Sensitivity; 71-100% Specificity. Only after 3 months post-surgery
  - Native valves: 6-39% Sensitivity



Gomes, A. *et al* 2017 Lancet Infect Dis

Rouzet, F. *al;* J Nucl Med. 2014

Diagnosis: imaging

(^{18}F-FDG) PET/CT and Implantable cardiac electronic device

21 patients with suspected infection vs 14 controls

- Ss, Sp, PPV and NPV: 80%, 100%, 100% and 84.6%
- Generator: 100%
- Leads: 60%, 100%, 100% and 73%

→Importance of antibiotic treatment prior to **(^{18}F-FDG) PET/CT**

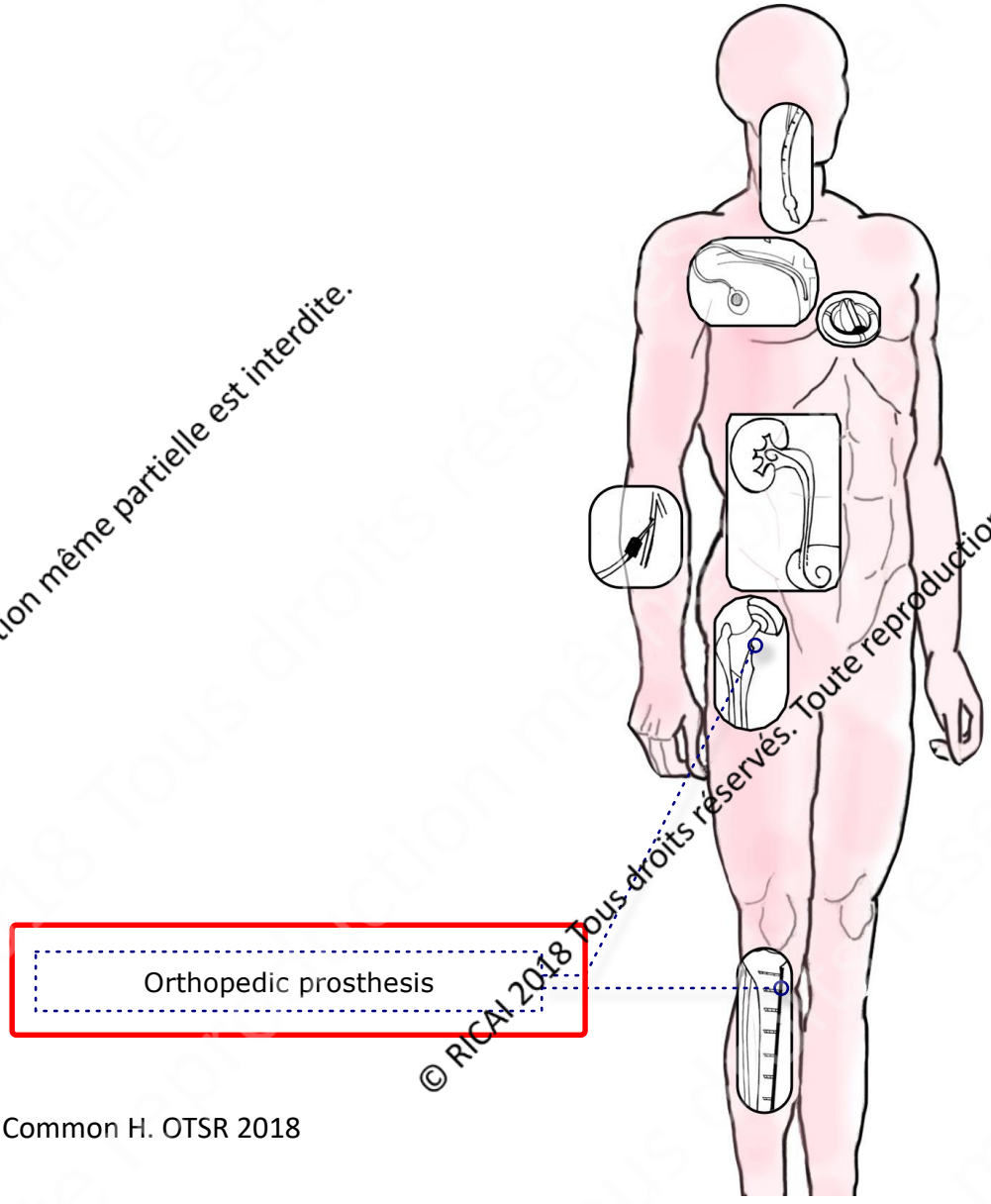
→20 days (false-negative) vs 3 days (true-positive)

Bensimhon *et al* Clin Microbiol Infect. 2011

Diagnosis: upgraded microbiology

- Use of beads-containing vials

  +/- Blood culture bottles



Arvieux, C. and Common H. OTSR 2018

Diagnosis: upgraded microbiology

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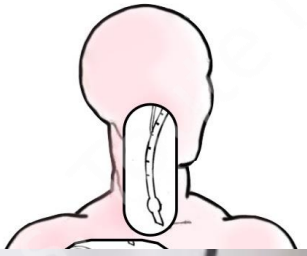
  +/- Blood culture bottles



Arvieux, C. and Common H. OTSR 2018

Diagnosis: upgraded microbiology

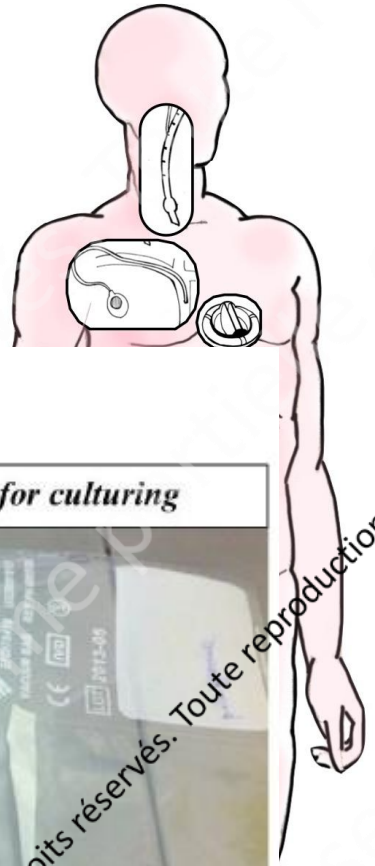
- Use of beads-containing vials

  +/- Blood culture bottles

- Sonication bath

Sonication bath

Collection of a sonication fluid sample for culturing



Diagnosis: upgraded microbiology

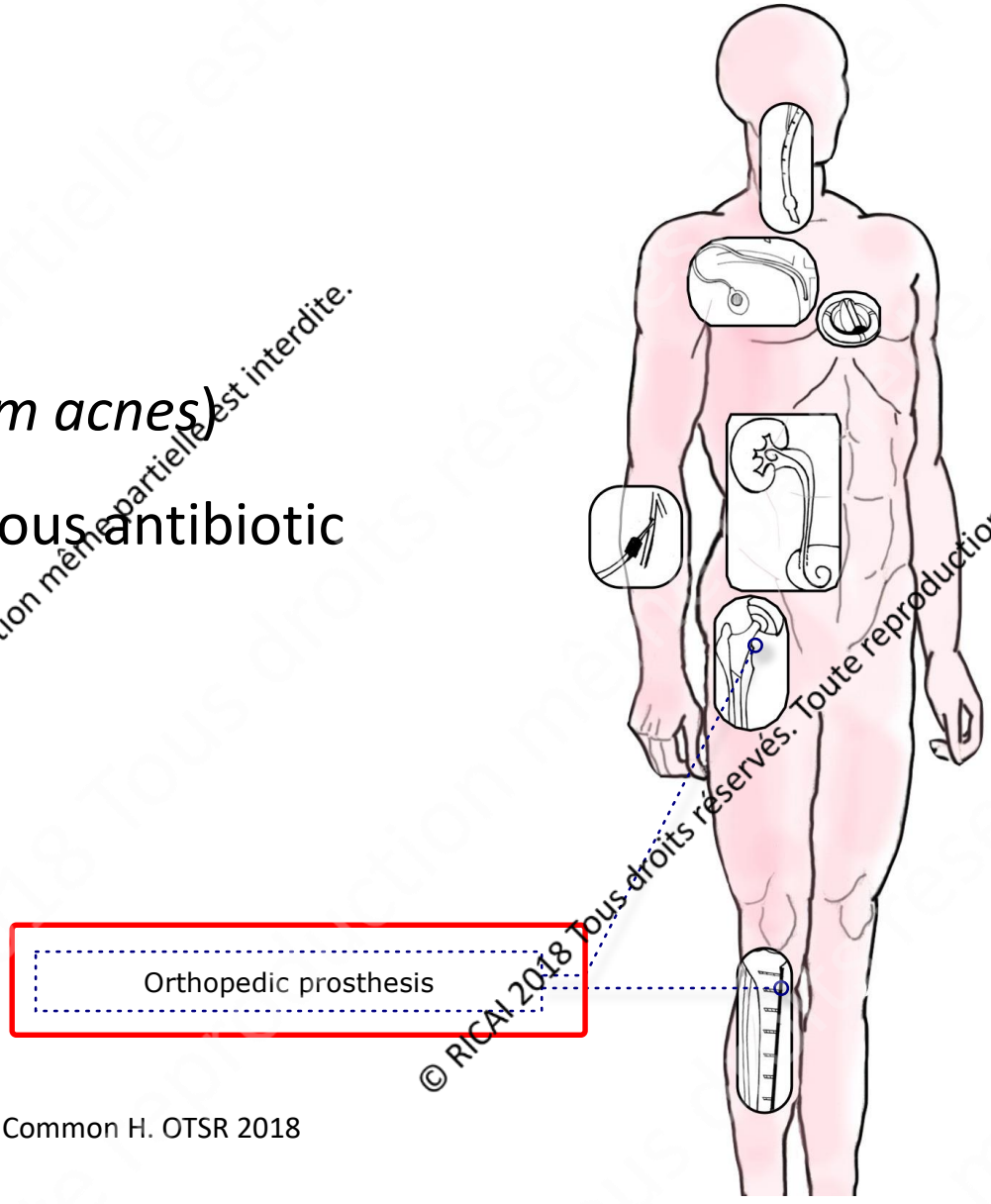
- Use of beads-containing vials

  +/- Blood culture bottles

- Sonication bath

  14 days culture (*Cutibacterium acnes*)

- +/- Molecular biology if previous antibiotic treatment

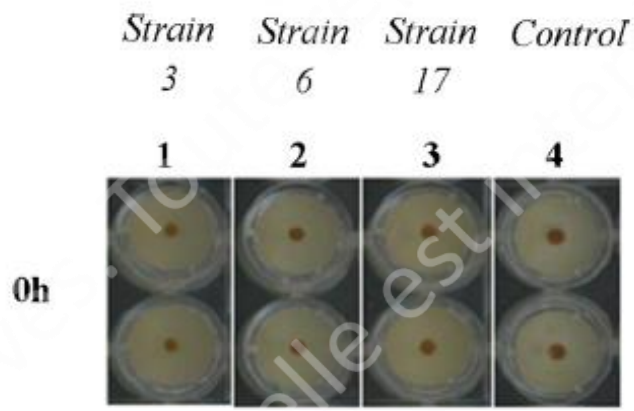


Arvieux, C. and Common H. OTSR 2018

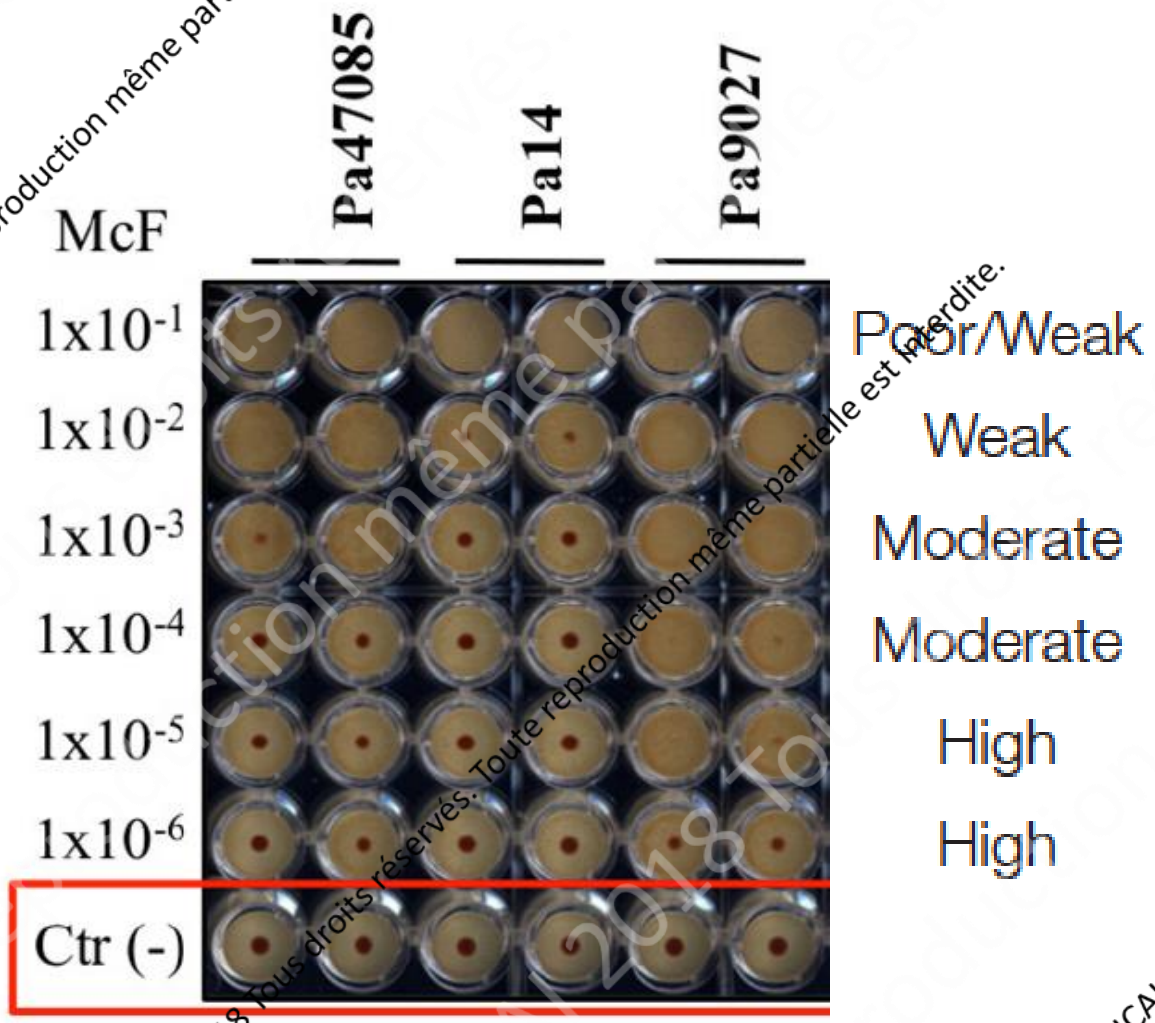
In vitro method to assess biofilm formation

- BioFilm Ring Test ®
- Standardized method to assess the *in vitro* biofilm formation a strain

Di Domenico, E.G. *et al* Frontiers Microbiol Infect 2016
Olivares, E. *et al* J Clin Microbiol 2016



In vitro method to assess biofilm formation



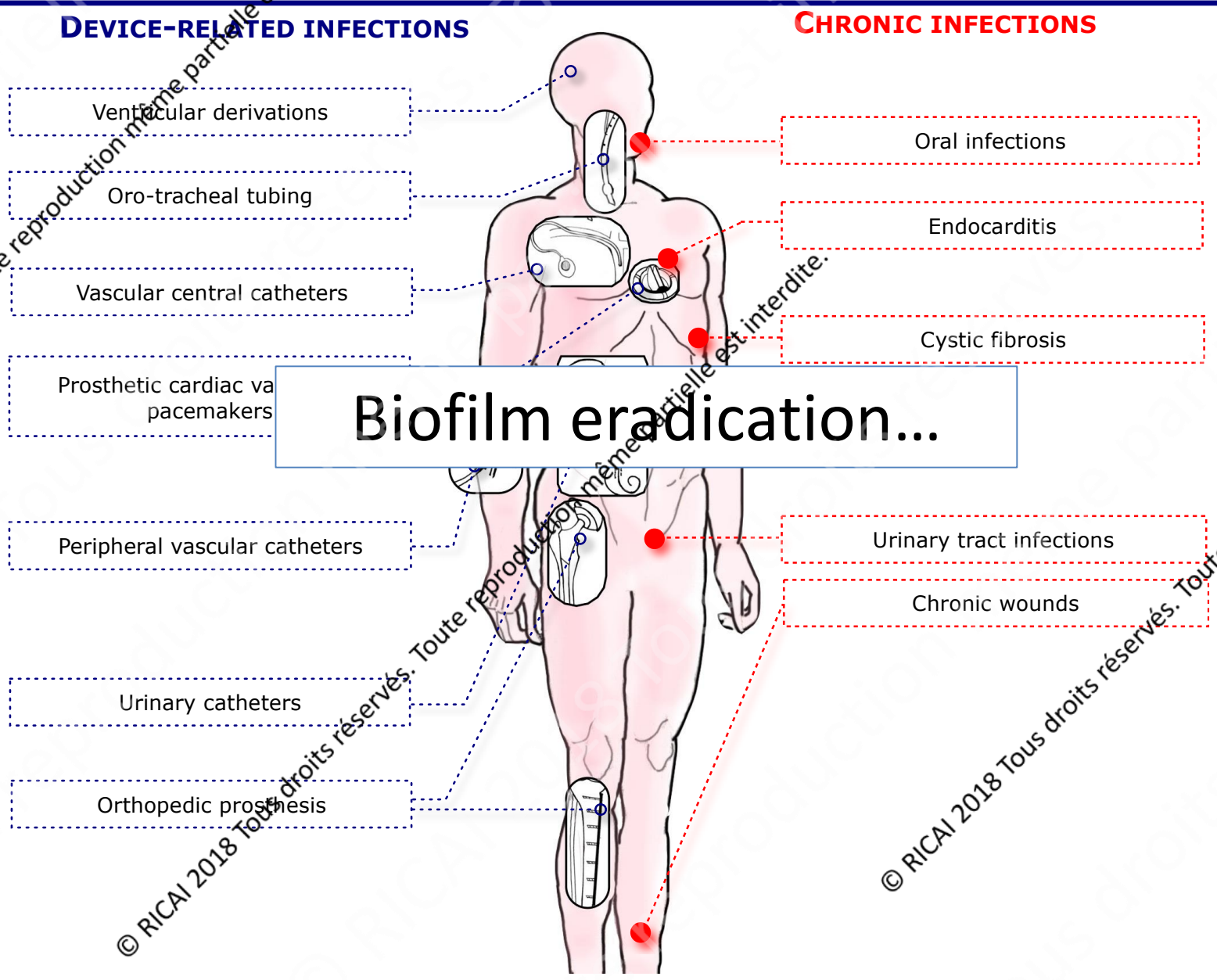
Di Domenico, E.G. *et al* Frontiers Microbiol Infect 2016
Olivares, E. *et al* J Clin Microbiol 2016

In vitro method to assess biofilm formation

- BioFilm Ring Test ®
- Standardized method to assess the in vitro biofilm formation a strain (1-2)
- *In vitro* assessment of antibiotics for prevention or eradication (3-4)
- Clinical impact → to be studied +++++

1-Di Domenico, E.G. *et al* Frontiers Microbiol 2016
2-Olivares, E. *et al* J Clin Microbiol 2016
3-Tasse, J. *et al* Pathogens and Disease 2016
4-Olivares, E. *et al* Frontiers Microbiol 2017

Medical complications of biofilm lifestyle

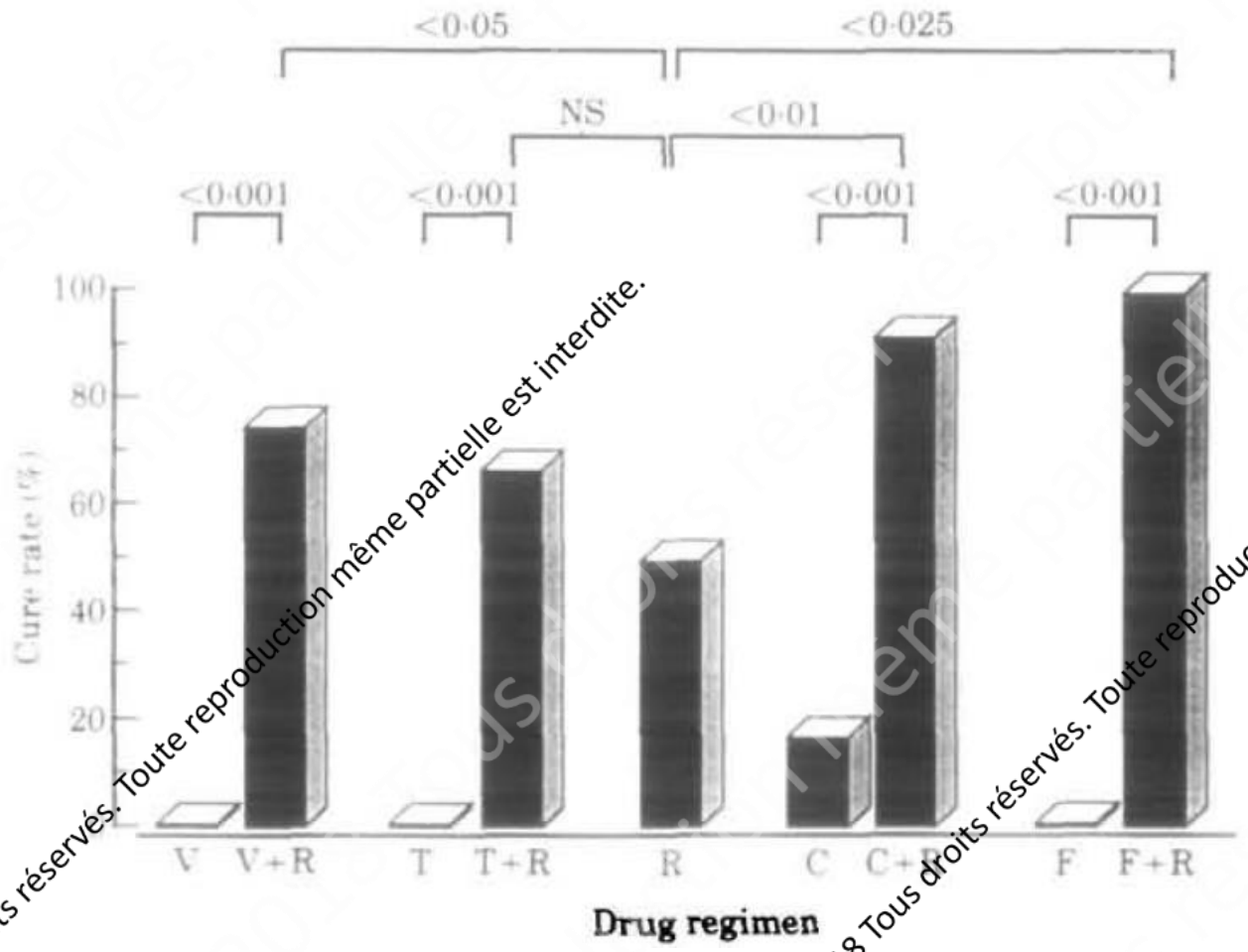


Biofilm eradication?

- Choose the right antibiotics (molecule, dose, duration)
- Removal of the device (not always feasible…)
- Or at least local treatment (debridement, ALT)

Anti-biofilm antibiotics? Rifampin

Perforated teflon tubes



V : vancomycine
T : teicoplanin
R : rifampicin
C : ciprofloxacin
F : penicillin M

Zimmerli, W. *et al* J Antimicrob Chemother 1994

Anti-biofilm antibiotics? Rifampin

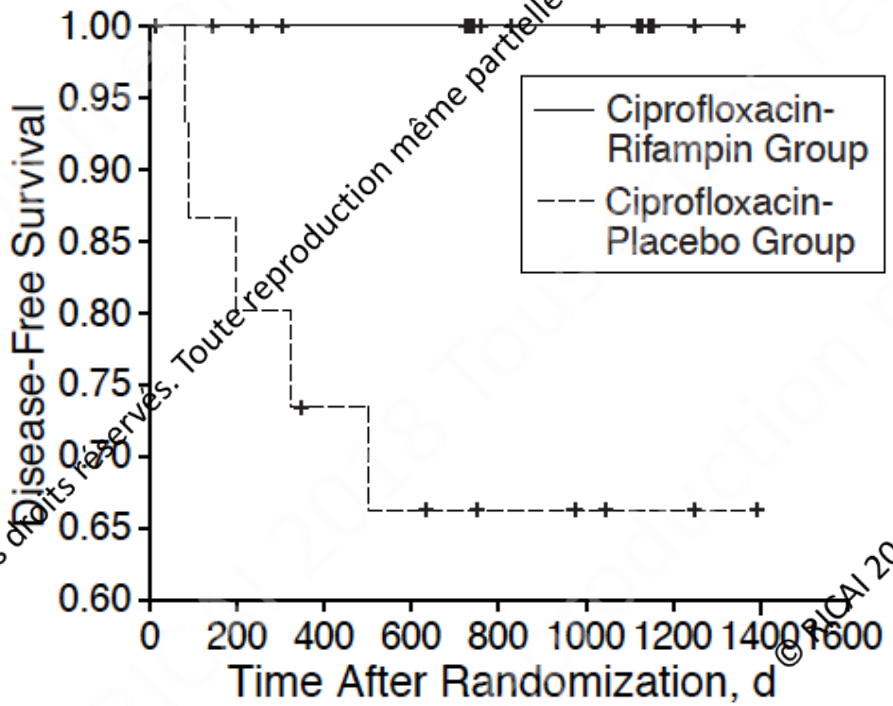
Prospective study

Orthopedic implant–related staphylococcal Infections

Initial debridement (implants left in place)

Cip/Rif (18 patients) VS Cip/placebo (15 patients)

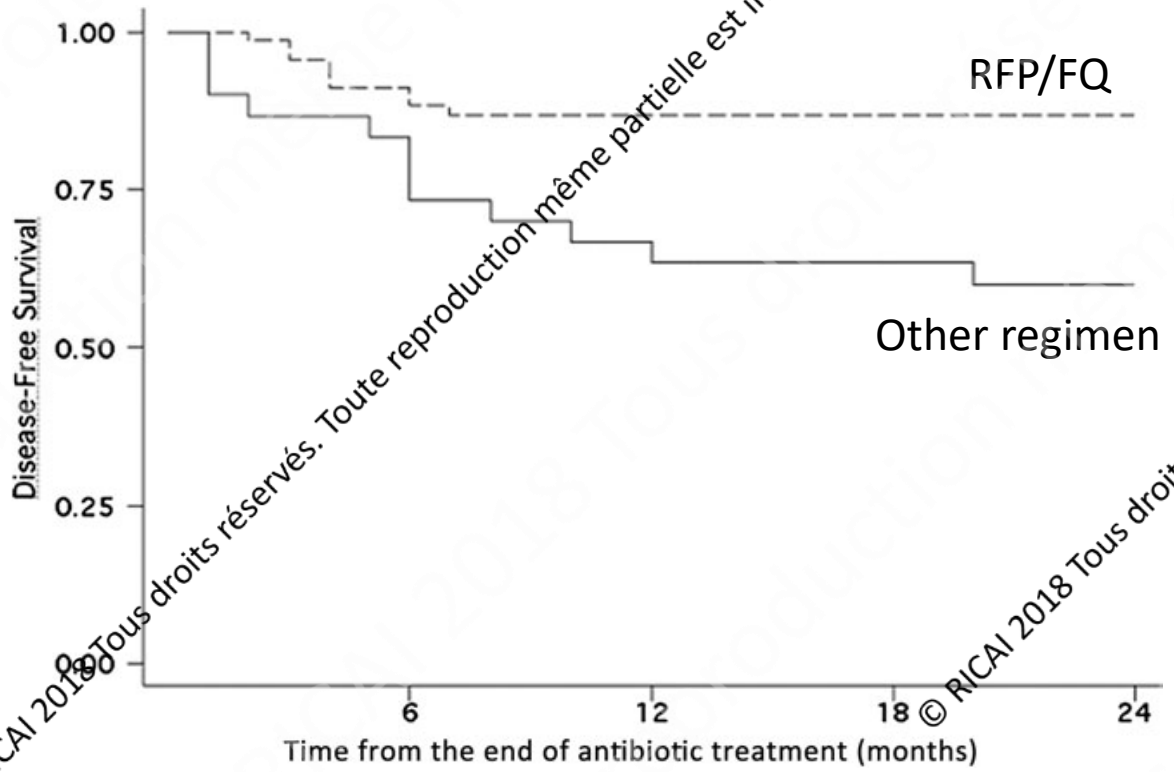
2w IV, 3-6 months treatment



Zimmerli *et al* JAMA 1998

Anti-biofilm antibiotics? Rifampin/quinolones

Retrospective study
Total hip/knee prosthetic joint-related infections
S. aureus
98 patients (100% surgery)



Senneville, E. *et al* Clin Infect Dis 2011

Anti-biofilm antibiotics? Rifampin in other settings

Legout *et al. BMC Infectious Diseases* 2014, **14**:228
http://www.biomedcentral.com/1471-2334/14/228

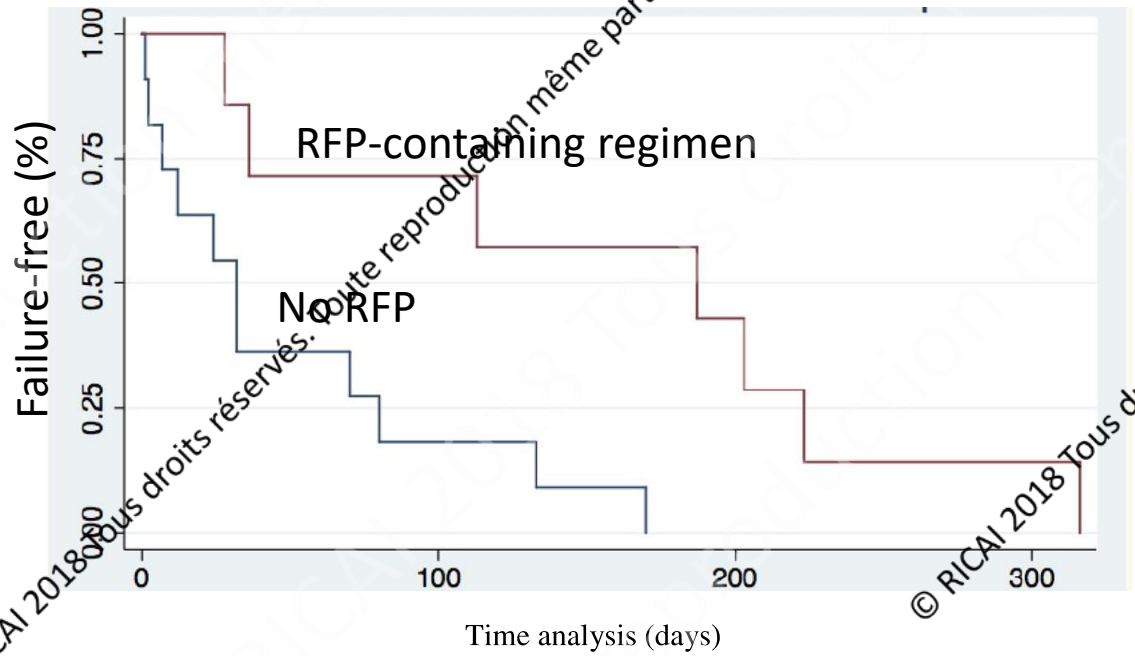
BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Factors predictive of treatment failure in staphylococcal prosthetic vascular graft infections: a prospective observational cohort study: impact of rifampin

Laurence Legout[1*], Piervito Delia[3,4], Béatrice Sarraz-Bournet[3], Cécile Rouyer[1], Massongo Massongo[1], Michel Valette[1], Olivier Leroy[2], Stephan Haulon[4] and Eric Senneville[1]

Prospective study
84 PVGI (**71 surg**)
S. aureus (65) or CoNS (22)



Legout, J, *et al* BMC Infect Dis 2014

Anti-biofilm antibiotics? Rifampin in PVIE

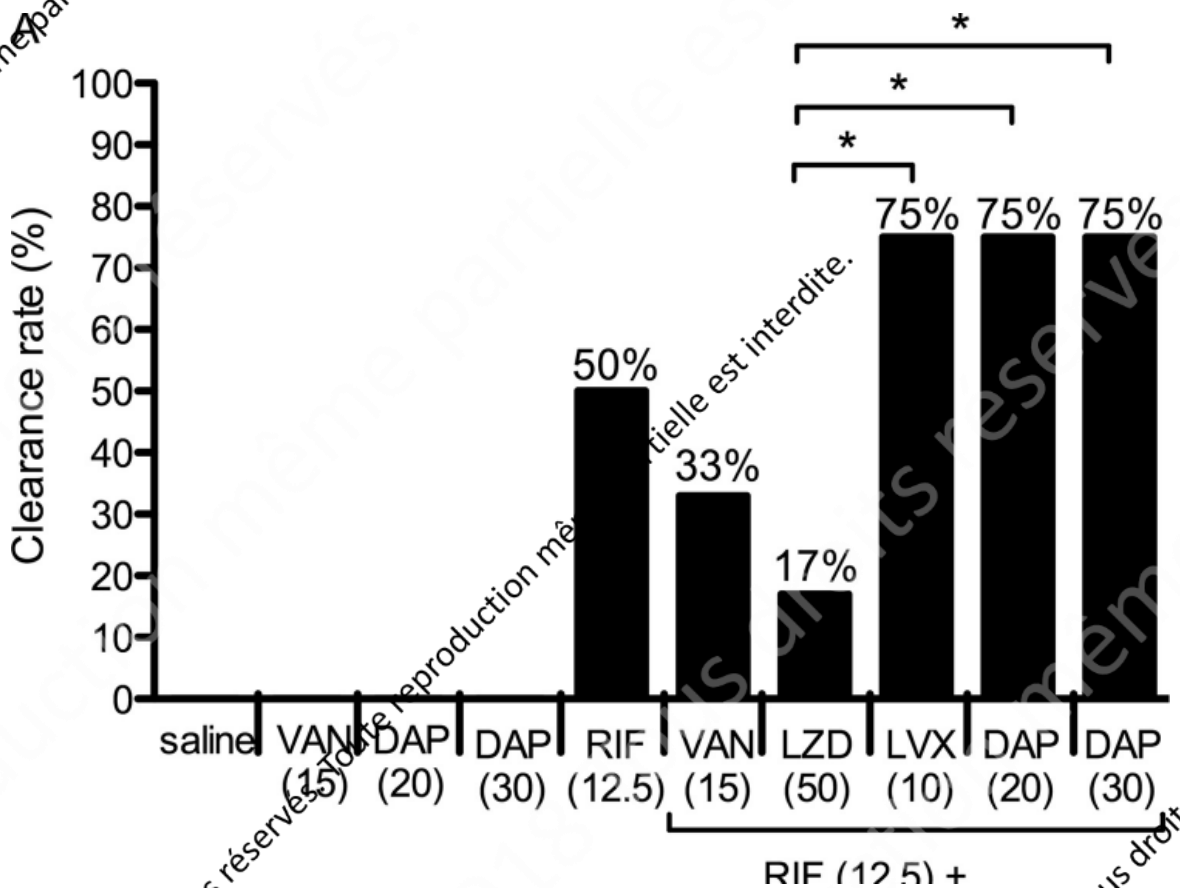
Prosthetic valves						
Methicillin-susceptible staphylococci						
(Flu)cloxacillin *or* oxacillin with Rifampin[e] and Gentamicin[f]	12 g/day i.v. in 4–6 doses	≥ 6	I	B	6,8, 135, 136	Starting rifampin 3–5 days later than vancomycin and gentamicin has been suggested by some experts. Gentamicin can be given in a single daily dose in order to reduce renal toxicity
	900–1200 mg i.v. or orally in 2 or 3 divided doses	≥ 6	I	B		
	3 mg/kg/day i.v. or i.m. in 1 or 2 doses	2	I	B		
	Paediatric doses:[g] Oxacillin and (flu)cloxacillin as above Rifampin 20 mg/kg/day i.v. or orally in 3 equally divided doses					

Previous guidelines….
1 case report of *S. capitis* PVE…
A series of 42 native valve *S. aureus* endocarditis (VS 42 controls without)

RFP group:
56% (9/16) of RFP-R *S. aureus* if introduction before BSI clearance
Longer duration of BSI
Higher mortality

Habib, G *et al* Eur Heart J 2015
Riedel, D.J. *et al* Antimicrob Agents Chemother 2008

Daptomycin against MRSA biofilms



Tissue-cage model
Against MRSA

John, A.-K. *et al* Antimicrob Agents Chemother 2009

Daptomycin against MRSA biofilms

in vivo rabbit model of catheter-related infections

1 lock and then sacrifice

Treatment	*S. aureus* MSSA ATCC 6538P		*S. aureus* MRSA 16	
	negative cultures / total (%)	log_{10} total cfu median (IQR)	negative cultures / total (%)	log_{10} total cfu median (IQR)
Control	0/12 (0)	6.07 (5.55-6.73)	0/14 (0)	6.59 (6.19-7.40)
Daptomycin 50	9/12 (75) [a]	0 (0-0.58) [a]	11/13 (84) [a]	0 (0-0) [a]
Daptomycin 5	3/11 (28)	1.27 (0.07-2.18) [b]	0/8 (0)	3.36 (2.66-4.83) [b]
Vancomycin 10	1/12 (8)	3.61 (2.56-4.43) [c]	0/13 (0)	4.80 (3.82-5.90)

Meije, Y. *et al* 2014 J Infect

Clinical data with Daptomycin against biofilms

Daptomycin as lock therapy…

Variable	Result
Age in years, median (range)	61.5 (35–75)
Gender (male), no. (%)	4 (50)
Underlying disease	
Solid neoplasia, no. (%)	6 (75)
Hematological neoplasia, no. (%)	1 (12.5)
Short bowel syndrome, no. (%)	1 (12.5)
Type of catheter (port-a-cath/Hickman/Groshong)	6/1/1
Clinical presentation, no. (%)	
Fever	8 (100)
Microorganisms	
MRSE[a], no.	5
MSSE[b], no.	1
Enterococcus faecium, no.	1
Polymicrobial infection[c], no.	1
ALT[d] duration in days, mean (range)	13 (7–16)
Outcome	
Success, no. (%)	6 (75)
Time to microbiological response in days, mean (range)	2 (1–6)
Failure, no. (%)	2 (25)

Efficacy of daptomycin lock therapy in the treatment of bloodstream infections related to long-term catheter

P. Tatarelli · A. Parisini · V. Del Bono · M. Mikulska · C. Viscoli

Comparative studies are needed

Tatarelli, P. *et al* 2014 Infection

Daptomycin in endocarditis…

Native valves							
Methicillin-susceptible staphylococci							
(Flu)cloxacillin *or* oxacillin	12 g/day i.v. in 4–6 doses	4–6	I	B	6,8, 128, 135, 136, 158	Gentamicin addition is not recommended because clinical benefit has not been demonstrated and there is increased renal toxicity
	Paediatric doses:[g] 200–300 mg/kg/day i.v. in 4–6 equally divided doses					
Alternative therapy* Cotrimoxazole[a]	Sulfamethoxazole 4800 mg/day and Trimethoprim 960 mg/day (i.v. in 4–6 doses)	1 i.v. + 5 oral intake	IIb	C		*for *Stahylococcus aureus*
with						
Clindamycin	1800mg/day i.v. in 3 doses	1	IIb	C		
	Paediatric doses:[g] Sulfamethoxazole 60 mg/kg/day and Trimethoprim 12 mg/kg/day (i.v. in 2 doses) Clindamycin 40 mg/kg/day (i.v. in 3 doses)					
Penicillin-allergic patie						
Vancomycin[b] **	40 mg/kg/day i.v. in 3 equally divided doses					ay
Alternative therapy:** Daptomycin[c,d]	10 mg/kg/day i.v. once daily	4–6	IIa	C		**Daptomycin** is superior to vancomycin for MSSA and MRSA bacteraemia with vancomycin MIC > 1 mg/L
	Paediatric doses:[g] 10 mg/kg/day i.v. once daily					

Some experts recommend adding cloxacillin (2 g/4 h i.v.) or fosfomycin (2 g/6 h i.v.) to daptomycin in order to increase activity and avoid the development of daptomycin resistance;

Habib, G *et al* Eur Heart J 2015

Daptomycin in endocarditis…

- ICE cohort : 29 Left-sided endocarditis treated with dapto (9.2 mg/kg)
- 18% PVIE
- Combination = 31% (fosfo, rifampin, …)
- 149 controls
- Comparable mortality
- Faster bloodstream clearance (1 VS 5d, P<0.01)
- Discontinuation of daptomycin: n=4 (14.3%)

Carugati, M *et al* Antimcrob Agents Chemother 2013

Daptomycin in endocarditis…

- Retrospective study (2005-2011), *S. aureus* (84%)
- 70 right or left-sided endocarditis treated with dapto (≥8 mg/kg)
- 11% PVIE
- Combination = 34,3% (fosfo, rifampin, …)

Table 2. Patients with MRSA IE developing non-susceptibility to daptomycin

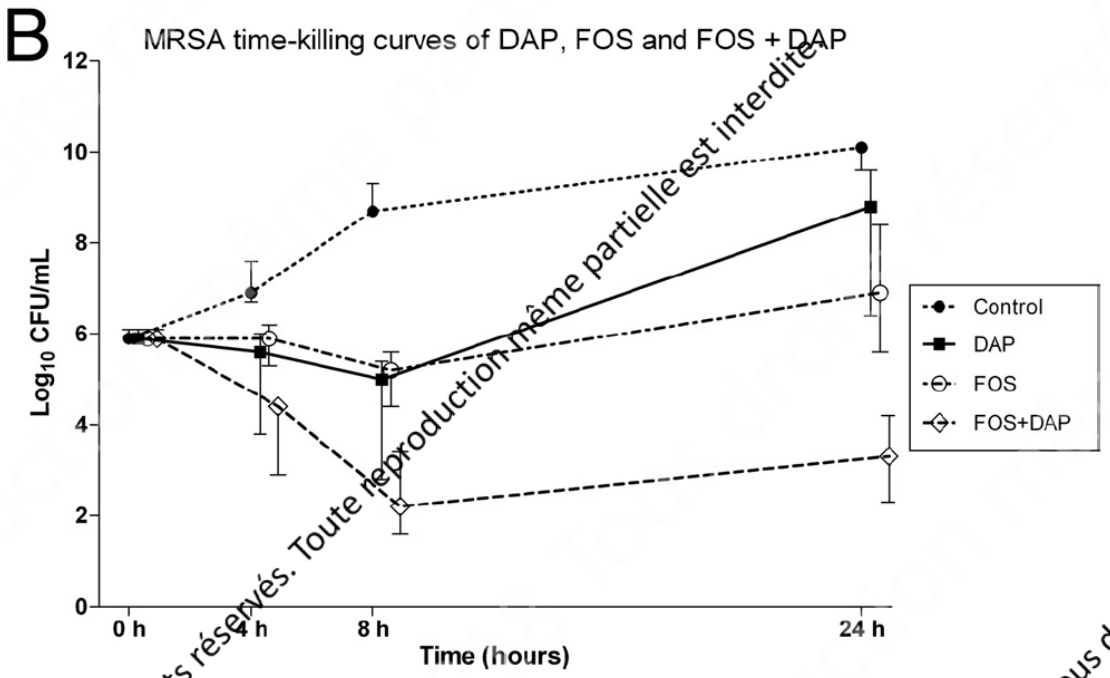
IE	DAP MIC (mg/L)	DAP MIC change	VAN MIC (mg/L)	VAN exposure (days)	Outcome
RIE	0.38→4	day 7 HD DAP	1.5→2	17	cleared on SXT
RIE	1→4	day 1 HD DAP	2→2	5	cleared on SXT
RIE	0.5→4	day 21 HD DAP	1→2	≤30 days prior to admission	organism persisted
LIE	1→4	day 5 HD DAP	2→2	2	cleared on HD DAP
RIE/LIE	0.5→4	day 11 HD DAP	hVISA 2→4	prior to admission VAN ×6 weeks	cleared on HD DAP
RIE/LIE	1→2	day 18 HD DAP	1.5→2	20	cleared on HD VAN

DAP, daptomycin; VAN, vancomycin; HD, high-dose; hVISA, heterogeneous vancomycin-intermediate *S. aureus*; SXT, trimethoprim/sulfamethoxazole.

Kullar, R *et al* J Antimcrob Chemother 2013

Daptomycin + fosfomycin in endocarditis…

- *In vitro* and *in vivo* data: synergistic in 11/14 strains
- Few reported cases



In vitro

Miro, J.M. *et al* Antimcrob Agents Chemother 2012

Daptomycin + fosfomycin in endocarditis…

- *In vitro* and *in vivo* data: synergistic in 11/14 strains
- Few reported cases

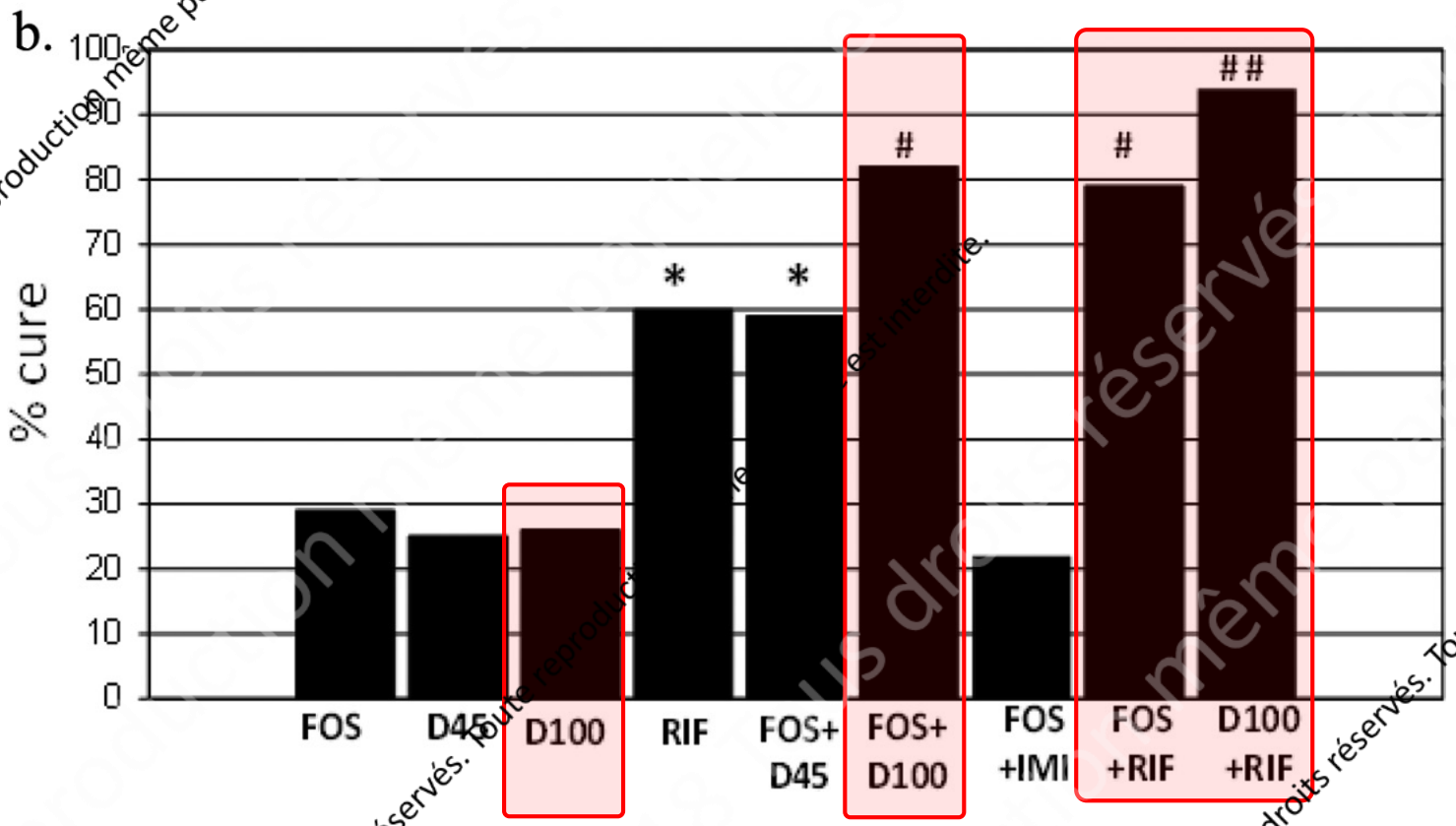
Treatment group	No. of rabbits with sterile vegetations/ total no. of rabbits (%)
Control	0/15 (0)
Daptomycin (simulating 6 mg/kg/day)	13/18 (72)[B,C,D]
Daptomycin (simulating 10 mg/kg/day)	14/15 (93)[B,E]
Daptomycin plus fosfomycin (simulating 6 mg/kg/day plus 2 g/6 h)	16/16 (100)[C,E,F]
Daptomycin plus cloxacillin (simulating 6 mg/kg/day plus 2 g/4 h)	14/16 (88)[D,F]

[a]Significance is indicated by superscript capital letters: AD, $P = 0.40$; E, $P = 1$; F, $P = 0.48$; G, $P = 0.025$; J, $P = 0.15$. NA, not applicable (the control animals wer

In vivo (rabbit IE model)

Miro, J.M. *et al* Antimcrob Agents Chemother 2012

Fosfomycin against MRSA biofilms



Tissue-cage model
Against MRSA

Garrigos, C. *et al* Antimicrob Agents Chemother 2013

Daptomycin in other settings

- Vascular graft infections: retrospective study, 11 patients (1)
  - 5/11 combinations (rifampin)
  - 67% were cured
- Complex bone and joint infections, 43 patients (2)
  - 86% combination (fosfo, rifamp, clinda)
  - 77% favorable outcome
- Device-related osteomyelitis, 82 patients (3)
  - ? % combination
  - 82% clinical success

1-Arnaiz de la Revillas, F. *et al* Int J Infect Dis 2018
2-Roux, S. *et al* BMC Infect Dis 2016
3-Hermsen, E.D. *et al* BMC Infect Dis 2016

Removal of colonized devices?

Implantable cardiac electronic device infection

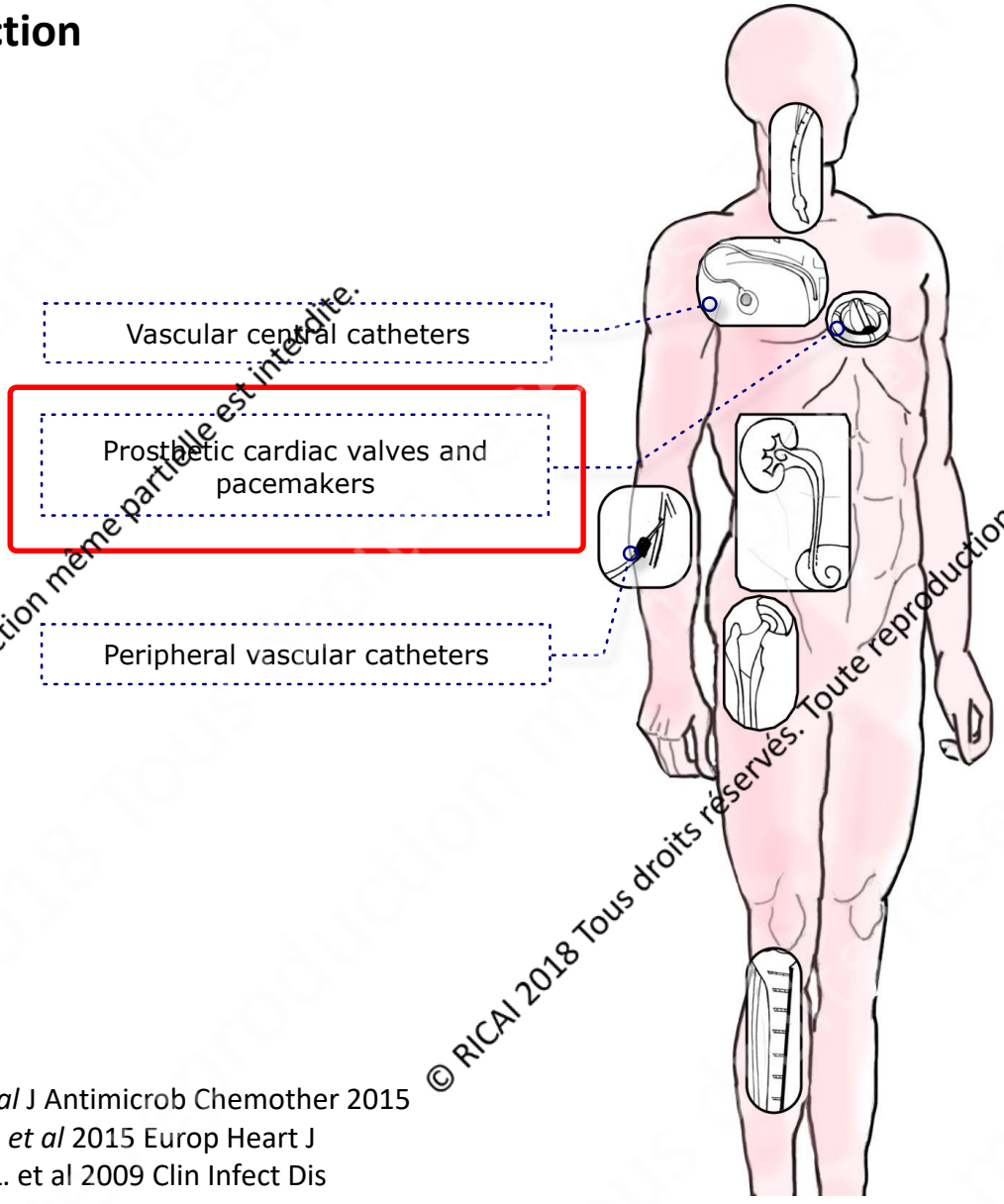
9.1.2 In generator pocket infection, ICED-LI and ICED-IE?

Summary:

- **Recommendation 9.1.2: Complete and early (as soon as possible, but not more than 2 weeks after diagnosis) removal of an infected ICED system (generator and all leads) combined with appropriate antimicrobial therapy is the most effective, safe and efficient treatment option. [B]**

Prosthetic valve IE, if:

- Heart failure
- Abscess, fistula
- Fungi
- Persisting positive BC
- Prevention of embolism



Sandoe, J.A.T *et al* J Antimicrob Chemother 2015
Habib, G. *et al* 2015 Europ Heart J
Mermel, L. et al 2009 Clin Infect Dis

Removal of colonized devices?

- Duration of symptoms?
- Stable implant?
- Absence of sinus tract?
- Susceptibility to antibiotics with activity against biofilms?

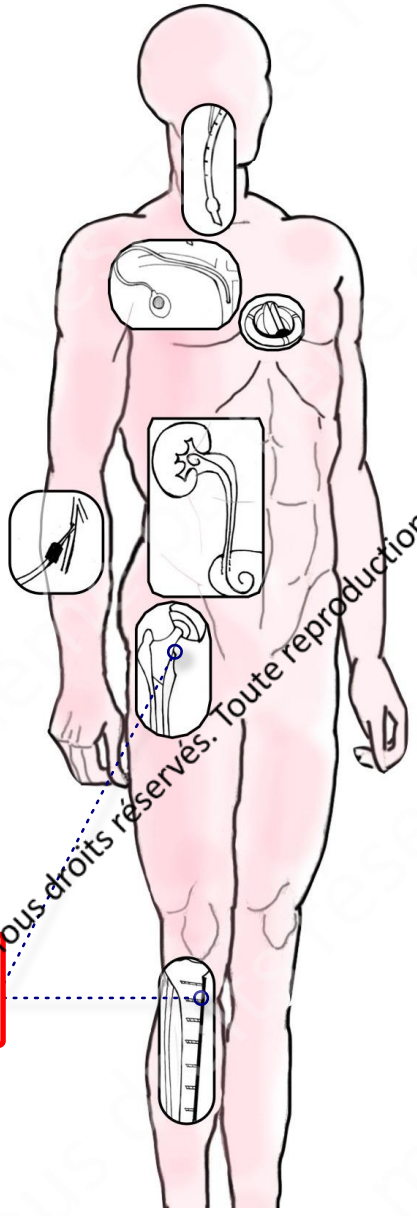
→ Debridement with retention

→ One-stage exchange

→ Two-stage exchange

→ Long-term suppressive antimicrobial treatment

Orthopedic prosthesis



Zimmerli, W. and Sendi, P. APMIS 2017

Eradication of catheter-associated biofilms

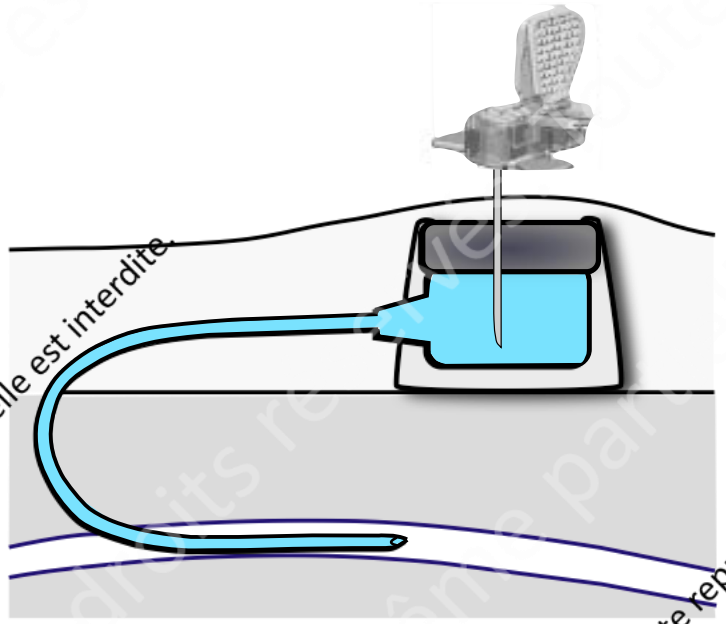
Antibiotic lock technic (ALT) for catheter-related infections

Local / high concentration of antibiotics

(up to 1,000 x MIC)

Long dwelling time (12-24 hours)

Associated with systemic antibiotics



IDSA 2009: « Conservative treatment of **Uncomplicated** long-term intravenous catheter-related BSI caused by CoNS or enterobacteria »

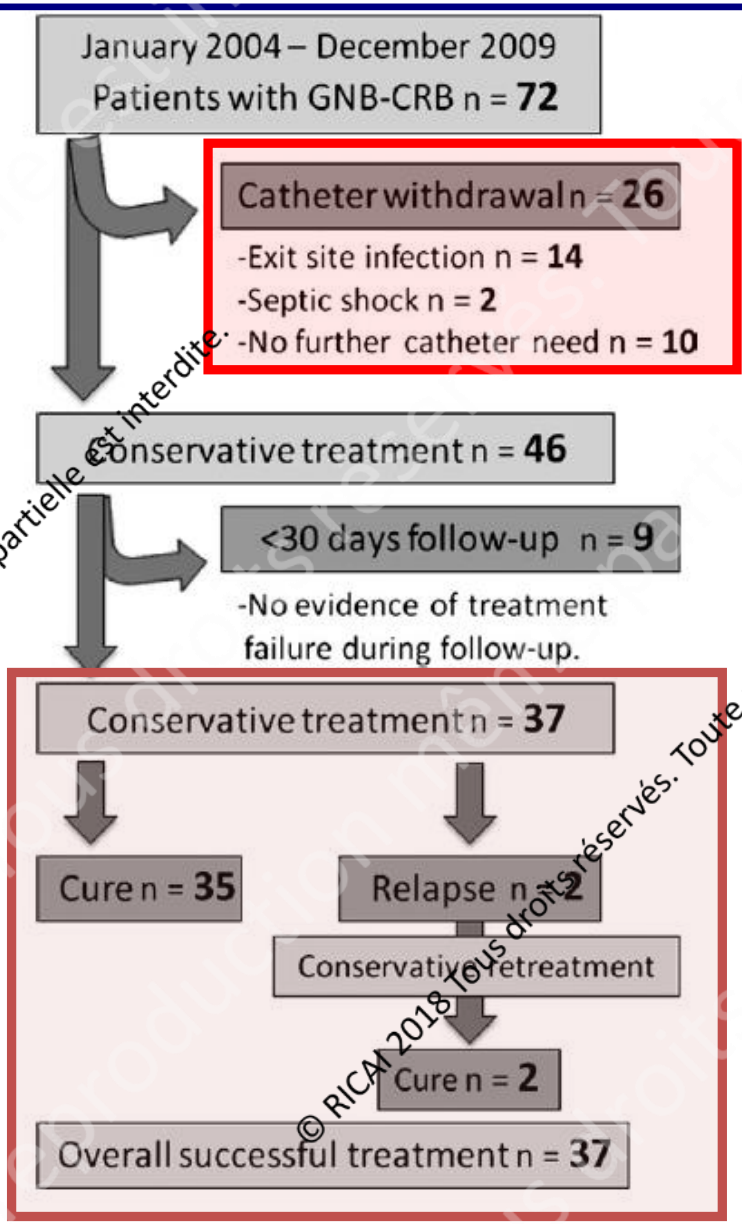
Messing, B. J Parenter Enteral Nutr 1988
Rijnders, B. J. *et al* 2005 J Antimicrob Chemother 55, 90
Mermel, L.A. *et al* 2009 Clin Infect Dis 49, 1
Lebeaux, D. *et al* 2014 Lancet Infect Dis

Lock therapy against Gram-negative bacteria…

Effectiveness of Antibiotic-Lock Therapy for Long-Term Catheter-Related Bacteremia Due to Gram-Negative Bacilli: A Prospective Observational Study

Ciprofloxacine or amikacine
2000 µg/ml

CRB etiologies	
Single GNB microorganism, n (%)	36 (78)
Pseudomonas spp.[b], n	1
Escherichia coli, n	6
Enterobacter cloacae, n	5
Klebsiella pneumoniae, n	4
Acinetobacter baumannii, n	3
Proteus spp., n	3
Others[c], n	4
Polymicrobial GNB infection[d], n (%)	10 (22)



Funalleras, G. *et al* 2011 Clin Infect Dis

ALT with ethanol, why not

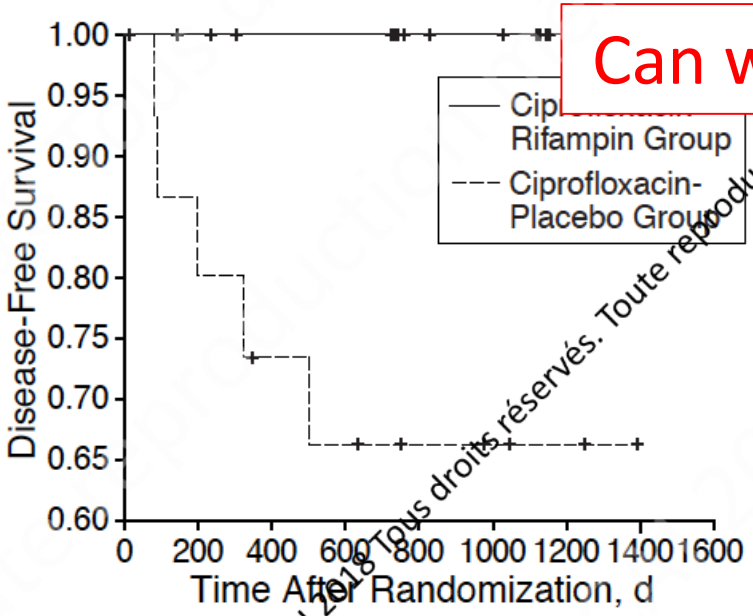
- Ethanol has a wide antimicrobial spectrum
- Active against *in vitro* biofilms
- Clinical data (catheter-related infections)
  - Between 75-92% success
  - Shorter treatment length (1 to 5 days)
  - Can be used against polymicrobial infections
- But
  - Many pediatric studies
  - Tolerance/thrombosis issues
  - Direct comparison: ETHALOCK (O. Lesens), JNI 2018

Valentine, K.M. *et al* Pediatr Crit Care Med 2011
McGrath, E.J. *et al* Clin Pediatr (Phila) 2011

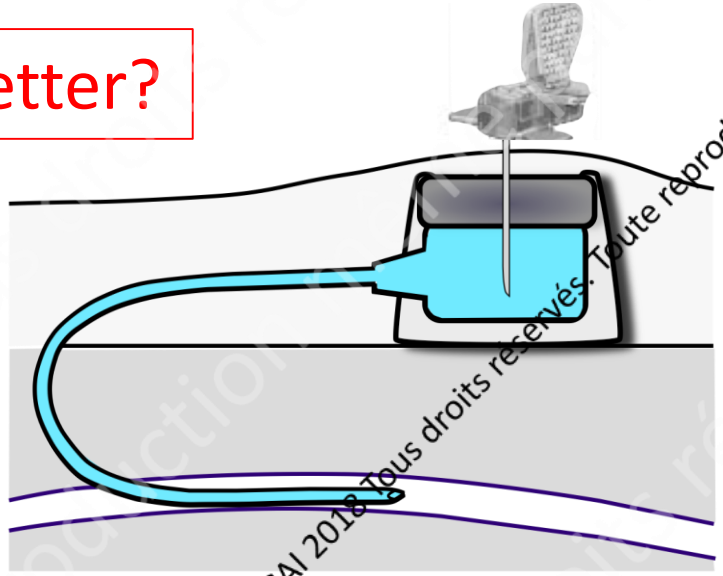
Broom, J. *et al* Scand J Infect Dis 2008
Onland, W. *et al* Arch Pediatr Adolesc Med 2006

Biofilm eradication?

- Choose the right antibiotics (molecule, dose, duration)
- Removal of the device (not always feasible…)
- Or at least local treatment (debridement, ALT)



Can we do better?

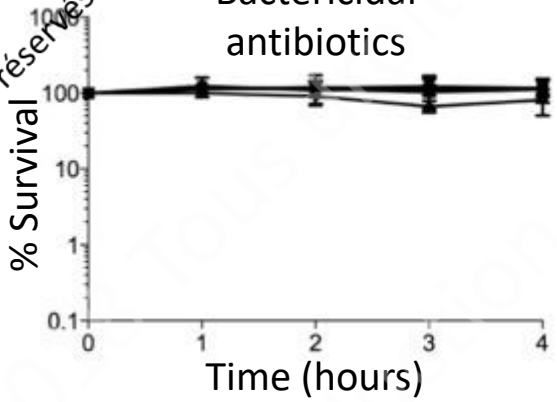


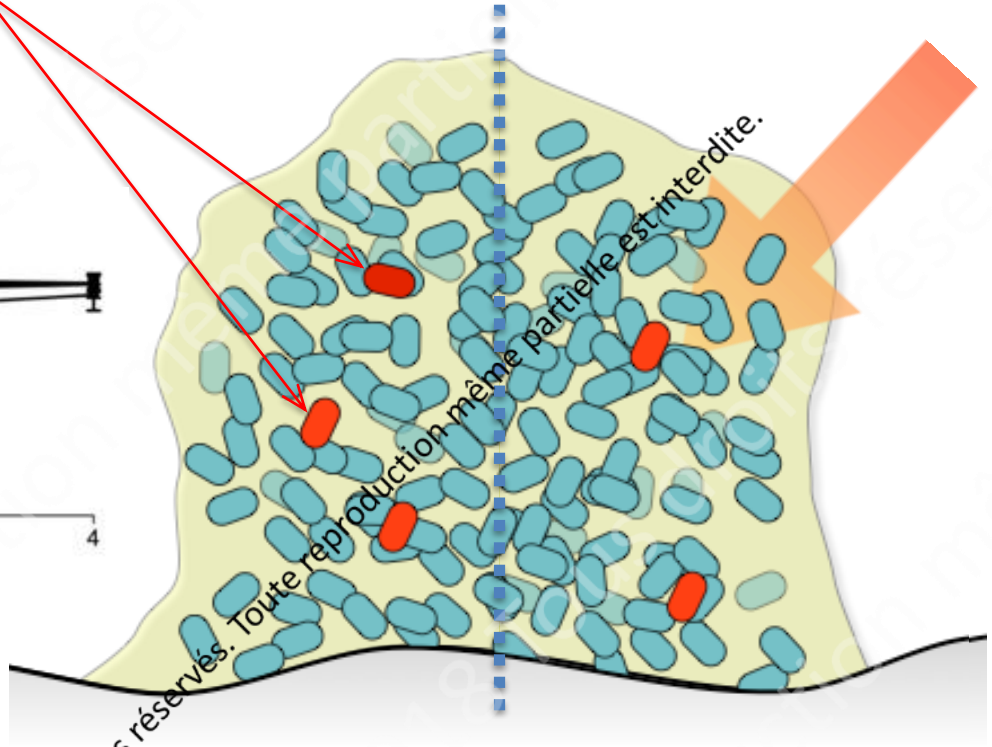
Zimmerli *et al* JAMA 1998

Biofilms are tolerant towards antibiotics

Persisters

Bactericidal antibiotics





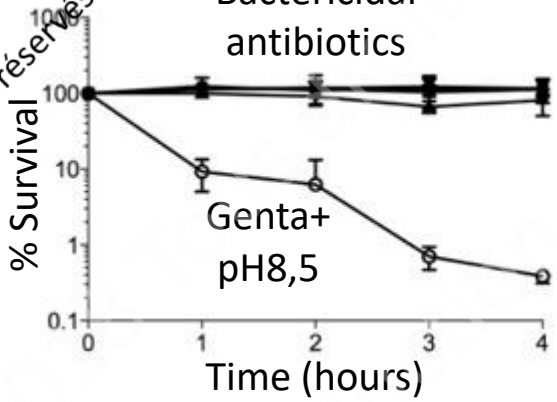
Impaired antibiotic diffusion

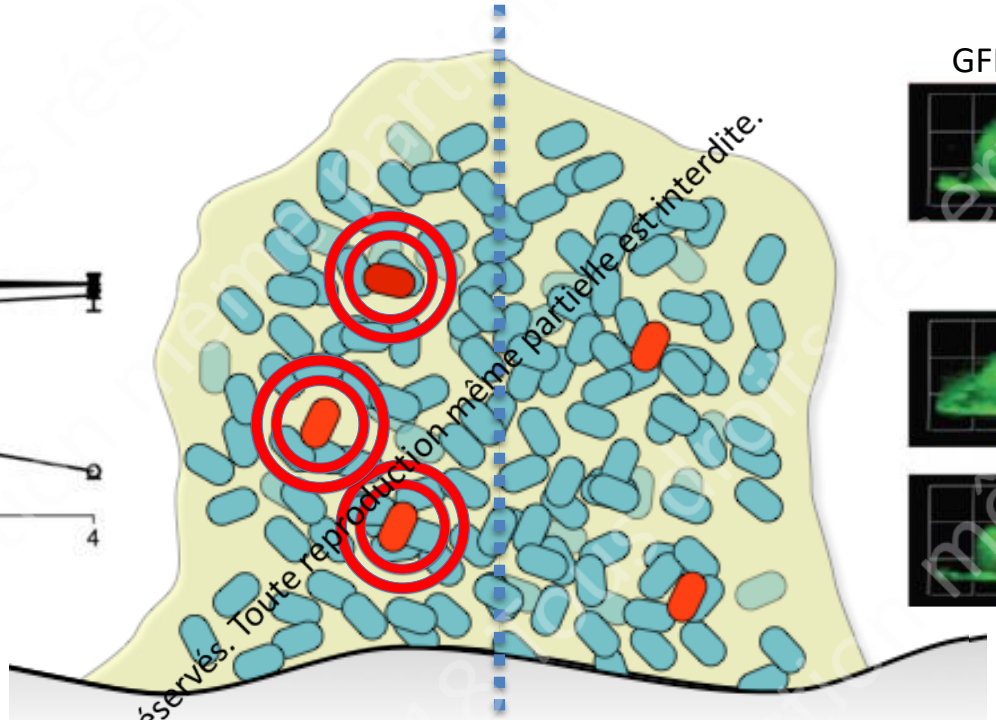
Lebeaux, D. *et al* 2015 J Antimicrob Chemother
Lebeaux, D. *et al* 2014 J Infect Dis
Chauhan A., Lebeaux, D. *et al* 2012 Antimicrob Agents Chemother
Chauhan A., Lebeaux, D. *et al* 2012 PLoS One

Biofilms are tolerant towards antibiotics

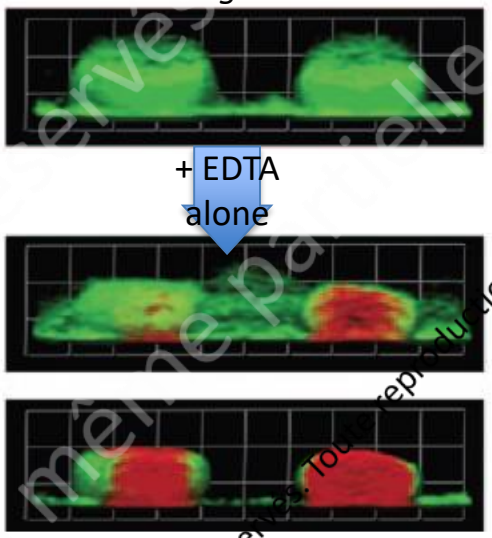
Anti-persister compounds?

Anti-matrix + antibiotics?





GFP *P. aeruginosa* biofilm



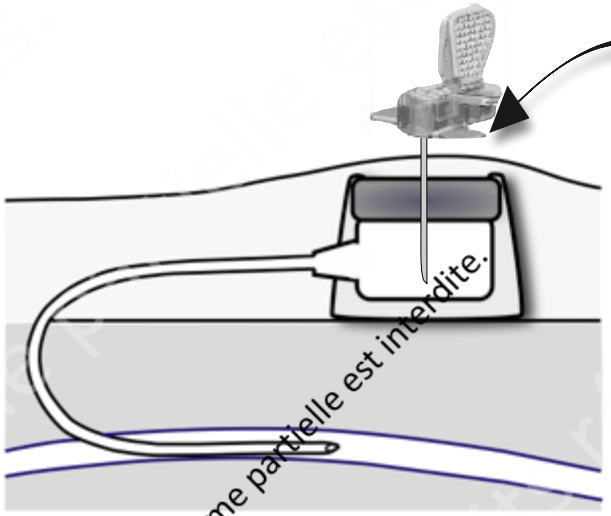
propidium iodide = Dead bacteria

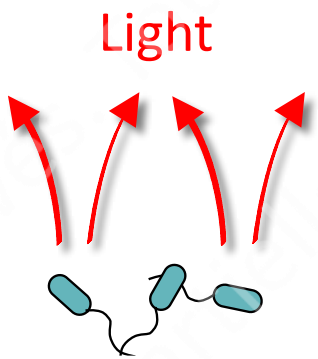
Gentamicin + EDTA

Lebeaux, D. *et al* 2015 J Antimicrob Chemother
Chauhan A., Lebeaux, D. *et al* 2012 Antimicrob Agents Chemother
Chauhan A., Lebeaux, D. *et al* 2012 PLoS One

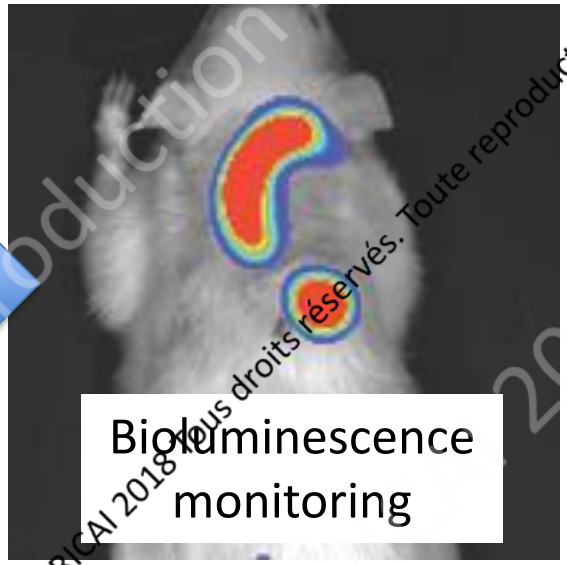
Banin, E. *et al* AEM 2006
Turakhia, M.H. *et al* AEM 1983

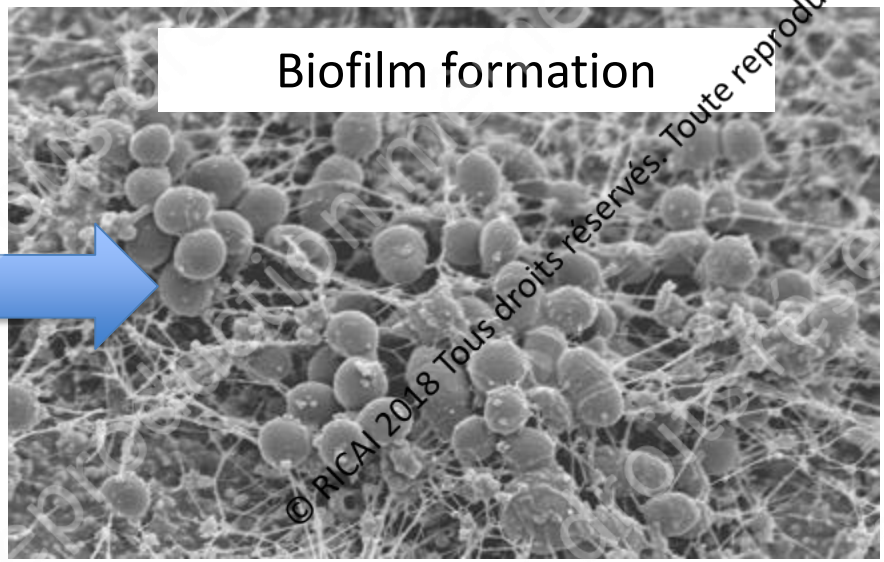
In vivo model of catheter-associated biofilm





Luminescent bacteria





Lebeaux, D. *et al* 2014 J Infect Dis / Chauhan A., Lebeaux, D. *et al* 2012 PLoS One

Anti-biofilm strategy: gentamicin + EDTA

- Staphylococcus aureus -

in vivo

- *S. aureus*
- *S. epidermidis*
- *E. coli*
- *P. aeruginosa*

in vitro

- 18 clinical strains

Lebeaux, D. *et al* 2015 J Antimicrob Chemother
Chauhan A, Lebeaux D *et al* 2012 Antimicrob Agents Chemother
Lebeaux, D. *et al* 2012 Medicine (Baltimore)

Antibiotic lock therapy (ALT)

Antibiotic lock technic (ALT) for catheter-related infections

Local / high concentration of antibiotics

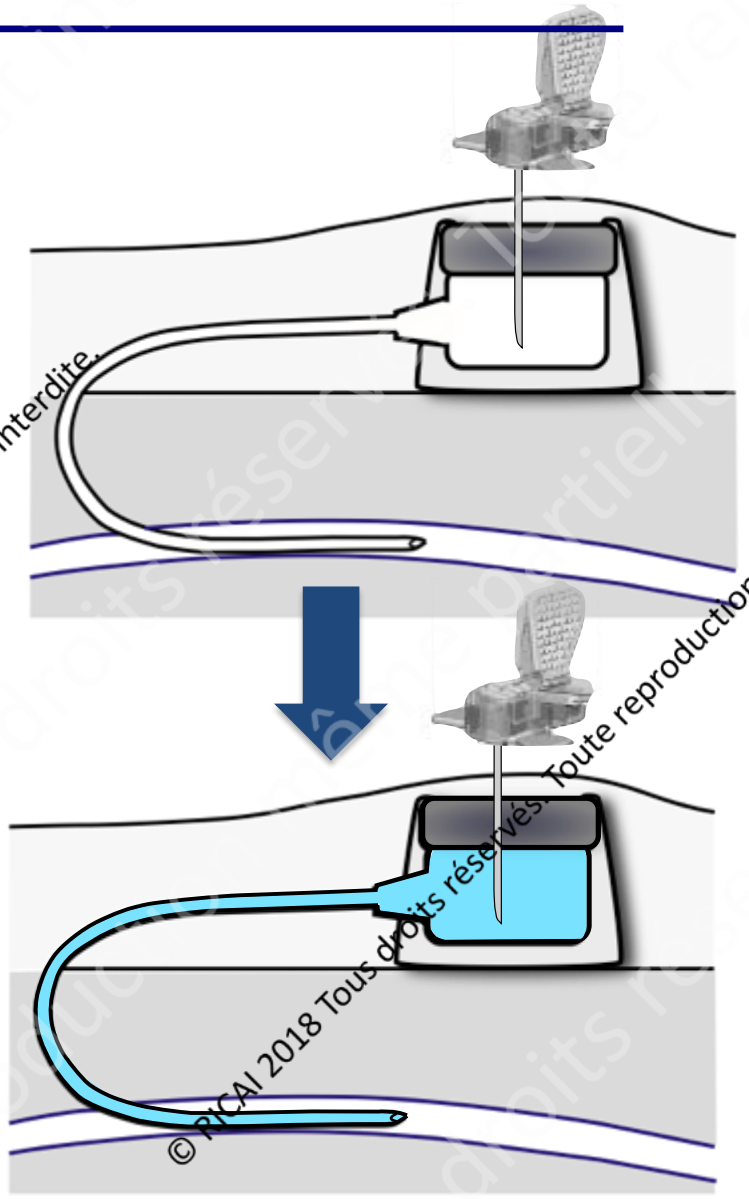
Long dwelling time (12-24 hours)

Already used in clinics

Uncomplicated C-RBSI caused by coagulase-negative staphylococci or enterobacteria

Success ~ 50% → 80%??

With Gentamicin + EDTA



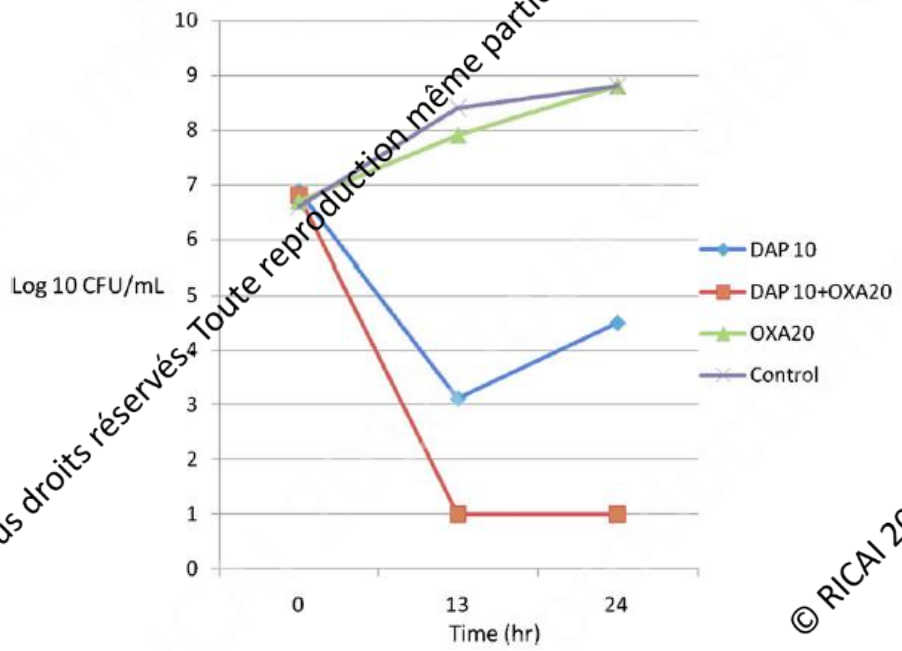
For long-term catheters

Conclusions

- Diagnostic improvement: imaging, microbiology
- A lot to be done with standardized tests
- Rifampin/fluoroquinolones for orthopedic implant–related staphylococcal Infections +++
- Beside, lower level of evidence
- A large room for improvement….

Daptomycin + oxacillin/nafcillin…

- MRSA persisting bloodstream infection
- Antistaphylococcal beta-lactam increases:
  - Daptomycin bactericidal activity
    - Daptomycin membrane binding
- Seven patients: cured, 2 relapsed



Dhand, A. *et al* Clin Infect Dis 2011

Daptomycin as lock therapy…

Table 1 Characteristics and outcome of patients included in the study

Age in years, median (IQR*)	64 (53–71)
Gender (male), no. (%)	7 (54)
Underlying disease	
Solid neoplasia, no. (%)	3 (23)
Haematological neoplasia, no. (%)	3 (23)
Renal failure, no. (%)	7 (54)
Neutropenia at diagnosis, no. (%)	1 (7)
Type of catheter (port/Hickman)	6/7
Catheter life span in days until infection episode, median (IQR)	282 (53–750)
Microorganisms	
Staphylococcus epidermidis, no.	6
Staphylococcus hominis, no.	2
Staphylococcus haemolyticus, no.	1
Enterococcus faecalis, no.	2
Polymicrobial (CNS), no.	2
ALT† duration in days, median (IQR)	14 (10–14)
IV antibiotic use, no. (%)	11 (85)
IV Daptomycin use, no. (%)	9 (69)
Outcome	
Success, no. (%)	11 (85)
Relapse, no. (%)	0
Failure, no. (%)	2 (15)
Infection attributable mortality, no.	0

*Interquartile range. †ALT, antimicrobial lock therapy.

ORIGINAL PAPER

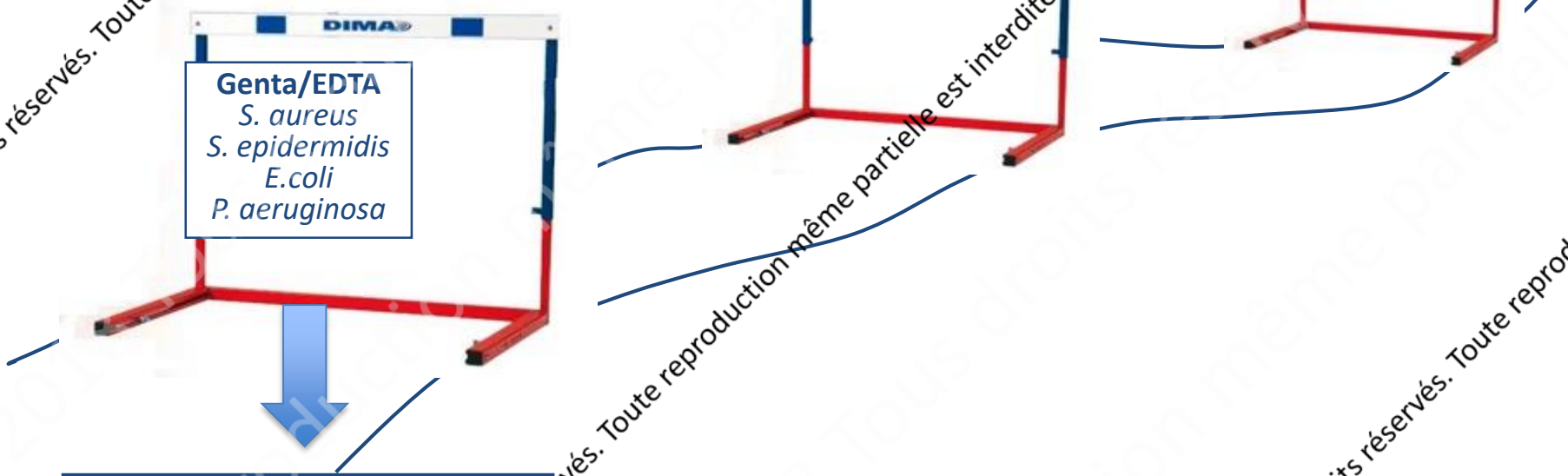
Daptomycin lock therapy for grampositive long-term catheter-related bloodstream infections

J. L. Del Pozo,[1,2] R. Rodil,[3] A. Aguinaga,[2] J. R. Yuste,[1,3] C. Bustos,[1] A. Montero,[3] G. Espinos N. García-Fernández[5]

Del Pozo, J.L. *et al* 2012 Int J Clin Practice

Toward a clinical study: a steeplechase run

Microbiology
Is it strain specific ?

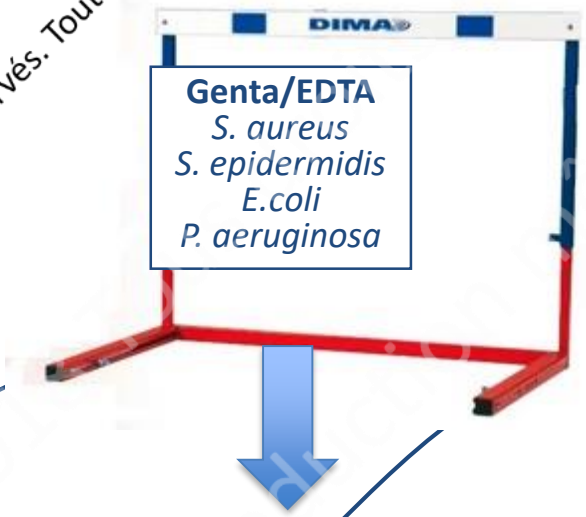


18 clinical strains
C-RBSI, Beaujon

Lebeaux, D. *et al* 2015 J Antimicrob Chemother
Lebeaux, D. *et al* 2012 Medicine (Baltimore)

Toward a clinical study: a steeplechase run

Microbiology ✓
Is it strain specific ?



Genta/EDTA
S. aureus
S. epidermidis
E.coli
P. aeruginosa

Pharmacy ✓

Institut Pasteur

Centre de Recherche Translationnelle

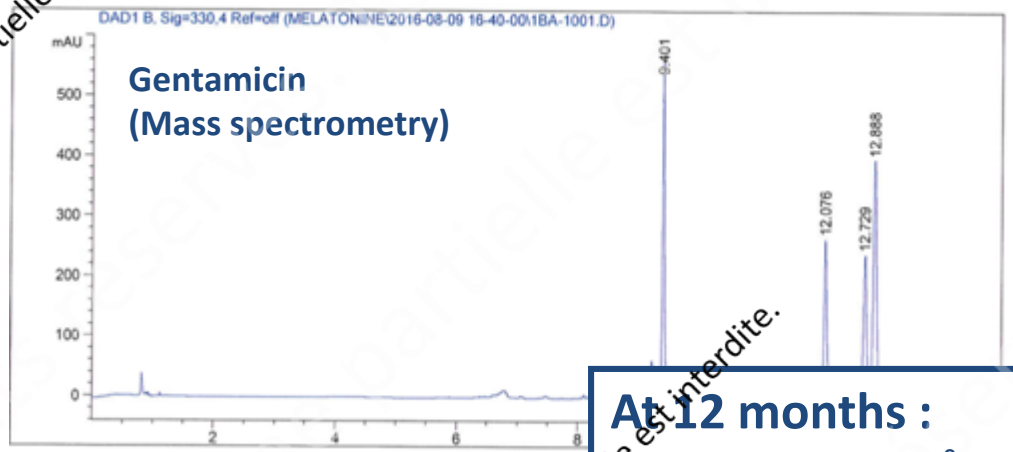
18 clinical strains
C-RBSI, Beaujon

EDTA + AB
Stability ?

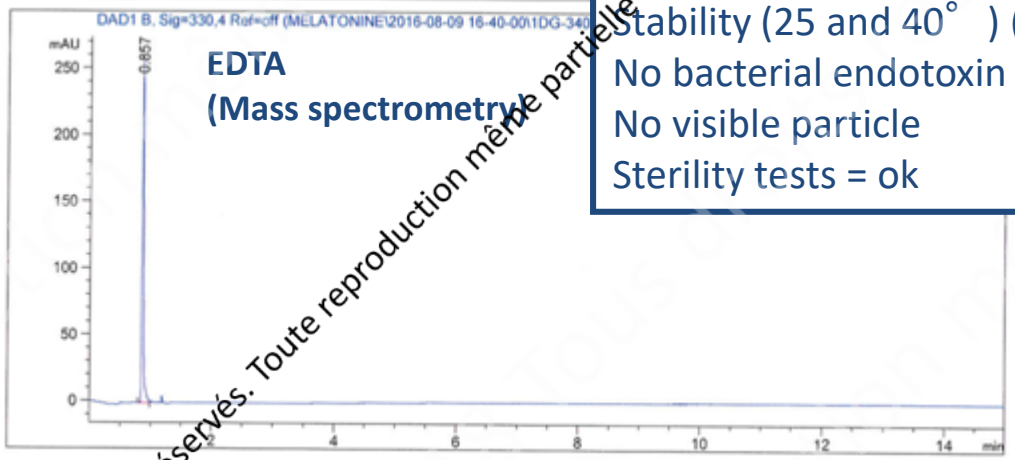
Pharmacie hôpital E. Herriot, Hospices Civils de Lyon, Plateforme FRIPHARM
Fabrice Pirot
Elise Jandot
Pauline Doucet
Anne-Sophie Fiolet

Lebeaux, D. *et al* 2015 J Antimicrob Chemother
Lebeaux, D. *et al* 2012 Medicine (Baltimore)

Toward a clinical study: pharmacology



Gentamicin
(Mass spectrometry)



EDTA
(Mass spectrometry)

At 12 months :

Stability (25 and 40°) (Mass-spec and UHPLC)
No bacterial endotoxin
No visible particle
Sterility tests = ok

Foliet, A.S. *et al* 2017 J Pharmaceutical Analysis

Pharmacie hôpital E. Herriot,
Hospices Civils de Lyon,
Plateforme FRIPHARM
Fabrice Pirot, Elise Jandot
Pauline Doucet, Anne-Sophie Fiolet

Toward a clinical study: a steeplechase run

Microbiology
Is it strain specific ?

18 clinical strains
CR-BSI, Beaujon

Pharmacy

EDTA + AB
Stable

Methodology for clinical study

Phase 2
Funding?

Lebeaux, D. *et al* 2015 J Antimicrob Chemother
Lebeaux, D. *et al* 2012 Medicine (Baltimore)

Toward a clinical study

- Phase 2 study
- 35 patients: HEGP, St-Louis, Mondor
- Adverse effects, efficacy
- Conservative treatment of uncomplicated LTIVC-related BSI
- Coagulase-negative staphylococci + *Enterobacteriaceae*
- Funding:
  - CRT Pasteur (technical/human support = 100 k€)
  - Labeix Pasteur (20 k€)
  - Fondation Descartes, Sauver la Vie (35 k€)
  - 25 k€ are missing: crowdfunding (Thellie) → November 2018

New approaches for biofilm eradication

Anti-persisters?

- Gentamicin
  - Sugar
  - ↑ pH (L-arginine)
- Rifampicin
  - ADEP4
- Antibiotic
  - Silver (ROS production)

Anti-matrix + antibiotics?

- Aminoglycosides
  - Chelators (EDTA)
- Antibiotics
  - **Pro-dispersal agents**
    Dispersin B, Dnase I, Autoinducing peptides (QS)

Clinical studies must now be performed

Others?

- Phages
- N-acetylcystein
- Mino/EDTA/EtOH

Lebeaux, D. *et al* 2014 J Infect Dis
Allison, K.R. *et al* 2011 Nature
Kim, J.-S. *et al* 2011 Antimicrob Agents Chemother
Chauhan, A., Lebeaux D. *et al* 2012 Antimicrob Agents Chemother
Morones-Ramirez, J.R. *et al* 2013 Sci Transl Med

Izano, E.A. *et al* 2008 Appl Environ Microbiol
Nijland, R. *et al PLoS One* 2010
Raad, I. *et al* 2013 Antimicrob Agents Chemother
Donlan, R.M. *et al* Clin Infect Dis 2011