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UNIVERSITE DE VERSAILLES
SAINT QUENTIN-EN-YVELINES

The role of animal models in the treatment of prosthetic joint infections

AC Crémieux

Financial disclosures

Grants for consultancies, workshops or travel to meetings from:

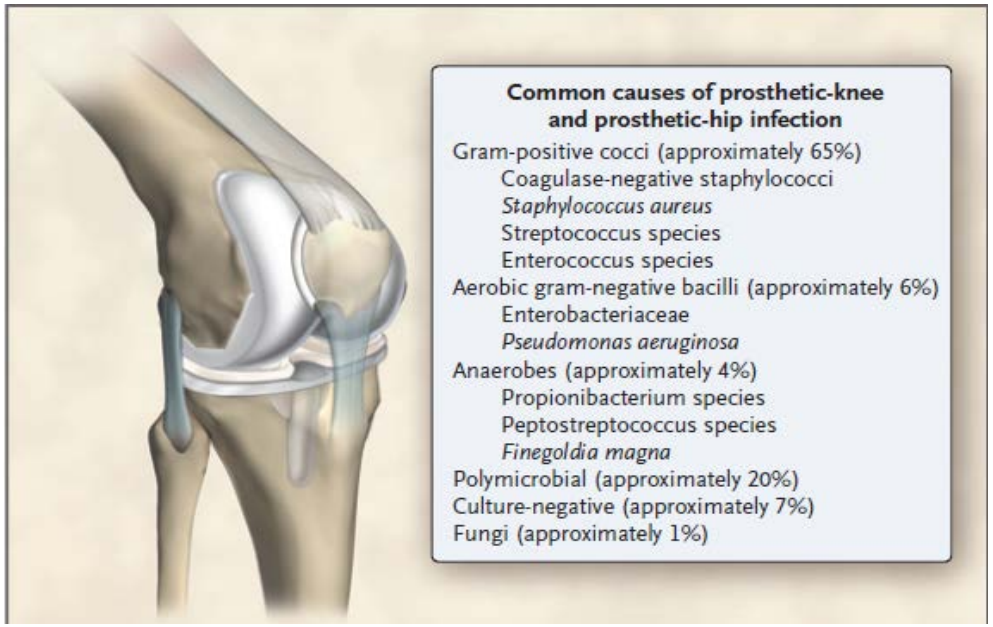
- **Novartis**
- **Janssen-Cilag**
- **Sanofi-Aventis**
- **Heraeus**
- **Astra-Zeneca**
- **Teva**

A challenge for antibiotics (1)

Common to biofilm-related infections

- **Endocarditis**
- **Osteomyelitis**
- **Implant-related infections**

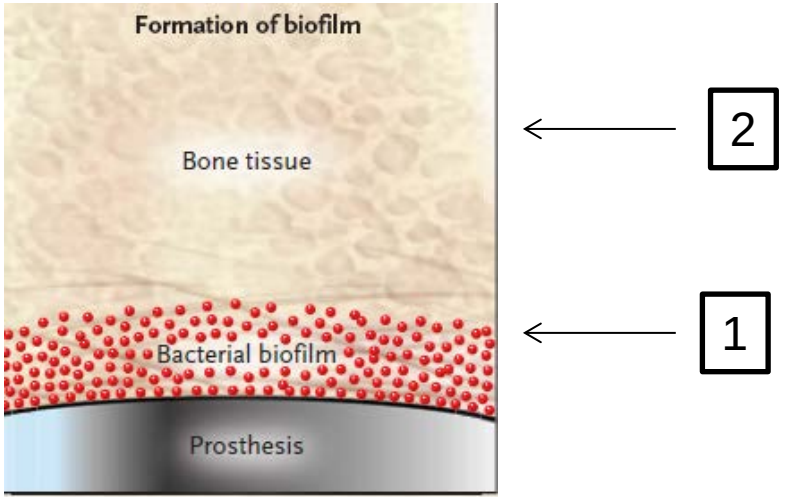
- **Difficult-to-cure/Recalcitrant infections**



Two factors in PJIs:

IMPLANT

BONE



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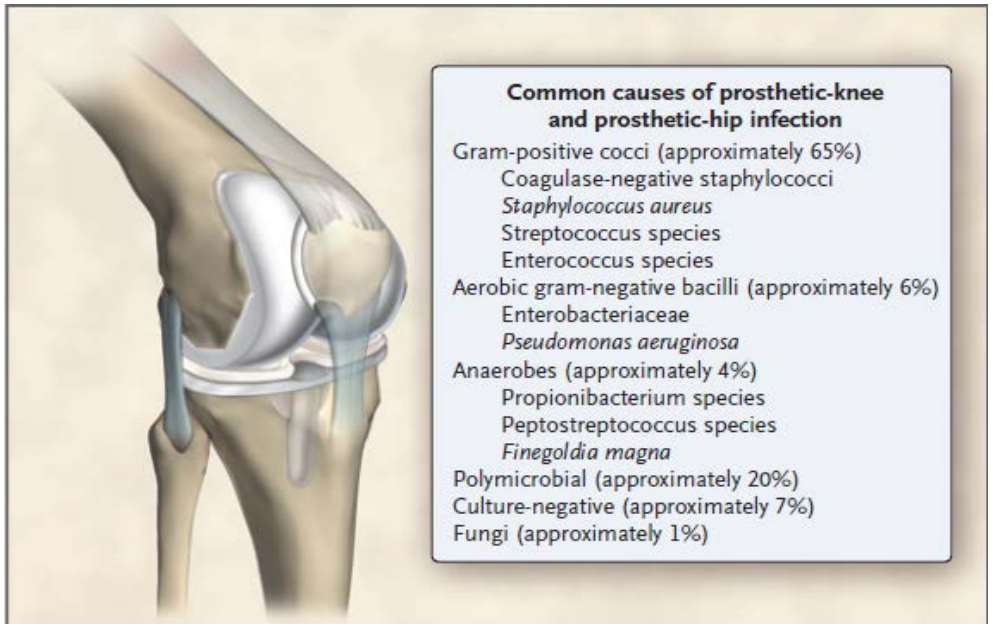
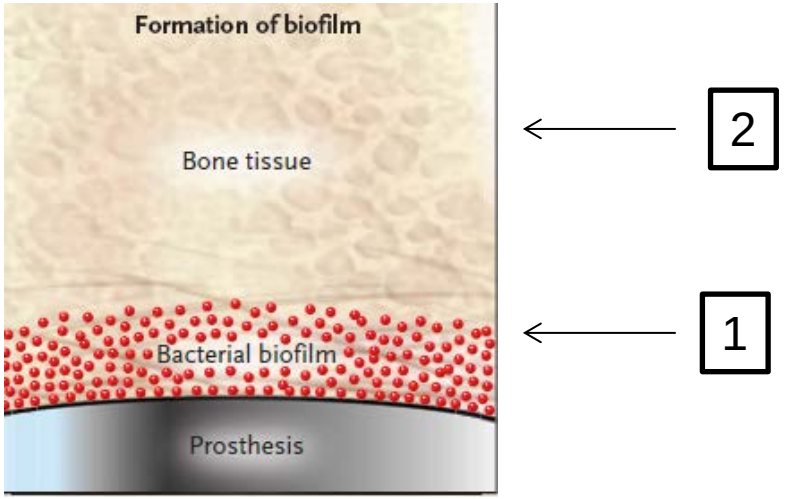


Two factors in PJIs:

IMPLANT

BONE



A challenge for antibiotics (2)

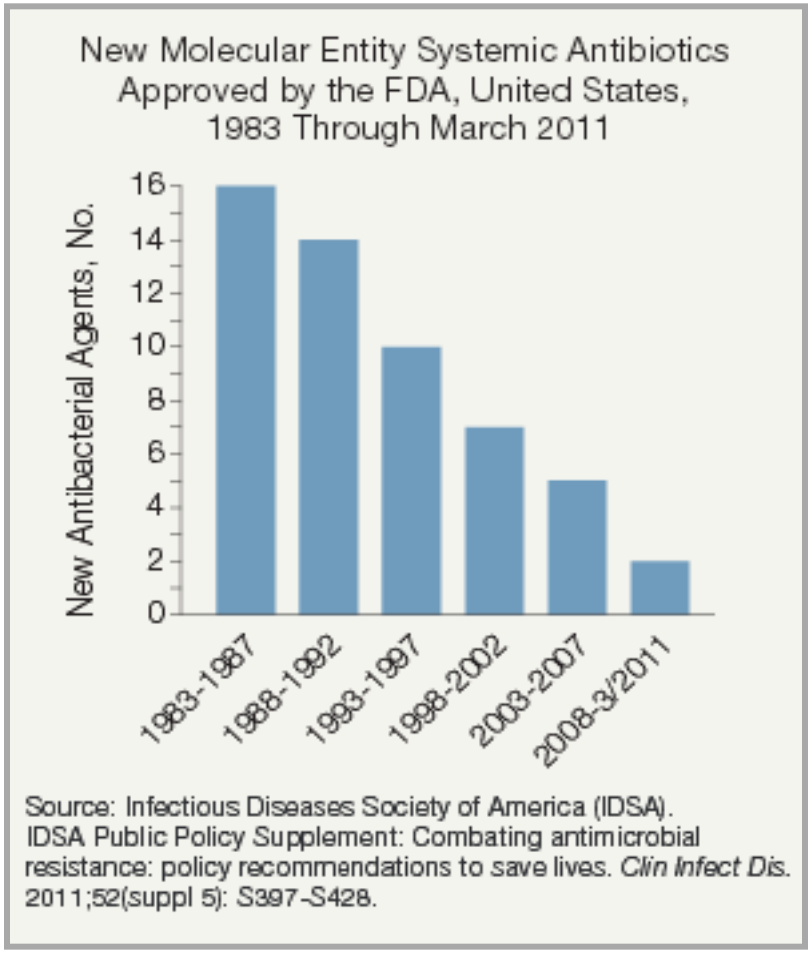
Antibiotic resistance in orthopaedic surgery: acute knee prosthetic joint infections due to extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*

LETTER TO THE EDITOR

Injured Libyan combatant patients: both vectors and victims of multiresistance bacteria?

Difficult-to-treat Gram-negative bone and joint infections: efficacy and safety of prolonged intravenous colistin☆

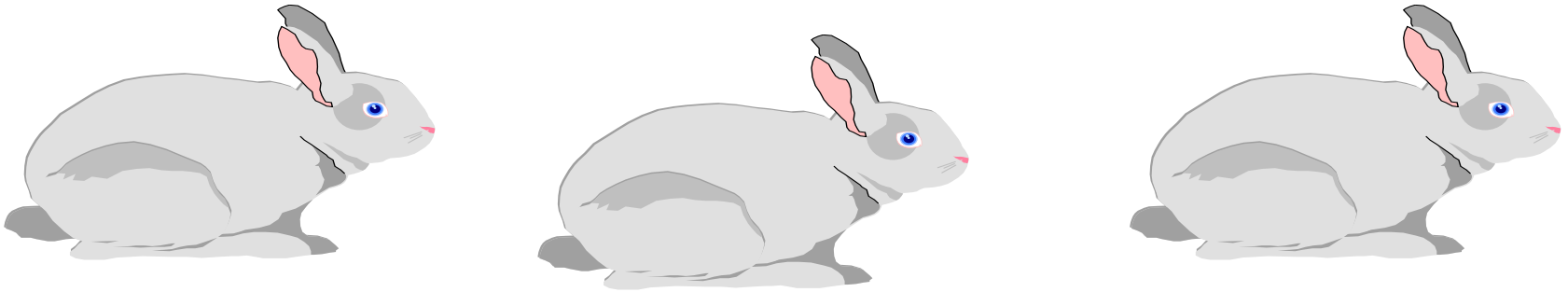
- **Multidrug-Resistant Bacteria**



Antibiotic crisis

Animal models of PJI (treatment)

- **Role**
  1. **Improve understanding of PJI recalcitrance & impact of virulence factors *in vivo***
  2. **Enables comparison of *in vivo* antibiotic efficacies**
- **Randomized prospective clinical trials are difficult to design because of the wide variability of factors conditioning the response to treatment**



We must question the relevance of our experimental models ++

There's more to life than rats and flies

BEST HIT

Choosing a research model should be more than a matter of convenience or convention. Scientists need to ask more questions — about the goals of a specific experiment, how suitable a given model is to reaching those goals, and what environmental or other external factors might be relevant to how well the model works. For a given

References

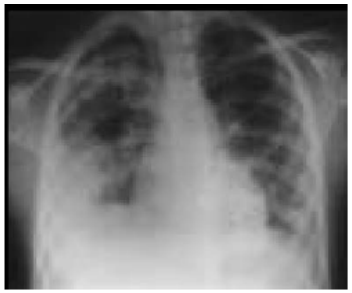
Bolker, Nature 2012
Wright III, Novick, PNAS 2005
Lebeaux, Pathogens 2015
Dastgheyb, Otto JID 2015

The role of Hla, PVL and PSM in experimental CA-MRSA osteomyelitis

D0 — D7 — D14

Acute initial phase

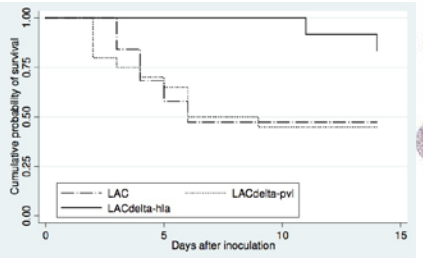
(severe sepsis with pulmonary involvment)

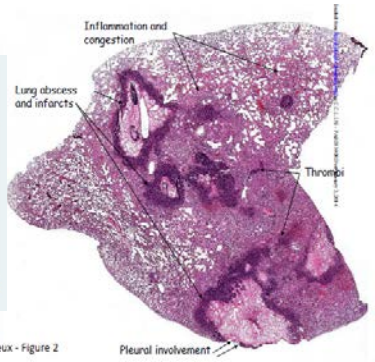


Lung X-ray shows bilateral, multifocal pneumonia

Hla

PSMs





Subacute phase

(osteomyelitis with extension to muscles)

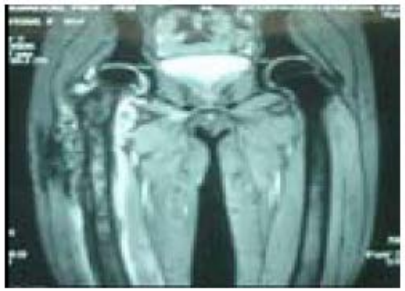
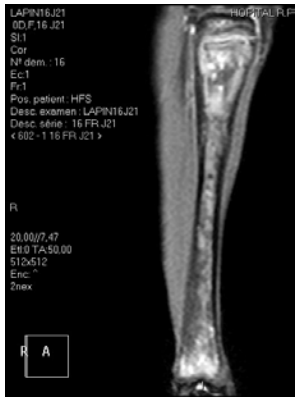
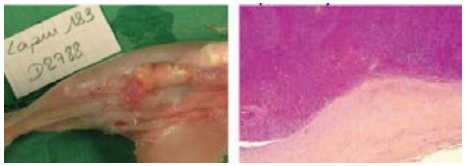


Fig. 3 MRI of right femur one month after onset of disease showing more extensive destruction of bone and its periosteum

PVL

~~PSMs~~





(Crémieux, Lina, Vandenesch et al, PloS One 2009, JID 2014)(Davido, Laurent et al PloS One 2016)

Staphylococcal quorum sensing in biofilm formation and infection

(Kong, Vuong, Otto IJMM 2006) (Dastgheyb, Otto Infect Immun 2015)

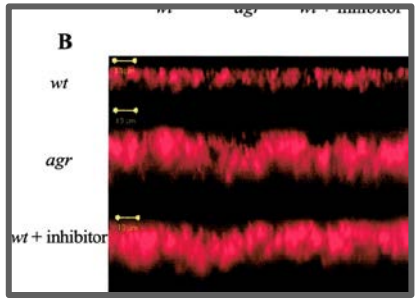
Acute/initial phase

"Significant QS activity in staphylococci is observed for actively growing cells at a high cell density, such as during the initial stages of an infection and under optimal environmental conditions."

High expression of agr-dependent cytolytic toxins: Hla, PSMs

Subacute/chronic phase characteristic of biofilm-related infections

"In contrast, the metabolically quiescent biofilm mode of growth appears to be characterized by an overall low activity of the staphylococcal QS systems."



Biofilm depth (confocal laser scanning microscopy

Lower expression of agr-dependent cytolytic toxins: Hla, PSMs

Messages: 1

- **Impact of infection duration on the metabolism of bacteria *in vivo* explains discordant results between acute and subacute/chronic models**

- **Anti-biofilm therapies must be tested in persistent subacute or chronic experimental models**

  Ex: Nature 21 Nov 2013: "Activated ClpP kills persisters and eradicates a chronic biofilm infection"

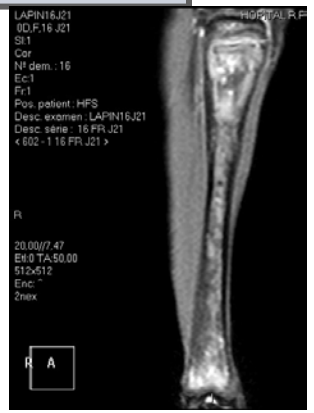
➔ **neutropenic thigh-infection model treated 24 h after inoculation of mice!**

Experimental models of bone and prosthetic joint infection

(AC Crémieux, C Carbon CID 1997)

1. Chronic osteomyelitis

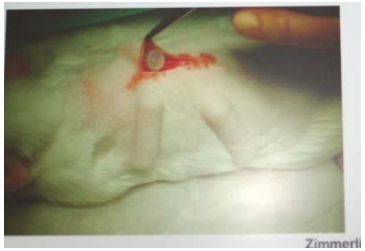
(Adapted from Norden's model JID 1970)



2. Animal models of foreign body infections

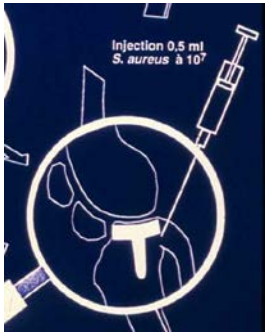
Subcutaneous tissue-cage model

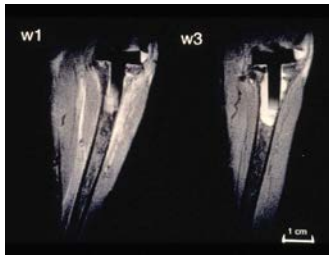
(Zimmerli, Waldvogel, Vaudaux JID 1982)



3. Prosthetic joint infection model

(AC Crémieux, A Saleh-Mghir, R Bleton, N Belmatoug, C Carbon, JID 1996)





AMERICAN SOCIETY FOR MICROBIOLOGY
50th ICAAC
September 12-15, 2010
Boston, MA
Poster B-698

COMBINING HIGH-DOSE DAPTOMYCIN AND RIFAMPIN IS CRUCIAL FOR THE TREATMENT OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* RABBIT PROSTHETIC JOINT INFECTION

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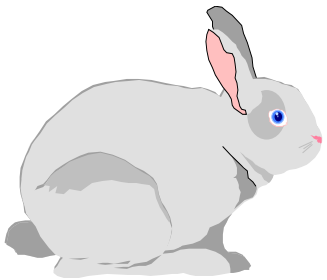
In vitro studies

DAP, VAN and RIF MIC were 0.125, 1.5 and 0.008 mg/L, respectively.

In vivo



Treatment on D7

Table2. Effect of antibiotic Rx on experimental MRSA prosthetic knee infection in rabbits

Rx[a]	No. of rabbits with sterile bone/Total	$\log_{10}$ CFU/g of bone mean ± SD	Decreased susceptibility to DAP/Total
None	0/9	5.93 ± 1.15	2/9
DAP	2/12	4.23 ± 1.44[b]	7/12
VAN	0/12	4.63 ± 1.08[b]	4/12
DAP+ RIF	11/11	1.47±0.04[c]	-
VAN+ RIF	6/8	1.50 ± 0.12[c]	0/2*

[a]Rabbits were treated for 7 days with DAP (22 mg/kg, iv., od.) or VAN (60 mg/kg, im, bid.) alone and combined with RIF (10 mg/kg, im, bid).
[b]Significantly different from untreated controls ($p<0.01$).
[c] Significantly different from monotherapy($p<0.01$).
* Other rabbits had sterile bones

DAP = 8 mg/kg OD

(*Antimicrobial Agents Chemother* 2011)

In vivo co-evolution of daptomycin resistance (DAP-R) and cationic host-defense peptide (HDP-R) cross-resistance profiles in untreated and DAP-treated animals with experimental MRSA PJI

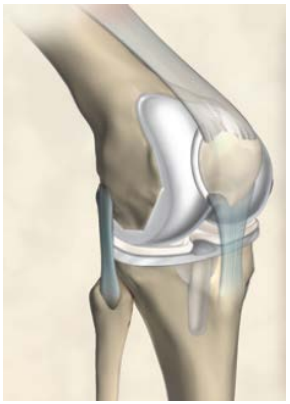
(Mishra, Bayer, *PLoS One* 2013)

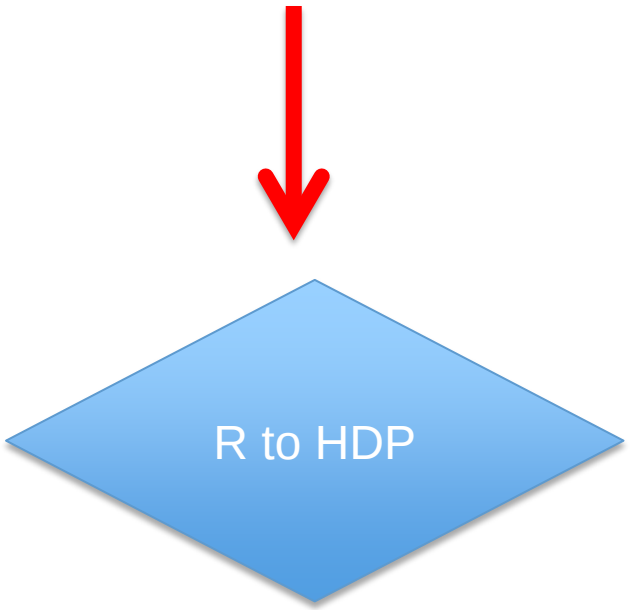
Strains	DAP (µg/ml)	tPMPs (2 µg/ml)	hNP-1 (10 µg/ml)	CW thickness (nm)	Total LPG
271 (Parent)	0.125	19±19	8±6	20.55±2.24	14.35±0.02
L16 untreated	0.75	58±13 *	17±4 *	21.37±2.67 *	31.01±1.82 *
L8 (DAP)	2	76± 27 *	38±27 *	24.98±2.94 *	31.75±0.54 *
L56 (DAP)	2	71±16 *	33±20 *	25.53±2.97 *	39.15±0.68 *

These data suggest the potential **that *S. aureus* strains may use adaptive mechanisms when encountering HDPs *in vivo*. DAP co-exposures may amplify those adaptive responses.**

*** P < 0.05 vs parental strain.**

Messages 2 : Natural resistance of MRSA to host-defense peptides in experimental PJI





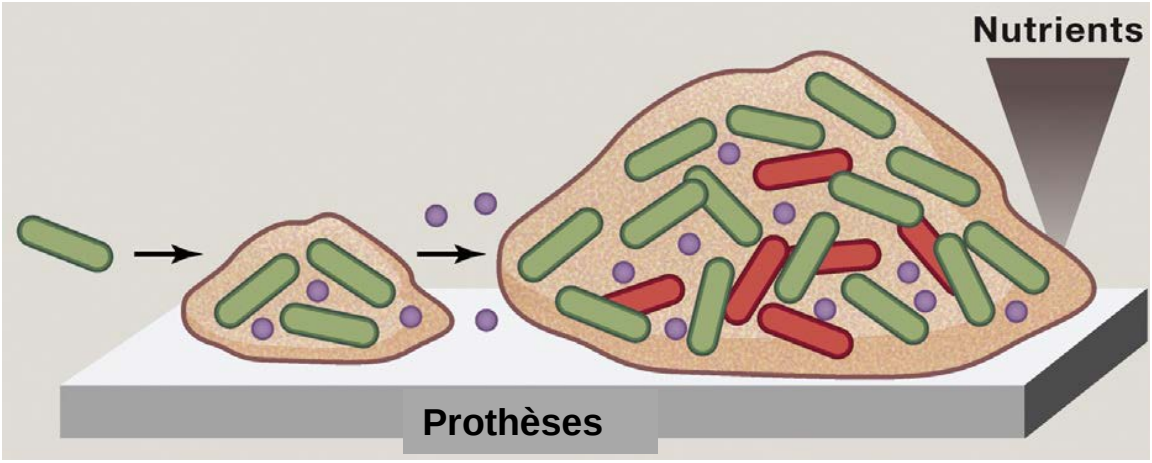
- **Could play a role in PJI persistence +++**
- **Daptomycin (lipopeptid AB) should be always used in combination therapy to avoid the emergence of resistant strains and therapeutic failures**
- **Prudent development of antimicrobial peptides (AMPs) as therapeutic agents**

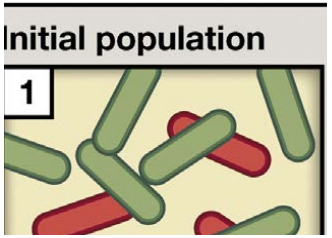
L'adaptation métabolique in vivo de S aureus à un environnement local défavorable lui permet de Persister et de Résister aux AB

D0 — D7 — D17 — AB

Host local environmement
- *Shortage of nutrients*
- *HDPs*

Biofilm
Dormant state (SCVs)
R to HDPs



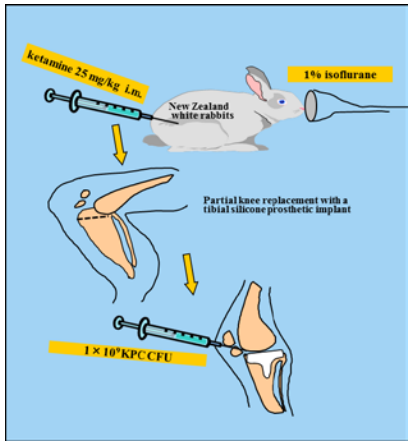


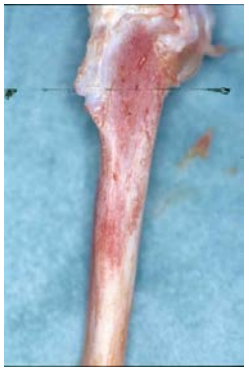
PERSISTANCE

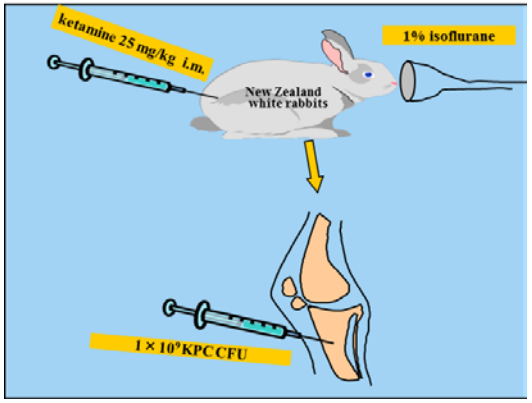
RESISTANCE

New experimental models of carbapenem-resistant *Klebsiella pneumoniae* prosthetic joint infections and post-traumatic osteomyelitis

L GATIN, A SALEH MGHIR, F LAURENT, MC VERDIER, I GHOUT, P TATTEVIN and AC CREMIEUX

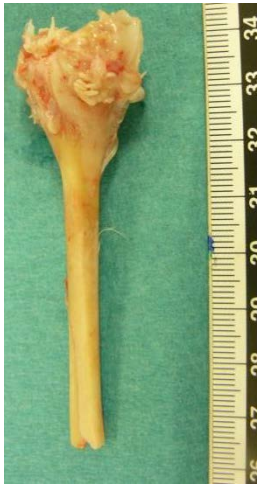


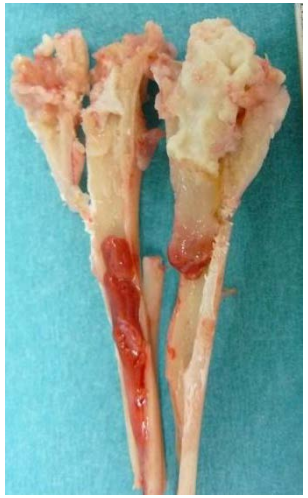


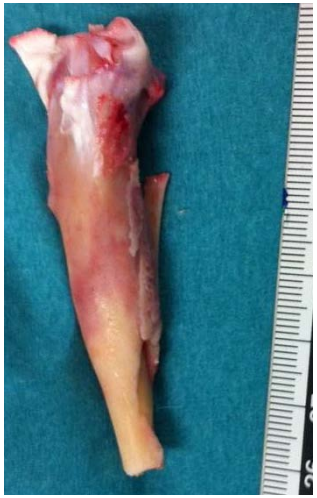


Experimental prosthetic joint infection (D31)

Experimental post-traumatic osteomyelitis (D31)







Souche KPC99YC

Résultats détermination CMI

Méthode	*Imipenem*	*Tigécycline*	*Fosfomycine*	*Gentamicine*	*Colistine*	*Meropenem*
E-Test	4 mg/L	1 mg/L	24 mg/L	1 mg/L	0,25 mg/L	4 mg/L
Macrodilution	32 mg/L	1 mg/L	32 mg/L	0,5 mg/L	1 mg/L	4 mg/L
		Susceptible	**Susceptible**	**Susceptible**	**Susceptible**	**Intermediate**

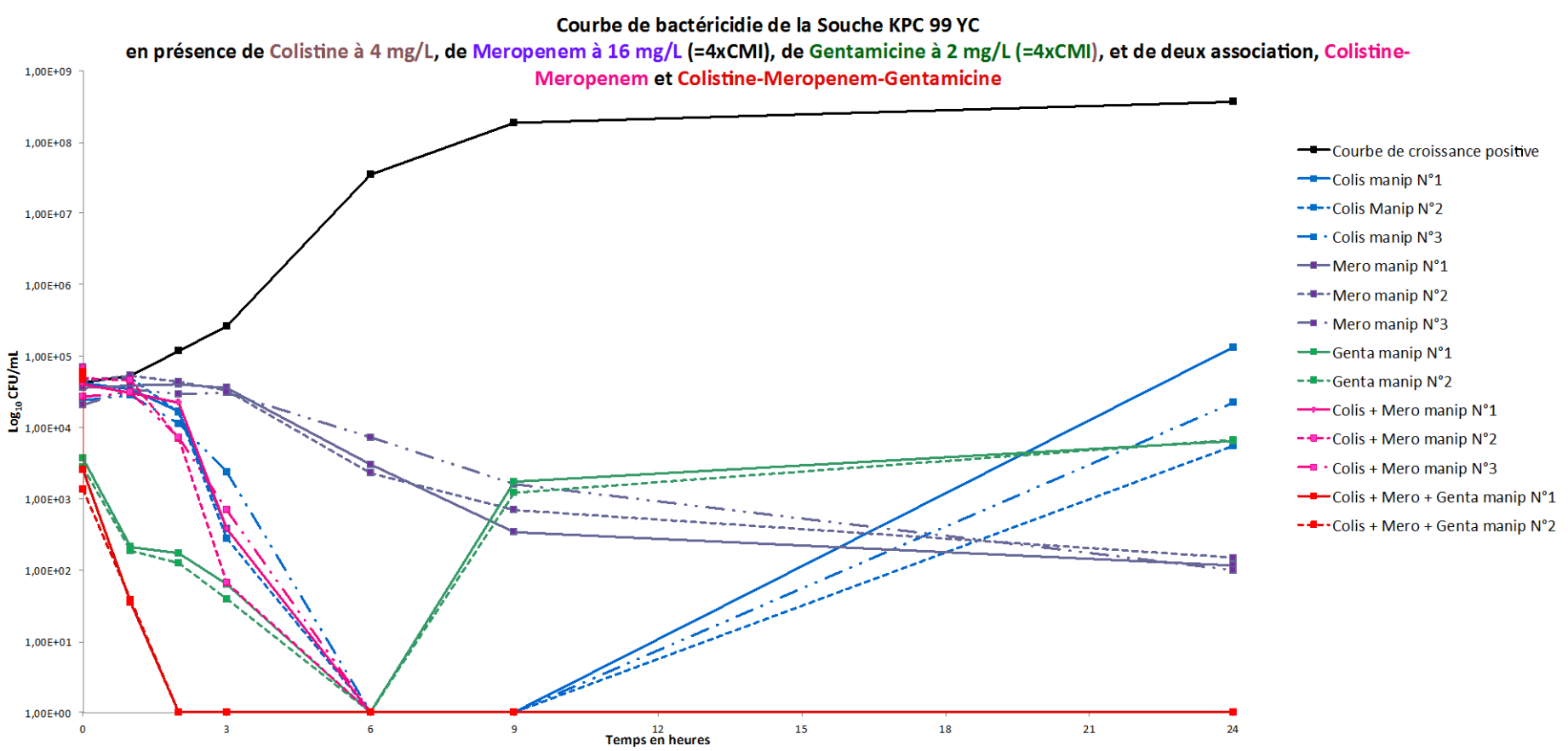
Inoculum à environ 5.10^4CFU/mL

Appartient au clone ST-258/Clonal Complex 292, le plus répandu

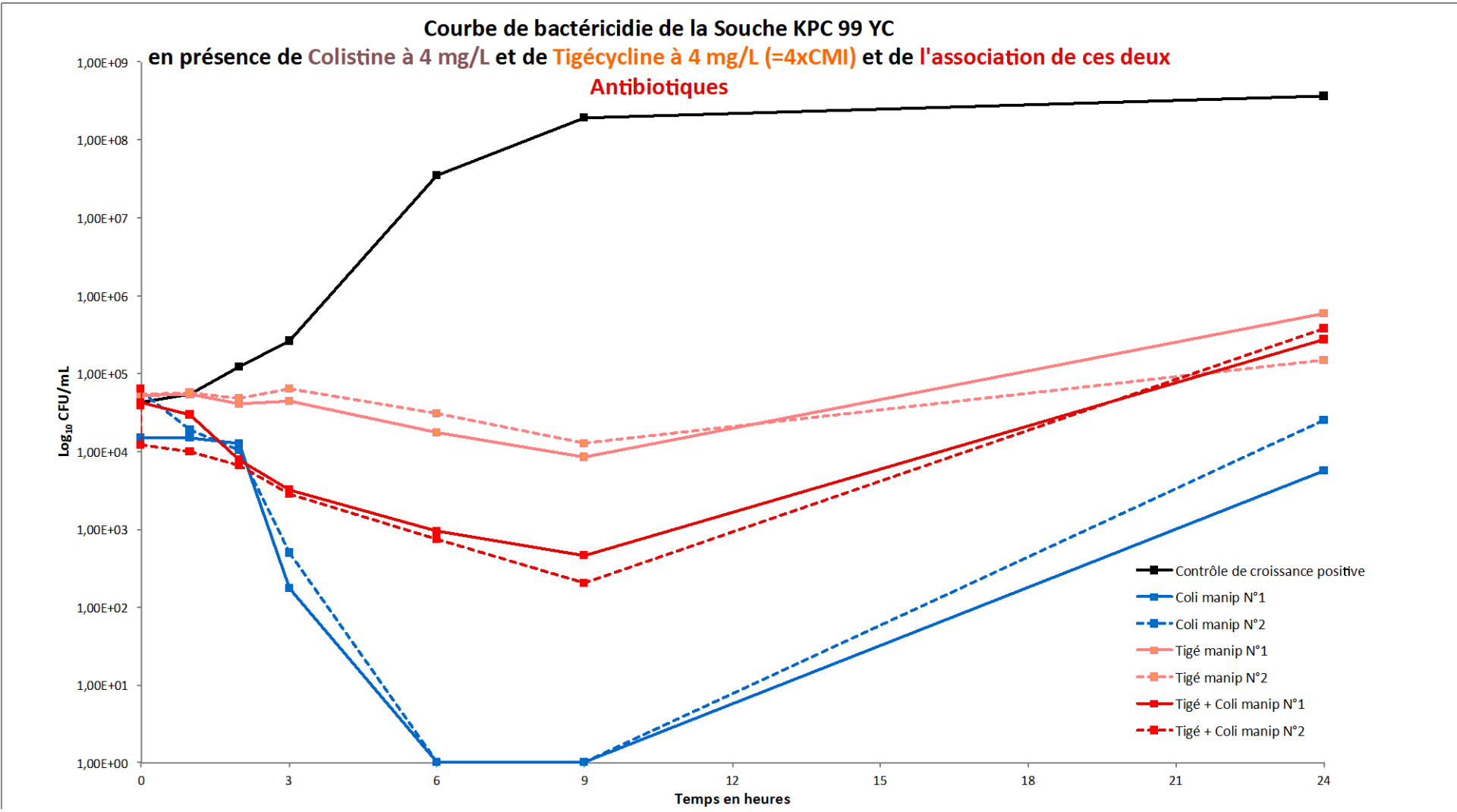
Ostéite expérimentale à KPC99YC

Groupe	CFU/g os à J15	P vs témoin	N os stérile J15	P vs témoin	N R coli	CMI coli
Témoin (N=10)	5.8 [4.9, 6.1]		0			
Coli (N=10)	5.7 [5, 6.2]	1	0	NS	3	4, 64, 64
Coli-Genta (N=11)	5 [4.6, 5.6]	0.179	1	NS	1	ND
Coli-Tige (N=11)	6.6 [6, 7.2]	0.023	0	NS	2	4, 32
Coli-Mero (N=11)	4.6 [4.1, 5.3]	0.019	0	NS	0	-
Coli-Mero-Genta (10)	1.5 [1.5, 4]	0.001	7	0.003	0	-
Mero-Fosfo (N=10)	6.3 [5.1, 6.5]	0.24	0	NS	-	-

Tx was started 14 days after, for 7 days; colistin (C) 150 000 IU/kg/12 mg/kg im tid (equivalent to 3 M IU tid in H), gentamicin (CG), 30 mg/kg im od (5 mg/kg od in H) ; tigecyclin 14 mg/kg im bid (50 mg bid in H) ; meropenem 80 mg/kg sc tid, (1 g tid in H), fosfomycine 150 mg/kg bid (16g/j)



EFFET SYNERGIQUE COLI-MERO±GENTA



EFFET ANTAGONISTE COLI-TIGE

Message 3

In this subacute model of carbapenem resistant osteomyelitis:

- Meropenem + coli ± gentamicin was the only effective therapy and prevent emergence of colistin-resistance,
- This result is in agreement with the concept that

  i) colimycin should be included in the regimen;

  ii) penems should be included whenever MIC<8 mg/L.

• Of note, the combination of colistin and tigecyclin was less effective than colistin alone, *suggesting an antagonistic* effect *in vitro and in vivo*.

Acknowledgments

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