Principes de traitement des infections à bactéries intracellulaires

Pr Amandine Gagneux-Brunon
Pr Elisabeth Botelho-Nevers

GIMAP (Campus Santé Innovations, UJM, UDL), CIRI
Service d'Infectiologie (CHU de Saint-Etienne)
Inserm CIC 1408- Axe Vaccinologie, I-Reivac, COVIREIVAC
Présage (Institut de prévention en santé globale)

Diplôme Universitaire de Thérapeutiques Anti-Infectieuses
Université Grenoble Alpes
1ère session – Janvier 2026

Plan

- Antibiotiques intracellulaires
- Principes de traitement des infections intracellulaires: *C. burnetii, Bartonella spp, Rickettsia spp, S. aureus*!

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- Antibiotiques intracellulaires
- Principes de traitement des infections intracellulaires: *C. burnetii, Bartonella spp, Rickettsia spp, S. aureus*!

Action intracellulaire des antibiotiques?

- Traitement des infections à bactéries intracellulaires strictes (obligatoires): *Coxiella burnetii, Rickettsia spp , Chlamydia spp, Ehrlichia spp, Anaplasma spp, Tropheryma whipplei, Mycobacterium spp*
- Traitement des infections à bactéries intracellulaires facultatives:
  - Les vraies: *Legionella spp, Mycoplasma spp, Bartonella spp, Francisella tularensis, Listeria monocytogenes, Samonella spp, Yersinia spp, Brucella*
  - Les « occasionnelles »: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes*

Cellules cibles

Souvent des macrophages… mais pas que

Main disease-causing intracellular bacteria and their preferential target cells.

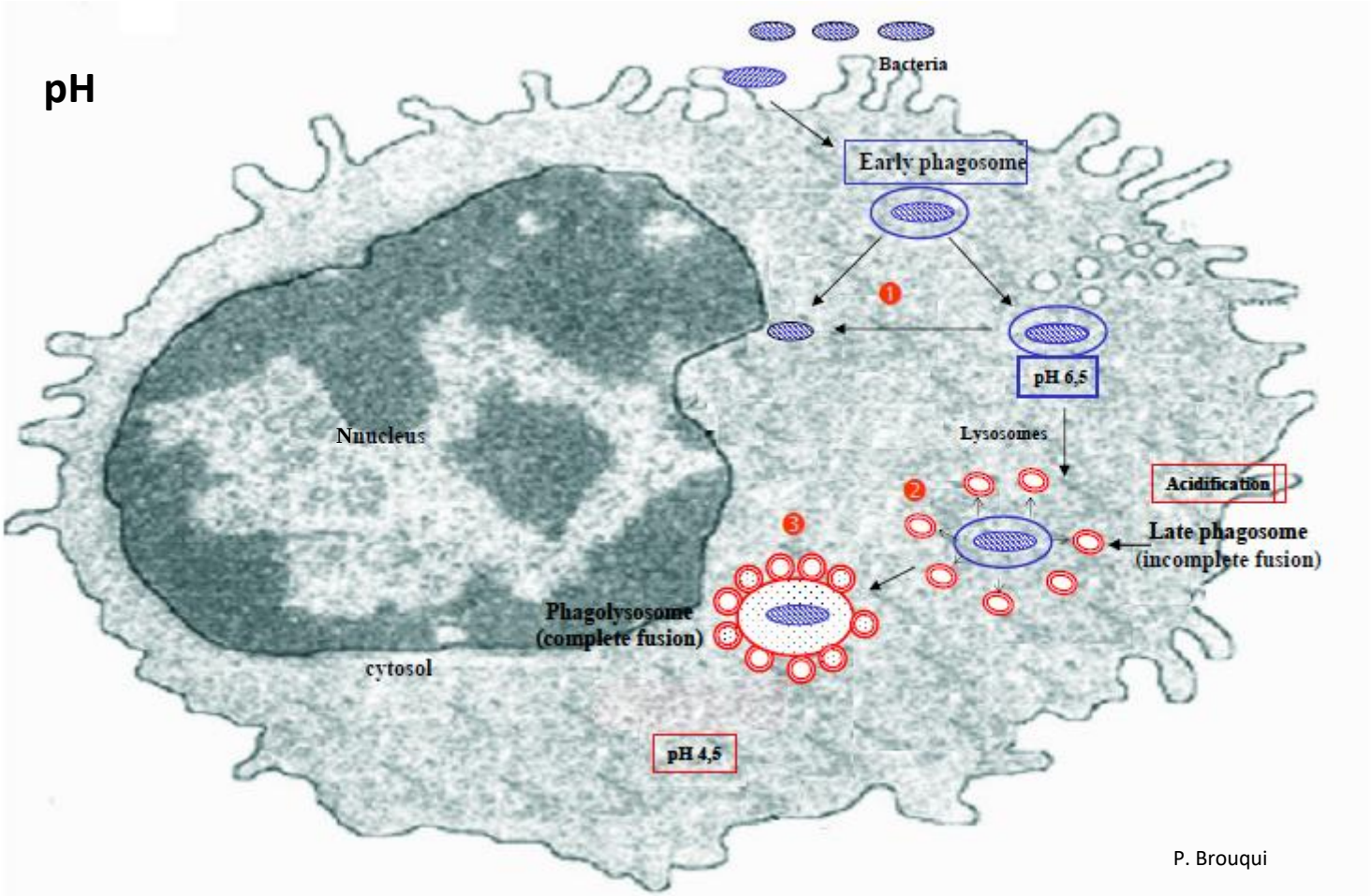
Bacterial pathogen	Associated disease(s)	Target cells	Reference(s)
Mycobacterium tuberculosis	Tuberculosis	Macrophages, hepatocytes	[13,14]
Mycobacterium avium complex	Pulmonary infections	Alveolar macrophages	[15]
Mycobacterium leprae	Leprosy	Macrophages, epithelial cells	[16]
Listeria monocytogenes	Listeriosis, meningitis, septicaemia	Macrophages, hepatocytes, enterocytes	[17,18]
Staphylococcus aureus	Pneumonia, mastitis, phlebitis, endocarditis, nosocomial infections, urinary tract infections, osteomyelitis	Macrophages, polymor-phonuclear neutrophils	[19]
Salmonella spp.	Salmonellosis, typhoid fever	Macrophages, enterocytes	[20,21]
Brucella spp.	Brucellosis	Macrophages	[22]
Yesinia pestis	Plague	Macrophages	[23]
Escherichia coli	Diarrhoeal illness, urinary tract infections, meningitis in neonates	Epithelial cells, macrophages	[24,25]
Pseudomonas aeruginosa	Pneumonia, endocarditis, meningitis, nosocomial infection	Macrophages, epithelial cells	[26,27]
Legionella pneumophila	Pneumonia	Macrophages	[28]

N Abed et al., IJAA 2014; 43: 485–496

Cellules cibles

- Les cellules cibles sont variables selon l'infection:
  - **Hématies:** *Bartonella spp*
  - **Monocytes-Macrophages:** *C. burnetii, Ehrlichia spp, L. monyctogenes, Legionella spp, Brucella spp, F. tularensis, Yersinia, Salmonella, S. aureus, Mycobacterium tuberculosis….*
  - **Cellules endothéliales***: Rickettsia spp., Bartonella spp.,*
  - **Cellules épithéliales**: *Chlamydia spp, S. aureus, E. coli*
  - **Ostéoblastes:** *S. aureus….*

pH

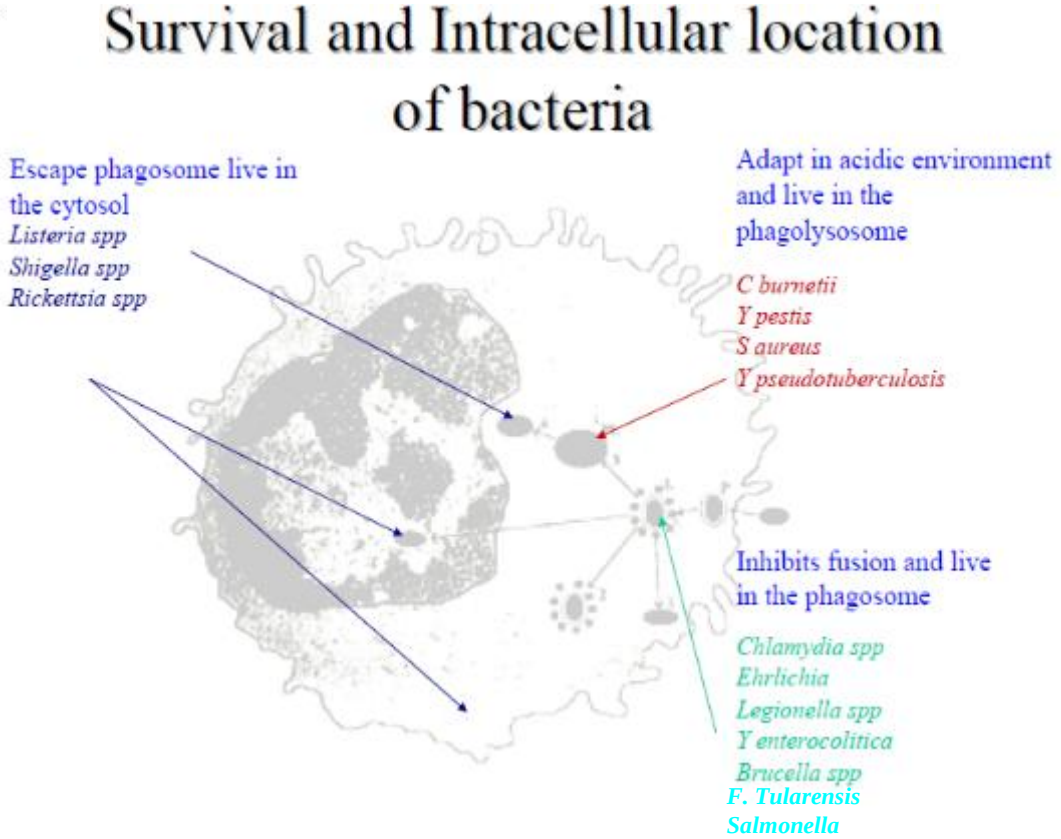

Bacteria
Early phagosome
1
pH 6,5
Nuucleus
Lysosomes
Acidification
2
3
Late phagosome
(incomplete fusion)
Phagolysosome
(complete fusion)
cytosol
pH 4,5

P. Brouqui

Table 1
Main intracellular bacteria with predominant target cells in humans and known subcellular localization of virulent forms

Organism	Type of parasite	Target cells	Subcellular localization	References
Brucella spp	Facultative	Macrophages	Phagosomes	[99]
Chlamydia spp	Obligate	Lung parenchyma cells	Inclusions	[100]
Coxiella burnetii	Obligate	Macrophages, lung parenchyma cells	Phagosomes, phagolysosomes	[101,102]
Francisella tularensis	Facultative	Macrophages	Phagosomes	[103]
Legionella pneumophila	Facultative	Macrophages	Endoplasmic reticulum, lysosomes	[102,104]
Listeria monocytogenes	Facultative	Macrophages, hepatocytes	Cytosol	[4]
Mycobacterium tuberculosis	Facultative	Macrophages	Early endosomes	[105]
Rickettsia spp	Obligate	Endothelial cells	Cytosol	[106]
Salmonella spp	Facultative	Macrophages	Phagosomes	[107]
Shigella flexeneri	Facultative	Macrophages	Cytosol	[108]
Staphylococcus aureus	Opportunist	Macrophages, PMNs	Phagolysosomes	[109–111]

Cibles cellulaires



Les bactéries vont se localiser dans des compartiments cellulaires différents

D'après P. Brouqui,
S. Carryn et al / Infect Dis Clin N Am 17 (2003) 615–634

« Pour vivre heureux, vivons cachés »

- Localisation intracellulaire:
  - évasion immune, protège la bactérie du système immunitaire
  - «Réservoir », infection chronique, rechutes
  - va permettre la dissémination de la bactérie dans les tissus, à distance (PNN, macrophages): «Cheval de Troie »
  - Protège de l'action des ATB

Thwaites GE, et al., Nat Rev Microbiol. 2011 Mar;9(3):215-22

Challenges des antibiotiques en intracellulaire

I. **Pénétration** de l'ATB en intracellulaire (phagocytes professionnels ou non professionnels)

Tulkens PM. Eur J Clin Microbiol Infect Dis. 1991 10:100-6
Butts JD, Clin . Pharmocokinet. 27 (1): 63-84.1994

Entrée dans la cellule

- Les molécules de petite taille (< 700 Da), lipophiles telles que les β lactamines, macrolides et quinolones rentrent par diffusion à travers la membrane lipidique
- L'entrée peut se faire par endocytose si la molécule est plus grande ou ne peut pas diffuser.
- L'endocytose nécessite une internalisation avec des vésicules à la membranes et une invagination de celle-ci. Les vésicules sont se diriger vers l'endosome. (Ex: aminosides, via la megaline (recepteur sur endosomes notamment au niveau tubule renal: d'où possible toxicité)
- **Une fois dans la cellule les ATB doivent être retenus et s'accumuler à une concentration suffisante pendant une période suffisante.**
- Des pompes à efflux vont pouvoir faire sortir les ATB (ex macrolides ou FQ)

- En fonction un ATB peut être bactéricide ou pas en intracellulaire (ex: tigecycline= bactériostatique sur *S. aureus* en intracellulaire et bactéricide sur Salmonella)

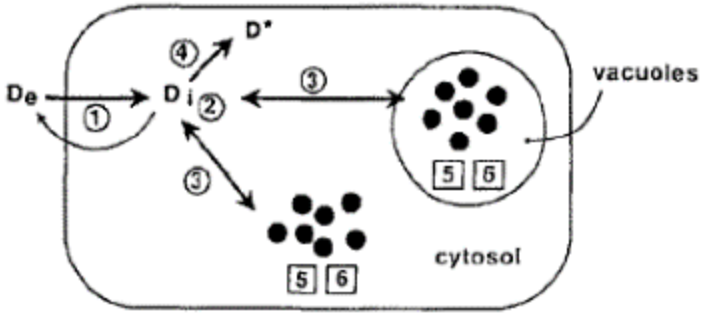
Challenges des antibiotiques en intracellulaire

I. **Pénétration** de l'ATB en intracellulaire (phagocytes professionnels ou non professionnels)
II. **Stabilité/ Maintien** de l'ATB dans le compartiment intracellulaire

III. **Hautes concentrations** d'ATB dans le compartiment intracellulaire
IV. **Bonne localisation**, au contact du pathogène dans la cellule (être dans le bon compartiment)
V. **Etre actif** sur bactérie en phase de multiplication et quiescente (ex: SCV *S. aureus* etc…)

Tulkens PM. Eur J Clin Microbiol Infect Dis. 1991 10:100-6
Butts JD, Clin . Pharmocokinet. 27 (1): 63-84.1994

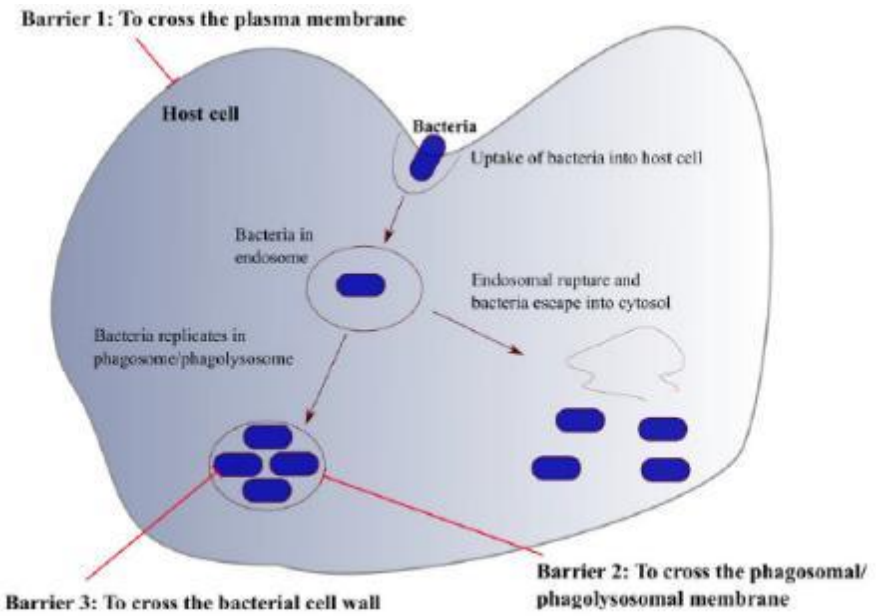
FACTORS AFFECTING THE ACTIVITY OF ANTIMICROBIALS AGAINST INTRACELLULAR BACTERIA



De = extracellular drug
Di = intracellular drug
D* = metabolites
● = bacteria

Pharmacokinetic parameters	Pharmacodynamic parameters
① Penetration and retention	5 Expression of activity
② Accumulation	6 Bacterial responsiveness
③ Subcellular disposition and bioavailability	
④ Melabolisation and inactivation	

Figure 1: Pharmacokinetic and pharmacodynamic parameters involved in the activity of antimicrobial drugs against intracellular microorganisms.



Tulkens PM. Eur J Clin Microbiol Infect Dis. 1991 10:100-6.

Challenges des antibiotiques en intracellulaire

VI. Certaines bactéries sont intracellulaires « facultatives » voire « opportunistes »: survie intra ET extracellulaire

- Donc ATB doivent **aussi** avoir une activité extracellulaire (concentrations)

Butts JD, Clin . Pharmocokinet. 27 (1): 63-84.1994

Table IV. Predictive activity of an antibacterial agent against *Staphylococcus aureus* on the basis of the intracellular and extracellular concentration to minimum inhibitory concentration (MIC) ratios after the addition of 1 and 4 times the minimum bactericidal concentration of the drug to the culture

Antibacterial agent	Intracellular concentration (IC) [mg/L] [a]	MIC (mg/L)[b]	IC : MIC ratio	Extracellular concentration (EC) [mg/L][a]	EC : MIC ratio
Azithromycin	0.42	0.25	1.68	Not detected	0
	1.78		7.12	0.23	0.92
Azithromycin	0.42	1.0	0.42	Not detected	0
	1.78		1.78	0.23	0.23
Azithromycin ratio range			**0.42-7.12**		**0-0.92**
Clarithromycin	0.34	0.12	2.83	0.15	1.93
	1.33		11.08	0.66	5.50
Clarithromycin	0.34	0.50	0.68	0.15	0.30
	1.33		2.66	0.66	1.32
Clarithromycin ratio range			**0.68-11.08**		**0.30-5.50**

a Data from Scaglione et al.[25] represent the resulting concentrations after addition of 1- and 4-times the minimum bactericidal concentration of drug.

b MIC data from the National Committee for Clinical Laboratory Standards.[17] The upper and lower limit of the range for MIC inhibitory to 90% of organisms were used to determine the intracellular and extracellular concentration to MIC ratios.

<1

Ratios intra/extracellulaire

- Variables selon les antibiotiques/ même famille
- Variables selon le compartiment cellulaire
- Variables selon le pH
- Variables selon la T°
- Variables selon que la cellule est ou non infectée

Butts JD, Clin . Pharmocokinet. 27 (1): 63-84.1994

Table III. Intracellular to extracellular concentration ratio (I/E ratio) for various antibacterial agents, including actual intracellular or extracellular concentrations (when available) for polymorphonuclear leucocytes and macrophages

Antibacterial agent	Assay type	Intracellular concentration (mg/L)	Extracellular concentration (mg/L)	I/E ratio	Reference
Polymorphonuclear leucocytes (PMNs)					
Human-PMNs					
Clindamycin	Radiolabelled		10	11.08	18
Erythromycin	Radiolabelled		18.4	13.32	
Ethambutol	Radiolabelled		6.9	4.83	
Rifampicin (rifampin)	Radiolabelled		20	2.33	
Chloramphenicol	Radiolabelled		10	2.23	
Lincomycin	Radiolabelled		10	1.78	
Isoniazid	Radiolabelled		3.3	1.04	
Gentamicin	Radiolabelled		18	0.84	
Cefalexin	Radiolabelled		10	0.55	
Benzylpenicillin (penicillin G)	Radiolabelled		10	0.16	
Cefamandole	Radiolabelled		10	<0.01	
Cefazolin	Radiolabelled		10	<0.01	
Sparfloxacin	Radiolabelled		0.5 to 25	>4	19
Lomefloxacin	Fluorometric		2, 5	7.9	20
Ofloxacin	Fluorometric		2, 5	7.1	
Ciprofloxacin	Fluorometric		2, 5	6.2	
Norfloxacin	Fluorometric		2, 5	5.1	
Lomefloxacin	Fluorometric		2-25	avg = 6.9	21
Ofloxacin	Fluorometric		2-25	avg = 6.1	
Ciprofloxacin	Fluorometric		2-25	avg = 5.5	
Norfloxacin	Fluorometric		2-25	avg = 4.5	
Temafloxacin	Fluorometric		2-25	avg = 3.6	

Antibacterial agent	Assay type	Intracellular concentration (mg/L)	Extracellular concentration (mg/L)	I/E ratio	Reference
Rabbit-AMs					
Benzylpenicillin	Radiolabelled		10	0.013	24
Cefazolin	Radiolabelled		10	0.07	
Cefamandole	Radiolabelled		10	0.39	
Cefalexin	Radiolabelled		10	0.44	
Rifampicin	Radiolabelled		20	1.78	
Ethambutol	Radiolabelled		6.9	6.83	
Chloramphenicol	Radiolabelled		10	2.07	
Gentamicin	Radiolabelled		18	0.56	
Isoniazid	Radiolabelled		3.3	0.55	
Tetracycline	Radiolabelled		10	0.86	
Lincomycin	Radiolabelled		10	1.64	
Erythromycin	Radiolabelled		18.4	20.64	
Clindamycin	Radiolabelled		10	42.18	
Human-AMs					
Rifabutin (ansamycin)		0.30	0.050-0.065	4.62-6.00	Unpublished data on file, Adria Laboratories
Infected Human-AMs					
Amoxicillin	Ultrasonification	ND	0.46	0	25
Amoxicillin	Ultrasonification	ND	0.94	0	
Amoxicillin	Ultrasonification	0.03	1.88	0.02	
Azithromycin	Ultrasonification	0.42	ND	?	
Azithromycin	Ultrasonification	0.88	0.08	11.00	
Azithromycin	Ultrasonification	1.78	0.23	7.74	

Butts JD, Clin . Pharmocokinet. 27 (1): 63-84.1994

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams *	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Daptomycine <1

* Sauf oxacilline *S. aureus* modèle macrophage

Tulkens, adapted from Van Bambeke *et al.*, Curr Opin Drug Discov Devel 2006;9:218-230

Rôle du pH sur l'activité des ATB

	pH<4	pH = 6-7	pH > 7
β-lactamines	-	++	+++
Aminosides	-	+	+++
Phenicolés	-	+	+++
Tétracyclines	-	+++	++
Macrolides	-	+	+++
Rifampicine	+++	++	+++
Fluoroquinolones	-	+	+++
Cotrimoxazole	-	+	+++
Glycopeptides	-	+	+++

Rôle du pH: pH intracellulaire varie de 7,4 dans le phagosome, à 6,8 dans le cytoplasme et 5,0 dans le lysosome

Table IV. Influence of pH on antibiotic activity.

Antibiotics	Activity at neutral pH	pH 6-7	pH <6	pH of optimum activity
β-Lactams	+++	++	+	7
Aminoglycosides	+++	+	–	7
Tetracyclines	++	+++	+	6.6
Chloramphenicol	+++	+	–	
Rifampicin (rifampin)	++	++	+++	<5
Erythromycin	+++	+	–	7.8
Clindamycin	+++	+	–	
Fluoroquinolones	+++	+	–	8
Ethambutol	+++	+	–	7
Pyrazinamide	–	–	+++	<5

Abbreviations: + = low activity; ++ = moderate activity; +++ = high activity; – = no activity.

Maurin M et al., Drugs 1996 Jul; 52 (1): 45~59

Objectif: co-localisation ATB et bactérie

- **Lysosomes et Phagolysosomes**

– Antibiotiques

Aminosides, macrolides, quinolones, rifampicine, clindamycine, cyclines ?

– Bactéries

C burnetii, Y pestis, S aureus, Y pseudotuberculosis

- **Cytosol**

– Antibiotiques

Cyclines, rifampicine, chloramphénicol, quinolones, ßlactamines

– Bactéries

Rickettsia spp, Shigella spp, Listeria spp

Objectif: co-localisation ATB et bactérie

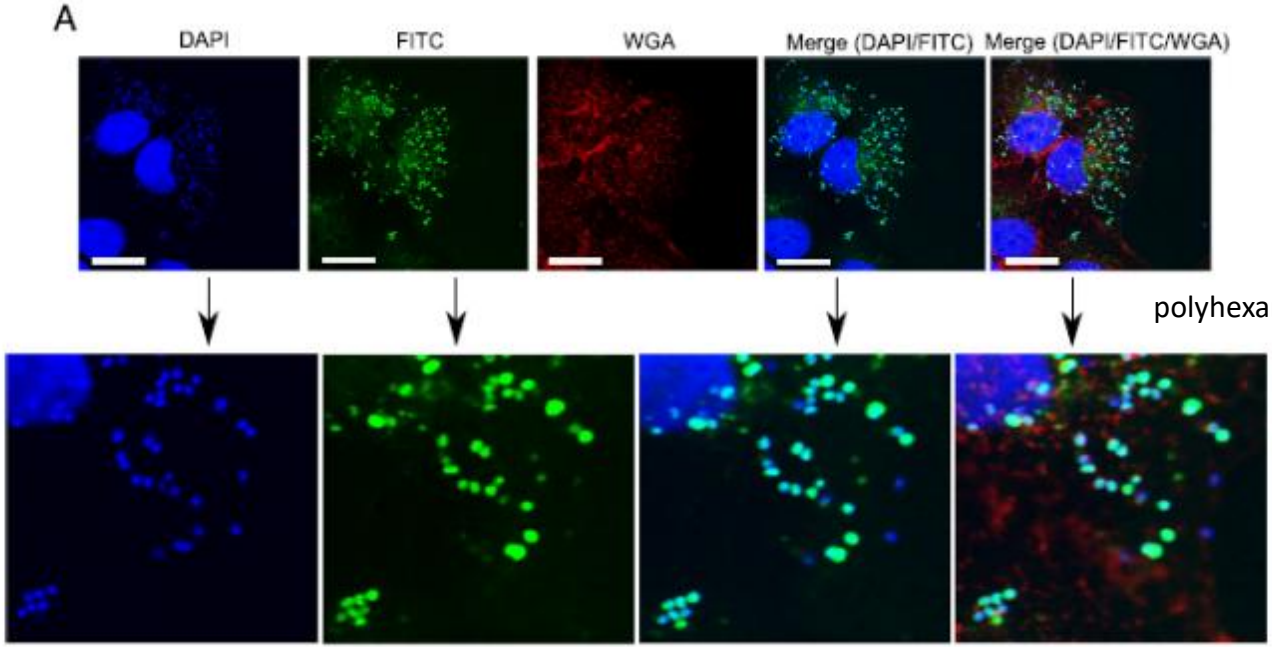
- **Phagosome**

– ATB ?

– Bactéries

Chlamydia spp, Ehrlichia spp, Legionella spp, Y. enterocolitica, Brucella spp.

Visualisation de la co-localisation



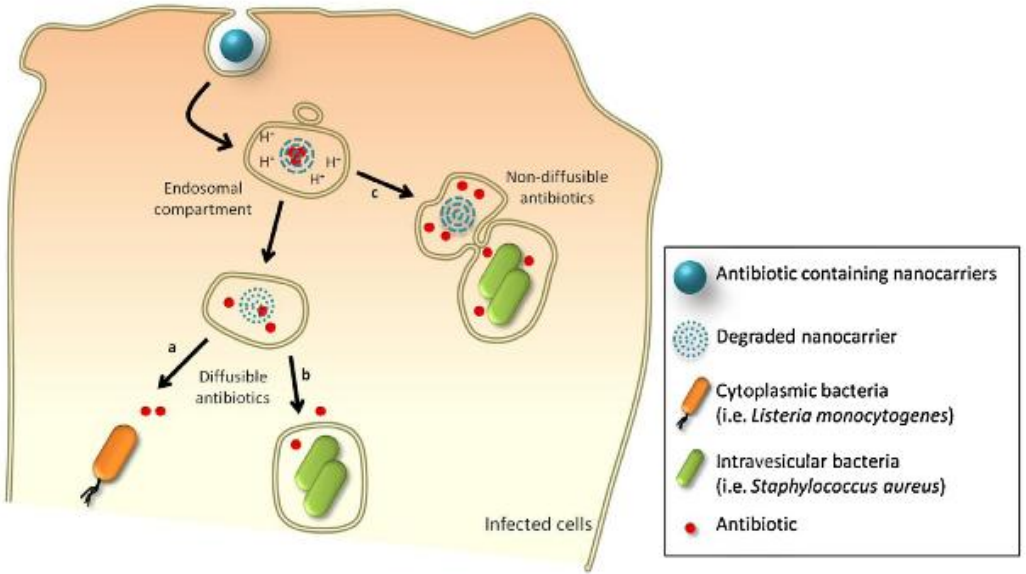
polyhexamethylene biguanide

Figure 3

Intracellular localization and bactericidal activities of nadifloxacin and PHMB against intracellular MRSA. (A) Colocalization of PHMB-FITC with epidemic methicillin-resistant *S. aureus* (EMRSA)-15 in keratinocytes. Keratinocytes were infected with EMRSA-15 followed by treatment with PHMB-FITC (green). Keratinocytes were labelled with DAPI (blue) for keratinocytes and EMRSA-15 nuclei staining and wheat germ agglutination (WGA) (red) for keratinocyte membrane stain. Upper panels are images of infected cells and merged images. Lower panels are enlarged images that clearly show colocalization between PHMB-FITC (green) and EMRSA-15 (blue). White scale bar is 25 μm. (B) Survival of EMRSA-15 within

Amélioration de la délivrance intracellulaire

N. Abed, P. Couvreur / International Journal of Antimicrobial Agents 43 (2014) 485–496



British Journal of Pharmacology

Themed Section: Drug Metabolism and Antibiotic Resistance in Micro-organisms

REVIEW ARTICLE

Targeting the hard to reach: challenges and novel strategies in the treatment of intracellular bacterial infections

Fig. 1. Intracellular delivery of antibiotics to treat intracellular infections. Following cell internalisation through one or several endocytic pathways, nanocarriers should be destabilised and/or degraded by hydrolytic enzymes and/or acidic pH to allow the antibiotic to be released. A diffusible antibiotic will easily escape from endosomes and reach cytoplasmic bacteria (a) or vesicles containing bacteria (b) to exert antimicrobial activity. In some cases, fusion between endosomes (with captured nanocarriers + antibiotic) and vesicles containing bacteria (c) are needed in order to allow non-diffusible antibiotics to exert their antimicrobial activity.

Diagnostic de ces infections

- Indirect++
- Direct:
  - Culture: rare
  - Biologie moléculaire+++

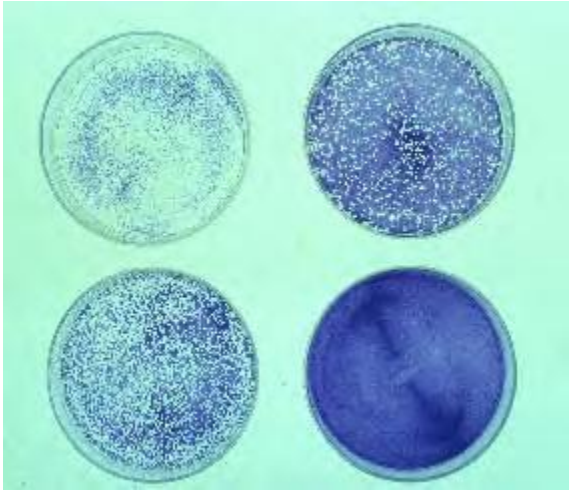
Tableau I. Approche diagnostique de certaines bactéries intracellulaires abordées dans cette revue
[1] Les anticorps de phase II (IgG ≥ 1 : 200 UI/ml; IgM ≥ 1 : 50 UI/ml) définissent la fièvre Q aiguë, les anticorps de phase I (IgG ≥ 1 : 800 UI/ml) définissent la fièvre Q chronique. TB: tuberculose.

Bactérie	Culture	Sérologie	PCR	Autres
M. tuberculosis et autres mycobactéries	• Recommandée dans tous les cas (test de référence) • Indispensable pour la détermination du profil de sensibilité antibiotique	Non disponible	Test commercialisé pour *M. tuberculosis*. Très utile pour le diagnostic précoce (TB pulmonaire ou méningite) en complément de l'examen direct	• Examen direct: très utile pour le diagnostic précoce, mais sensibilité limitée • Interferon-γ release assays (QuantiFERON-TB, T-SPOT.TB): non recommandé pour la TB active (TB latente uniquement)
Legionella spp.	• Recommandée pour la pneumonie (milieu spécial) • Très faible rendement dans les échantillons non respiratoires (hémocultures notamment)	• Peu utile pour la forme classique (pneumonie) • A considérer pour des formes atypiques (endocardite)	Utile (si disponible) en complément de la culture sur les échantillons respiratoires ou autres	• Antigène urinaire: recommandé (ne remplace cependant pas la culture, sensibilité limitée, détecte uniquement *L. pneumophila* sérogroupe 1) • Immunofluorescence directe: peu utile (peu sensible)
Chlamydia pneumoniae	Non effectuée de routine	Peu utile en raison du délai pour le diagnostic	Test de choix (rapide et sensible)	–
Mycoplasma pneumoniae	Non effectuée de routine	Peu utile en raison du délai pour le diagnostic	Test de choix (rapide et sensible)	–
Coxiella burnetii	Non effectuée de routine	• Recommandée (à répéter à un intervalle de 14 jours si négatif)[1] • Aussi utile pour le suivi	Utile, en complément de la sérologie	–
Bartonella spp.	• Test de référence pour la bactériémie chronique (*B. quintana*) • Peu utile dans les autres cas (peu sensible, incubation prolongée)	Test de référence. Bonne sensibilité pour la maladie des griffes du chat et l'endocardite	Utile, en complément de la sérologie (endocardite notamment)	–
Tropheryma whipplei	Non effectuée de routine	Non disponible (taux d'anticorps faibles ou absents)	Recommandée. Sur échantillons de selles et salive (± biopsie cutanée et autres sites selon la présentation clinique)	• Histopathologie (coloration PAS): pour la forme digestive classique • Immunohistochimie: sur échantillons ciblés

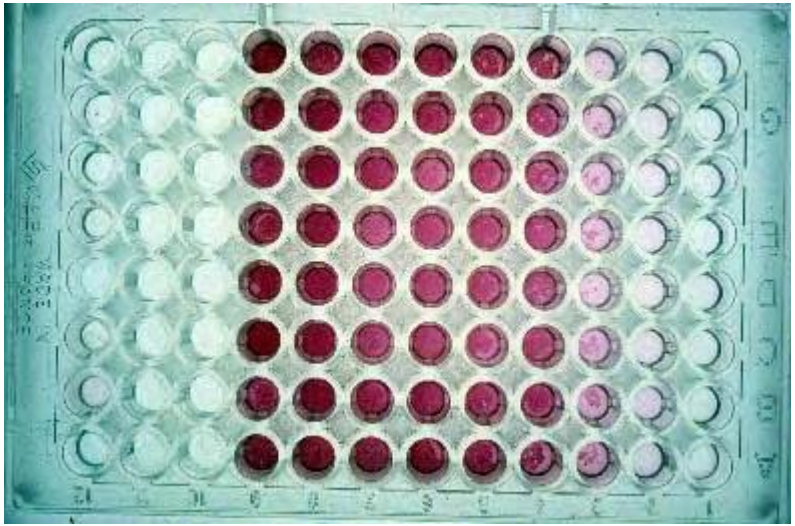
Etude de la sensibilité aux « ATB » en intracellulaire

- Complexe
- Fait souvent appel à la culture cellulaire
- Pas en routine
- « time consumming »
- Techniques ATBgramme différentes

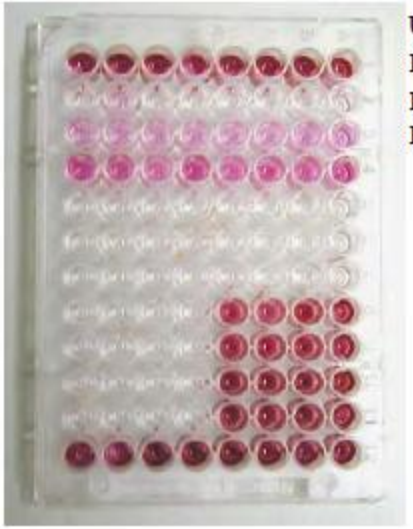
Bactéries intracellulaires strictes



Plaque assay



Dye uptake assay



Uninfected cells OD = 1

Infected cells 2000 PFU OD = 0

Infected cells 200 PFU OD = 0.150

Infected cells 20 PFU OD = 0.700

•The optical density at 492 nm of each well is determined using a spectrophotometer

The MIC corresponds to the lowest antibiotic concentration for which the mean OD at 492 nm is higher than that of the 20 PFU controls

Doxycycline 0.015 µg/ml OD =0

Doxycycline 0.03 µg/ml OD = 0

Doxycycline 0.06 µg/ml OD = 0

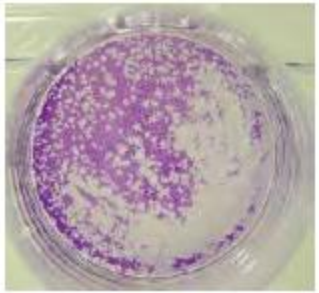
Doxycycline 0.125 µg/ml OD =0.89 MIC

Doxycycline 0.25 µg/ml OD = 0.95

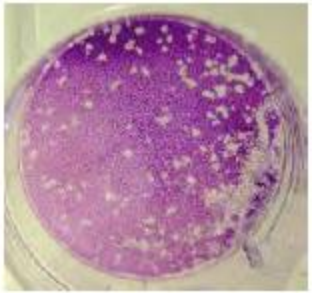
Doxycycline 0.5 µg/ml OD =1

Doxycycline 1 µg/ml OD = 1

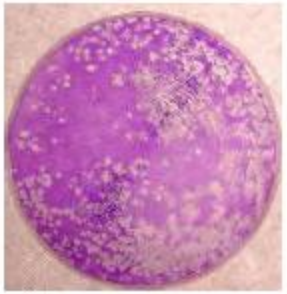
Doxycycline 0.015 µg/ml



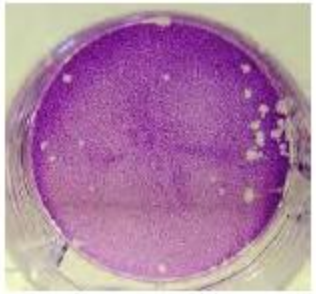
Doxycycline 0.03 µg/ml



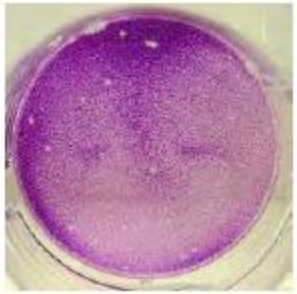
Doxycycline 0.06 µg/ml



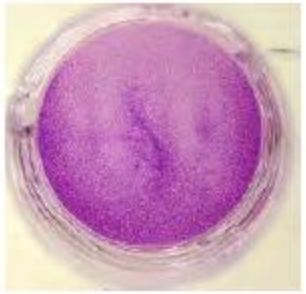
Doxycycline 0.125 µg/ml



Doxycycline 0.25 µg/ml



Doxycycline 0.5 µg/ml



The MICs are defined as the lowest antibiotic concentration allowing complete inhibition of plaque formation, as compared to a drug-free control

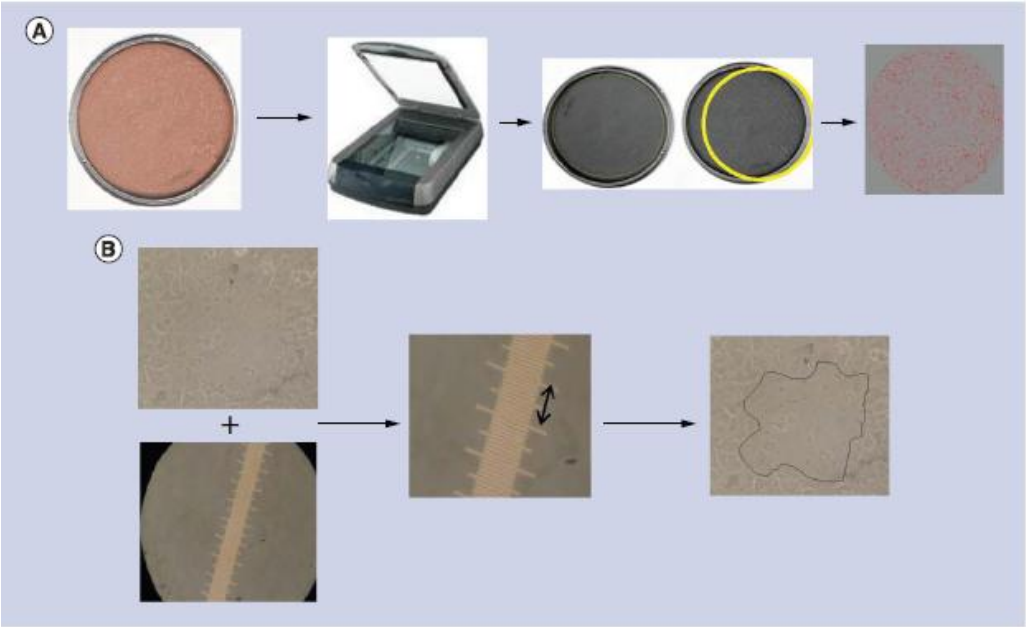


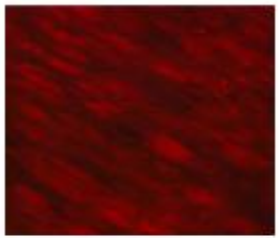
Figure 3. Two examples of informatics tools recently developed to measure the number and/or the size of the lytic plaque. (A) To study the effect of statins on *Rickettsia conorii*-infected cells, an image of the Petri dish was acquired with a photo scanner after staining with crystal violet, and the images were analyzed with the Image J software (MD, USA) using the 'local threshold' algorithm. This software counts and evaluates the size of the plaques in each Petri dish [7]. **(B)** To study the deleterious effects of antibiotics on *R. conorii*-infected cells, the lytic area was measured with the Image J software to more precisely determine the area of each individual lytic plaque (mm^2) by tracing the outline of each plaque [8,10]. The arrow in the centre image shows determination of the scale bar (number of pixels/mm).

Edouard S. *Future Microbiol.* (2013) 8(10), 1301–1316

Bactericidal assay: results

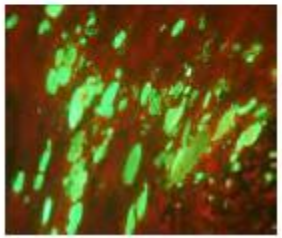
Bacteriocidal activity was deduced from the reduction of the Vacuole Forming Unit (VFU) in cultures receiving antibiotics as compared with drug-free controls

IF

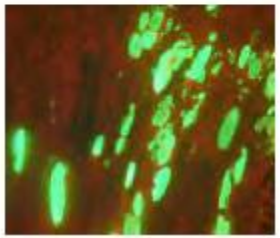


Negative control

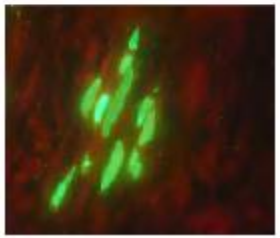
Pure



10^{-1} VFU

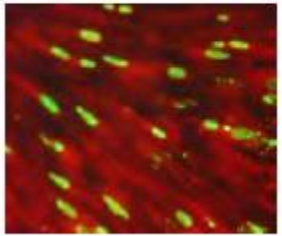


10^{-2} VFU

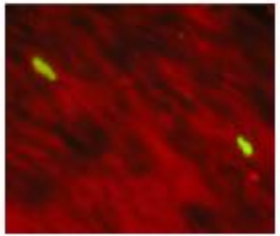


Doxycycline + hydroxychloroquine

10^{-3} VFU



10^{-4} VFU



10^{-5} VFU

P. Brouqui

Utilisation de la PCR en temps réel

Table 1
Comparison of the antibiotic MICs for two strains of. *C. burnetii* (Q212 and Nine Mile strains), using real time PCR and immunofluroscence assay

Antibiotics susceptibility	Q212, using real time PCR (mg/l)	Q212 using IF (mg/l)	Nine Mile, using real time PCR (mg/l)	Nine Mile, using IF (mg/l)	Previous reports using IFA
Doxycycline	4	2	2	1	[15,26]
Ofloxacin	2	2	2	1	[15,26]
Ciprofloxacin	4	8	2	4	[15,26]
Levofloxacin	2	2	2	1	[27,28]
Rifampicin	4	2	4	2	[25]
Erythromycin	4	8	2	4	[8]
Telithromycin	2	1	2	11	[8]
Thiamphenicol	32	64	32	32	This study
Gentamicin	>10	>10	>10	>10	[25]
Co-trimoxazole	16	8	8	8	[6]

ain as determined by

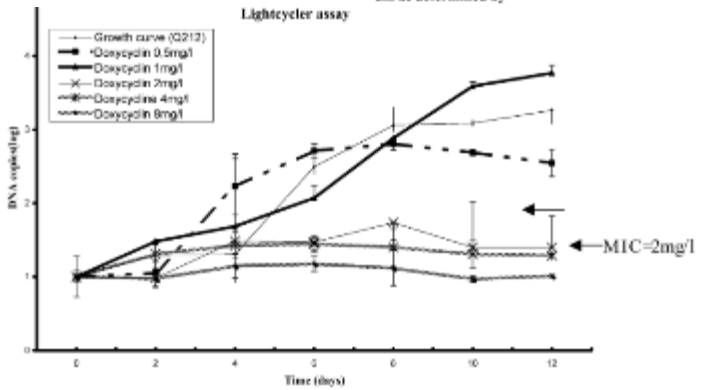


Fig. 3. Antibiotic susceptibility of *C. burnetii* (Q212) in cell culture with serial dilutions of doxycycline (0.5–8 mg/l), determined using real time PCR assay.

Boulos A, et al., Int J Antimicrob Agents. 2004;23(2):169-74

The Effect of pH on Antibiotic Efficacy against *Coxiella burnetii* in Axenic Media

(2019) 9:18132 | https://doi.org/10.1038/s41598-019-54556-6

Cody B. Smith[1], Charles Evavold[1,2] & Gilbert J. Kersh[1*]

International Journal of Antimicrobial Agents

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journal homepage: www.elsevier.com/locate/ijantimicag

Short Communication

Use of axenic media to determine antibiotic efficacy against *coxiella burnetii*

K.A. Clay [a,b], M.G. Hartley [a], P. Russell [a], L.H. Norville [a,*]

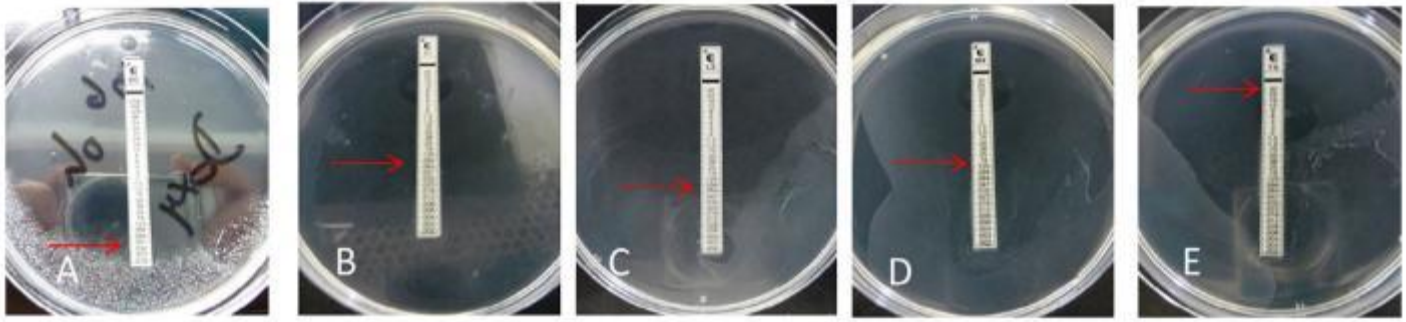


Fig. 1. *Representative image of Etests for C. burnetii NMII.* Red arrow indicates MIC. Etests were performed in triplicate and read by two independent observers. A—Doxycycline (0.032 μg/mL). B—Ciprofloxacin (0.25 μg/mL). C—Levofloxacin (0.064 μg/mL). D—Moxifloxacin (0.125 μg/mL). E—Co-trimoxazole (32 μg/mL).

Bactéries intracellulaires facultatives

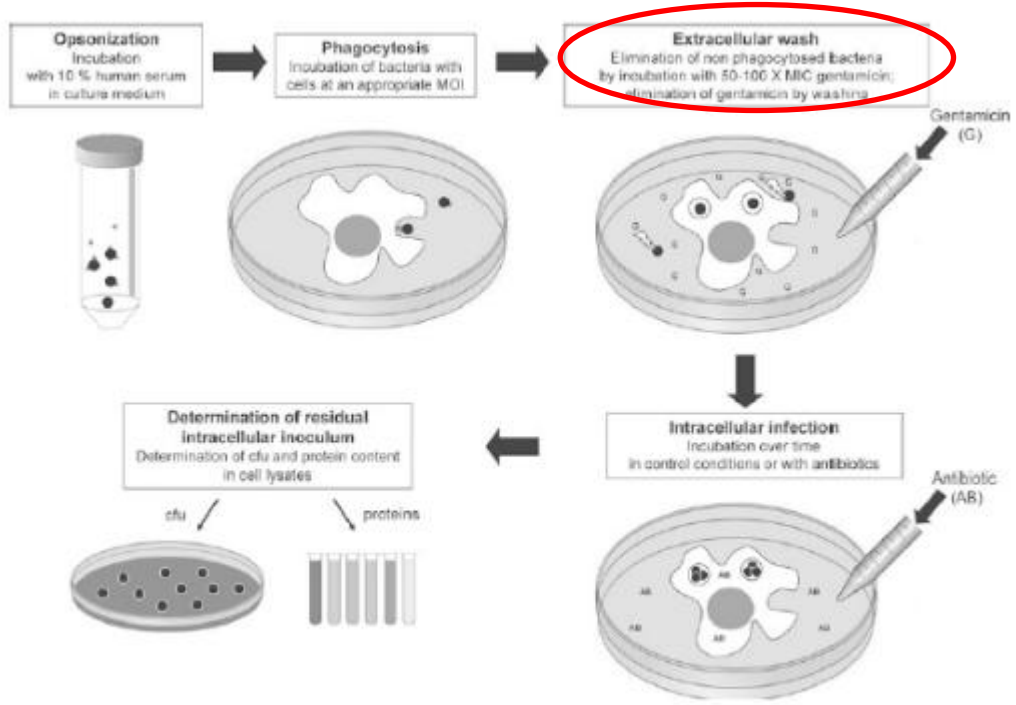
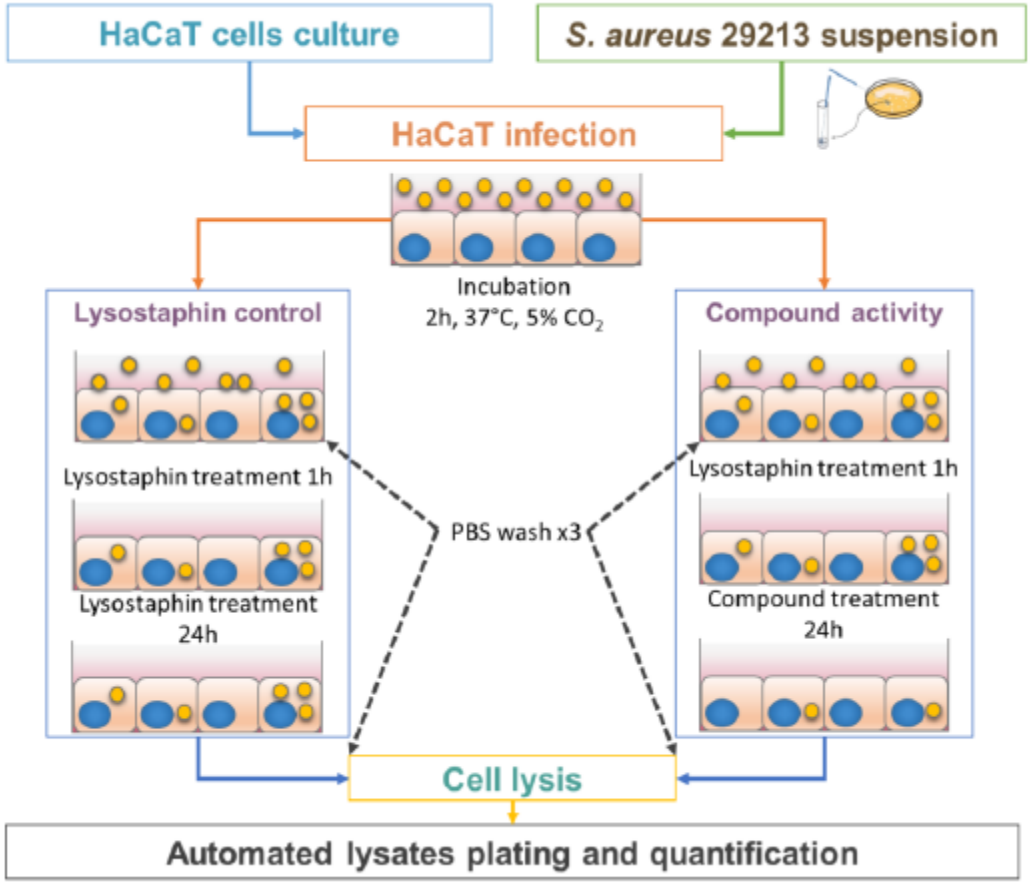


Fig. 1 In vitro model for the assessment of intracellular activity of antibiotics

Buycket al. In vitro Models for the Study of the Intracellular Activity of Antibiotics; In “Bacterial Persistence”, Molecular Biology Laboratory Protocols Series, J. Michielsand M. Fauvart, editors, 2016, p 147-157



Rigaill et al., J Antimicrob Chemother. 2018 Nov 1;73(11):3044-3048

- ATB à diffusion intracellulaire: à retenir
  - Selon le pathogène, localisation différente
  - Plusieurs barrières à la pénétration et au maintien dans les cellules
  - Impact du pH sur l'activité des ATB
  - Besoin d'avoir les bactéries **ET** l'ATB au même endroit
  - Besoin parfois d'avoir des ATB efficaces en intracellulaire ET en extracellulaire

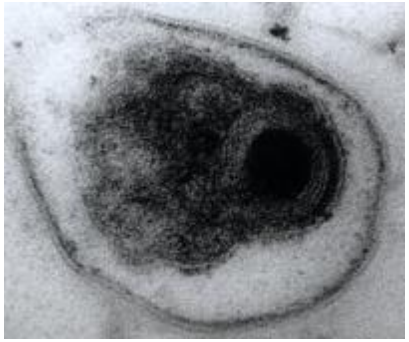
Plan

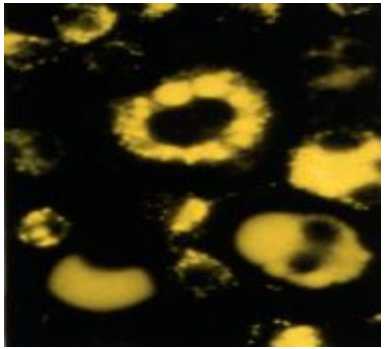
- Antibiotiques intracellulaires
- Principes de traitement des infections intracellulaires: *C. burnetii, Bartonella spp, Rickettsia spp, S. aureus*!

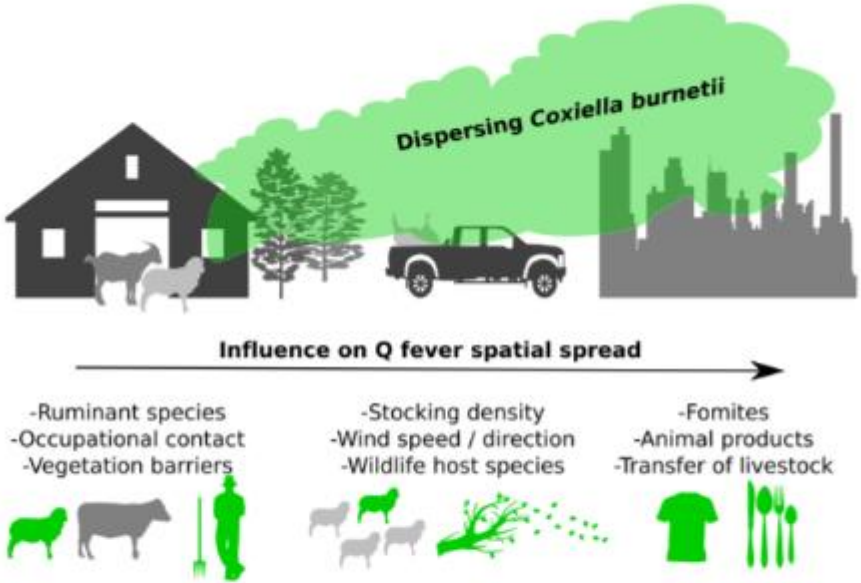
Coxiella burnetii

Coxiella burnetii : Bactériologie

- Coccobacilles gram -
- Bactérie intracellulaire stricte
- Zoonose
- Multiplication dans les phagolysosomes des macrophages
- Survit dans les vacuoles acides
- Extrêmement résistante dans l'environnement







Aérosol jusqu'à 30km

Fig. 1 Schematic representation of potential drivers of *Coxiella burnetii* spatial dispersal from livestock holdings. Green shading indicates potential human transmission pathways. The top section of the figure demonstrates how airborne dispersal and environmental contamination are proposed to contribute to the zoonotic exposure of human communities. This dispersal can be influenced over a range of spatial distances by factors represented in the bottom section of the figure

Présentation clinique

- Plus grande épidémie de FQ
- Histoire naturelle de l'infection
- 4026 cas humains notifiés

ELSEVIER MASSON

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Médecine et maladies infectieuses

General review

Q fever in the Netherlands – 2007–2010: What we learned from the largest outbreak ever

Fièvre Q aux Pays-Bas – 2007–2010 : les enseignements d'une épidémie d'une ampleur sans précédent

P.M. Schneeberger[a], C. Wintenberger[b,*], W. van der Hoek[c], J.P. Stahl[b]

Fever was the most frequent symptom, in more than 90% of notified cases. The other reported symptoms were: flu-like syndrome with malaise and headaches in 63 to 92% of cases, coughing in 66% of cases, nocturnal hyperhidrosis in 80% of cases. Pneumonia concerned 66% of notified cases, hepatic presentations were more rare (15%) than in previous outbreaks, especially compared to the French outbreak in Chamonix [4,9,15,23]. The male/female ratio of notified cases in 2007 and 2008 was 1.7/1, with an average age of 51 years [9].

Original Investigation | Infectious Diseases

Clinical Features and Complications of *Coxiella burnetii* Infections From the French National Reference Center for Q Fever

Cléa Melenotte, MD; Camélia Protopopescu, PhD; Matthieu Million, MD, PhD; Sophie Edouard, PharmD, PhD; M. Patrizia Carrieri, PhD; Carole Eldin, MD, PhD; Emmanouil Angelakis, MD, PhD; Félix Djossou, MD, PhD; Nathalie Bardin, PharmD, PhD; Pierre-Edouard Fournier, MD, PhD; Jean-Louis Mège, MD, PhD; Didier Raoult, MD, PhD

Figure 1. Study Flowchart

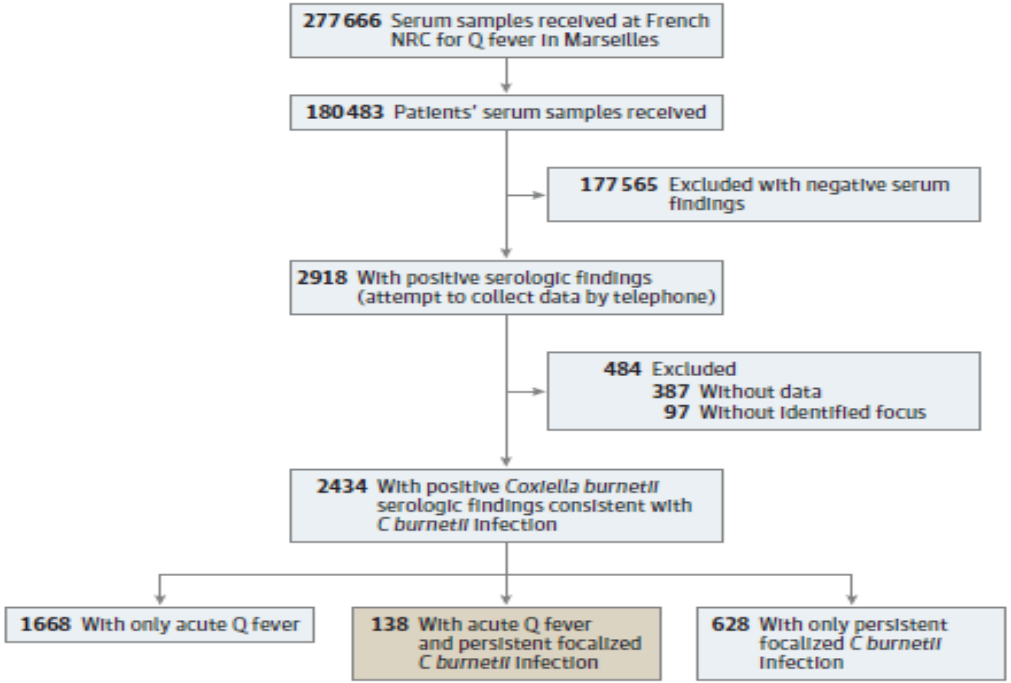
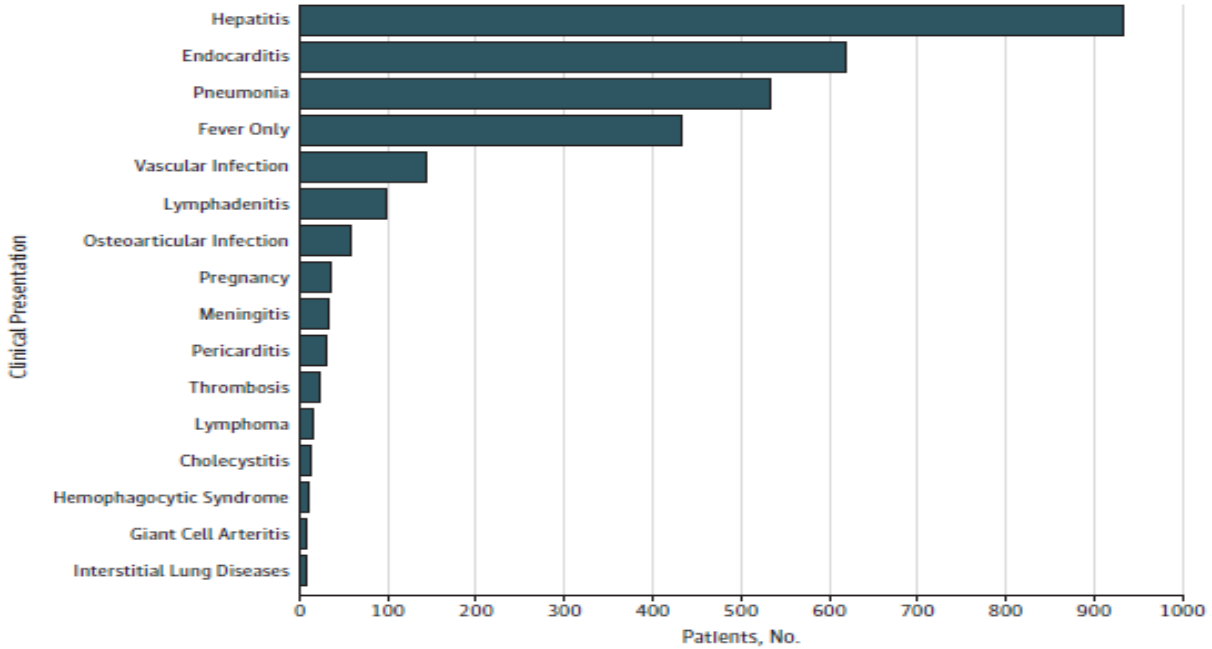
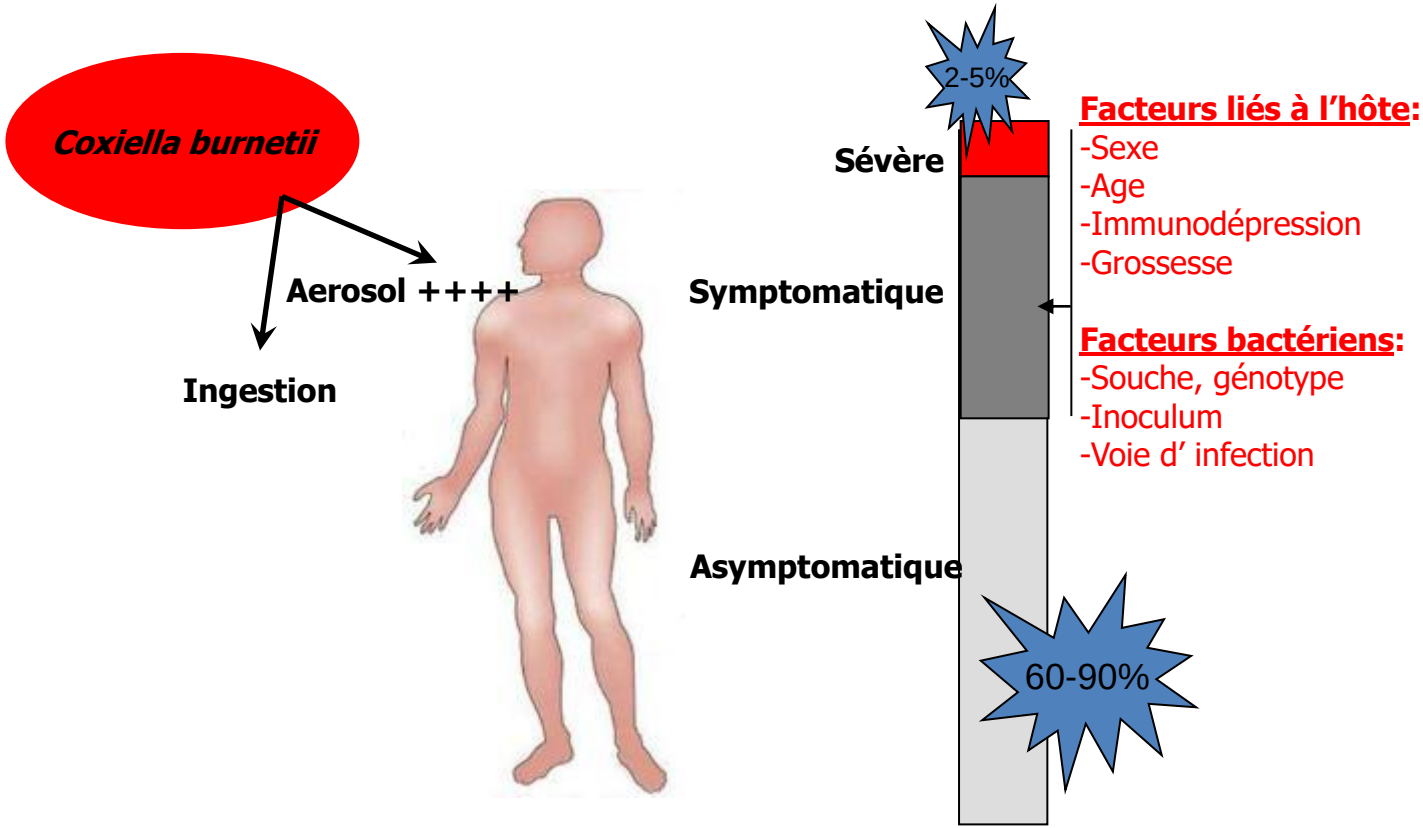


Figure 2. Clinical Presentations of *Coxiella burnetii* Infection



Includes a total of 2434 patients with positive *C burnetti* serologic findings consistent with *C burnetti* infection.



Fièvre Q Aigue

eTable 7. Clinical Presentation of Acute Q Fever in 1806 Patients

	n=1806	Percentage
Mean age	48.8 ± 16.5 years	-
Follow up duration	11.4 ± 21.8 months	-
Predisposing valvulopahy	255	10.4%
No predisposing valvulopathy	997	40.9%
Unknown predisposing valvulopathy	554	22.7%
Immunosuppression	66	3.6%
Hepatitis	836	46.3%
Pneumonia	480	26.6%
Hepatitis + pneumonia	141	7.8%
Flu like syndrome or isolated fever	350	19.3%
Lymphadenitis	66	3.7%
Lymphadenitis + hepatitis	24	1.3%
Lymphadenitis +pneumonia	16	0.9%
Acute Q fever endocarditis	50	2.8%
Thrombosis	16	0.9%
Pregnancy	16	0.9%
Meningitis and or encephalitis	25	1.4%
Meningoencephalitis	8	0.4%
Meningitis	16	0.6%
Encephalitis	1	0.05%
Alithiasic cholecystitis	11	0.6%
Pericarditis	23	1.3%
Hemophagocytic syndrome	9	0.5%
Myocarditis	7	0.4%
Imprecise	127	7%

Melenotte et al., JAMA Network Open 2018

Evolution vers forme persistante

Table 1. Evolution to Persistent *Coxiella burnetii* Infection in 1806 Patients With Acute Q Fever

Patient Characteristic	No. (%) of Patients: Acute Q Fever Without Persistent *C burnetii* Infection (n = 1668)	No. (%) of Patients: Acute Q Fever Progressing to Persistent *C burnetii* Infection (n = 138)	Univariate Analysis *P* Value[a]	Logistic Regression: Univariate OR (95% CI)	Univariate *P* Value	Multivariate OR (95% CI)	Multivariate *P* Value
Immunosuppression							
Valvulopathy							
No	1498 (89.8)	53 (38.4)	NA	1 [Reference]	NA	1 [Reference]	NA
Yes	170 (10.2)	85 (61.6)	<.001	14.1 (9.7-20.6)	<.001	9.8 (6.1-15.8)	<.001
Sex							
Male	1115 (66.8)	110 (79.7)	.002	1.9 (1.3-3.0)	.002	1.9 (1.1-3.1)	.01
Female	553 (33.2)	28 (20.3)	NA	1 [Reference]	NA	1 [Reference]	NA
Age at baseline, median (IQR), y	48 (37-59)	55.5 (46-68)	<.001	1.03 (1.02-1.04)	<.001	1.01 (1.00-1.03)	.03
Lymphadenitis							
No	1616 (96.9)	124 (89.9)	NA	1 [Reference]	NA	1 [Reference]	NA
Yes	52 (3.1)	14 (10.1)	<.001	3.5 (1.9-6.5)	<.001	3.3 (1.6-7.1)	.002
Thrombosis							
No	1657 (99.3)	133 (96.4)	NA	1 [Reference]	NA	1 [Reference]	NA
Yes	11 (0.7)	5 (3.6)	.005	5.7 (1.9-16.5)	.002	6.8 (1.9-24.8)	.004
Acute endocarditis							
No	1636 (98.1)	120 (87.0)	NA	1 [Reference]	NA	1 [Reference]	NA
Yes	32 (1.9)	18 (13.0)	<.001	7.7 (4.2-14.1)	.001	3.8 (1.5-9.8)	.006
IgG titer to phase I on first serologic analysis							
≤800	1476 (88.5)	84 (60.9)	NA	1 [Reference]	NA	NA	NA
>800	192 (11.5)	54 (39.1)	<.001	4.9 (3.4-7.2)	<.001	NR	NA
Maximum IgG titer to phase I							
≤800	1313 (78.8)	48 (34.8)	NA	1 [Reference]	NA	1 [Reference]	NA
>800	354 (21.2)	90 (65.2)	<.001	7.0 (4.8-10.1)	<.001	5.2 (3.3-8.1)	<.001
Positive *C burnetii* PCR							
No	1481 (91.3)	105 (77.8)	NA	1 [Reference]	NA	1 [Reference]	NA

3 mois – 3 ans
Après primoinfection
(symptomatique ou non)

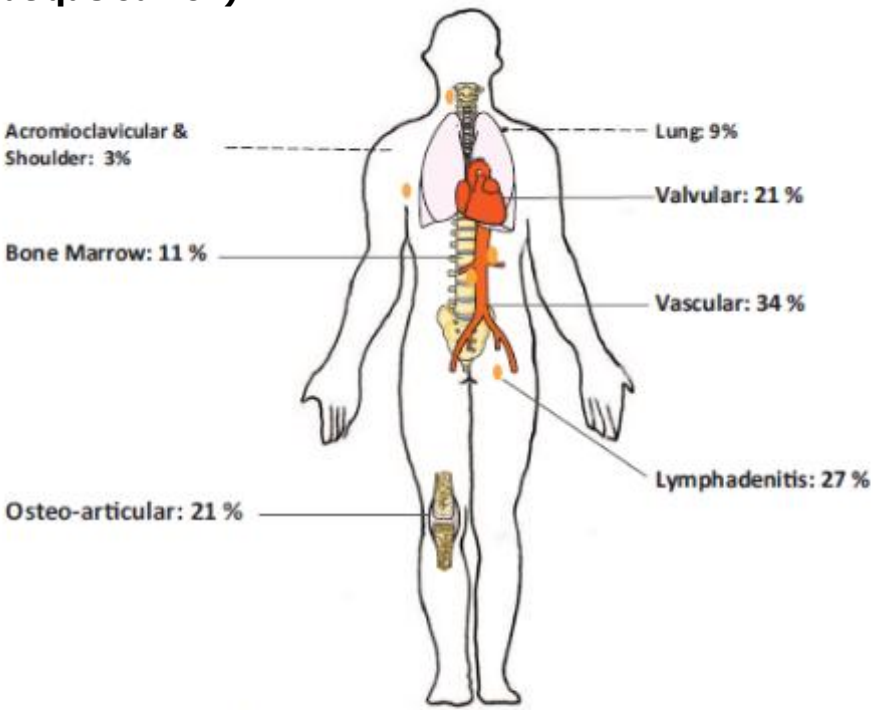
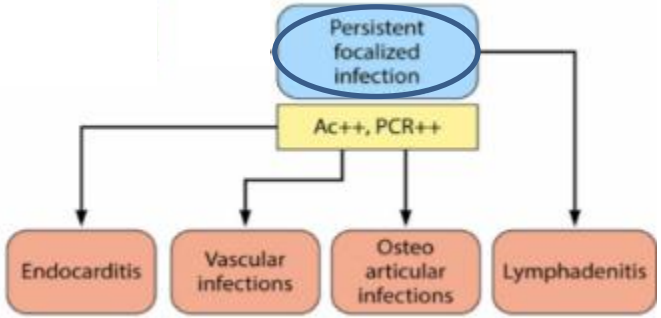


Figure 3. Distribution of Q fever foci identified by ^{18}F-FDG PET/CT. ^{18}F-FDG PET/CT = ^{18}F-fluorodeoxyglucose positron emission tomography.

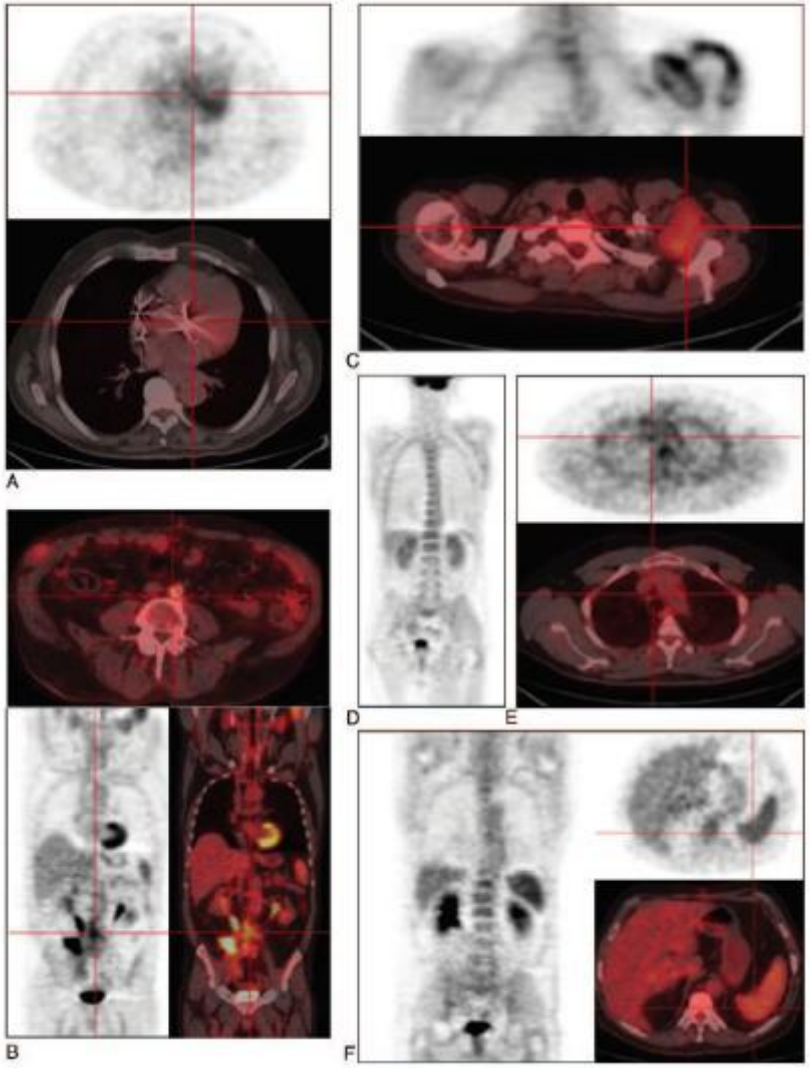
Titre élevé d'AC mais pas que

TABLE 2 The Dutch consensus guidelines criteria for chronic Q fever[a]

Category and criterion (criteria)[b]
Proven chronic Q fever
Positive *C. burnetii* PCR in blood or tissue **or**
IFA titer of ≥1:1,024 for *C. burnetii* phase I IgG **and**
Definite endocarditis according to the modified Duke criteria **or**
Proven large-vessel or prosthetic infection by imaging studies (18 F-FDG PET, CT, MRI, or AUS)
Probable chronic Q fever
IFA titer of ≥1:1,024 for *C. burnetii* phase I IgG **and** one or more of the following criteria:
Valvulopathy not meeting the major criteria of the modified Duke criteria
Known aneurysm and/or vascular or cardiac valve prosthesis without signs of infection by means of TEE/TTE, 18 F-FDG PET, CT, MRI, or abdominal Doppler ultrasound
Suspected osteomyelitis or hepatitis as manifestation of chronic Q fever
Pregnancy
Symptoms and signs of chronic infection such as fever, wt loss, and night sweats, hepatosplenomegaly, persistent elevated ESR and CRP
Granulomatous tissue inflammation, proven by histological examination
Immunocompromised state
Possible chronic Q fever
IFA titer of ≥1:1,024 for *C. burnetii* phase I IgG without manifestations meeting the criteria for proven or probable chronic Q fever



Fièvre Q infection focalisée persistante
« Chronique »



Observational Study

Medicine

OPEN

^{18}F-FDG PET/CT as a central tool in the shift from chronic Q fever to *Coxiella burnetii* persistent focalized infection

A consecutive case series

Cette dénomination a le mérite de faire rechercher des foyers
Dont certains nécessiteront une PEC autre qu'antibiotique

Eldin C, et al., Medicine (Baltimore). 2016 Aug;95(34):e4287.

eTable 9. Clinical Presentation of Persistent *C burnetii* Infections in 766 Patients

	Endocarditis N=581		Vascular infection N=145		Osteo articular infection N=56	
Age (mean±SD)	59.4±17.3	-	63.4±14.3	-	59.6±19.9	-
Sex (men)	419	72.1%	127	88.2%	37	66.1%
Immunosuppression	22	3.8%	6	4.2%	1	1.8%
Valvular predisposition	449	77.4%	57	39.6%	7	12.5%
Prosthetic material	204	35%	62	44%	10	17.8%
Endocarditis	-	-	49	34.0%	7	12.5%
Vascular infection	49	8.4%	-	-	11	19.2%
Osteoarticular infection	8	1.3%	11	7.5%	-	-
Hepatitis	123	21.2%	28	19.4%	6	10.7%
Pneumonia	52	8.9%	10	6.9%	2	3.6%
Lymphadenitis	26	4.5%	6	4.2%	4	7.1%
Acute endocarditis	13	2.2%	1	0.7%	0	0%
Lymphoma	10	1.7%	2	1.4%	0	0%
Meningitis	7	1.2%	0	0%	1	1.8%
Hemophagocytic syndrome	1	0.2%	1	0.7%	0	0%

Melenotte et al., JAMA Network Open 2018

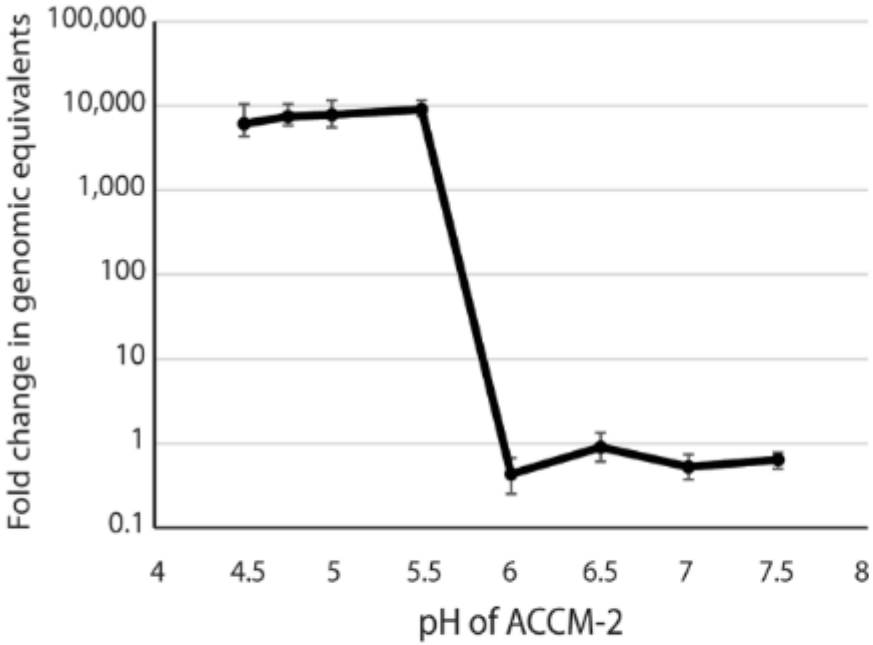


Figure 1. Growth of *Coxiella burnetii* Nine Mile Phase 2 in ACCM-2 medium. Genomes in the culture were calculated at the start of the culture and again on day 8 using qPCR. Robust growth of *C. burnetii* was observed at pH 5.5 and lower, but not at pH 6 and higher. Error bars represent 95% confidence intervals.

(2019) 9:18132 | https://doi.org/10.1038/s41598-019-54556-6

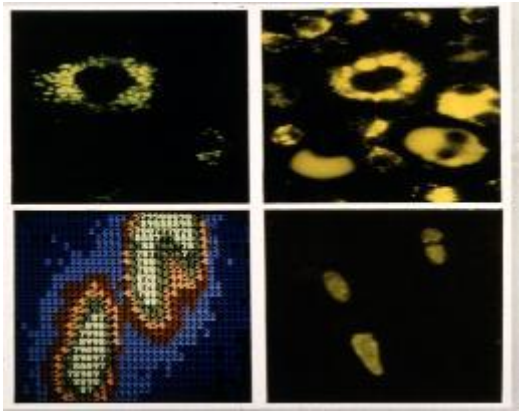
Traitement : Fièvre Q aigue

- **Evolution spontanément favorable en 15 jours**
- **Antibiotiques actifs *in vitro* et *in vivo* bactériostatiques**
  - Ofloxacine, Pefloxacine, Erythromycine, Chloramphenicol, Cotrimoxazole, Ceftriaxone
- **Doxycycline (200 mg/j) 14 j reste le traitement de choix, si possible débuté précocement**
- **ATB diminue le risque d'hospitalisation**
- **Quinolones: intérêt dans les méningo-encéphalites**
- **Alternatives à la doxycycline=** clarithromycine (500 mg twice daily), fluoroquinolones (ofloxacine 200 mgx3/j), ou co-trimoxazole (160 mg trimethoprime et 800 mg sulfamethoxazole 2x/j)

Eldin C, et al., Clin Microbiol Rev. 2017 Jan;30(1):115-190

Traitement : Fièvre Q focalisée persistante/chronique

- Etudes *in vitro* :
  - Alkalinisation des phagolysosomes: impact sur la croissance bactérienne? Amélioration de l'activité de la doxy? bactéricidie?
- Implications thérapeutiques:
  - Ajout d'Hydroxychloroquine à la doxycycline



→Endocardites: Doxycycline+ Hydroxychloroquine
18 mois si valve native, 24 mois si valve prothétique
→ Infections vasculaires:
Traitement prolongé et Retrait du matériel prothétique++++++++++
→ Autres infections persistantes: Doxycycline+ Hydroxychloroquine 18 mois

- Maurin M, et al. J. Infect. Dis. 1992; 166:1097-1102
- Smith, C.B., et al. Sci Rep 9, 18132 (2019)
- Million M et al., Lancet Inf Dis. 2010
- Botelho-Nevers E et al. Eur J Microbiol Inf Dis 2007
- Eldin C, et al., Clin Microbiol Rev. 2017 Jan;30(1):115-190

(2019) 9:18132 | https://doi.org/10.1038/s41598-019-54556-6

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2005, p. 2673–2676 Vol. 49, No. 7
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.7.2673–2676.2005

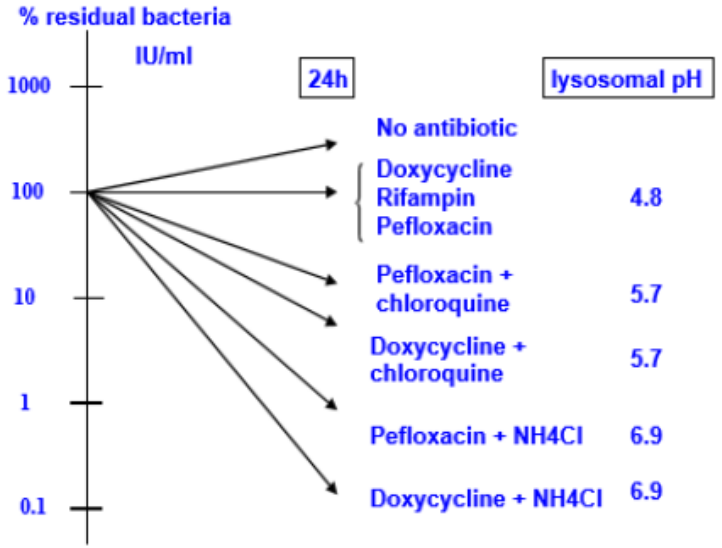
Correlation between Ratio of Serum Doxycycline Concentration to MIC and Rapid Decline of Antibody Levels during Treatment of Q Fever Endocarditis

Jea lt*
Unité d ranée,

**Endocardi th hydroxy-
chloroquine tions in the
rapidity of t tive exami-
nation of wh doxycycline
and respons from whom
Coxiella burn cycline and
hydroxychlo IC of doxy-
cycline for C PCR assay.
At the comp owed a low
decline of an ntigen) had
a ratio of se ratio of ≥1
showed a ra ad a serum
doxycycline- resistant to
doxycycline monitored
during the course of therapy in patients with Q fever endocarditis.**

- Doxycycline: taux sérique> 5µg/mL
- OH-chloroquine: 1+/- 0.2µg/mL
- Surveillance mensuelle
- Si possible tester la sensibilité de la souche
- Augmentation des souches avec sensibilité diminuée: CMI>2µg/mL

Role de l'Hydroxychloroquine

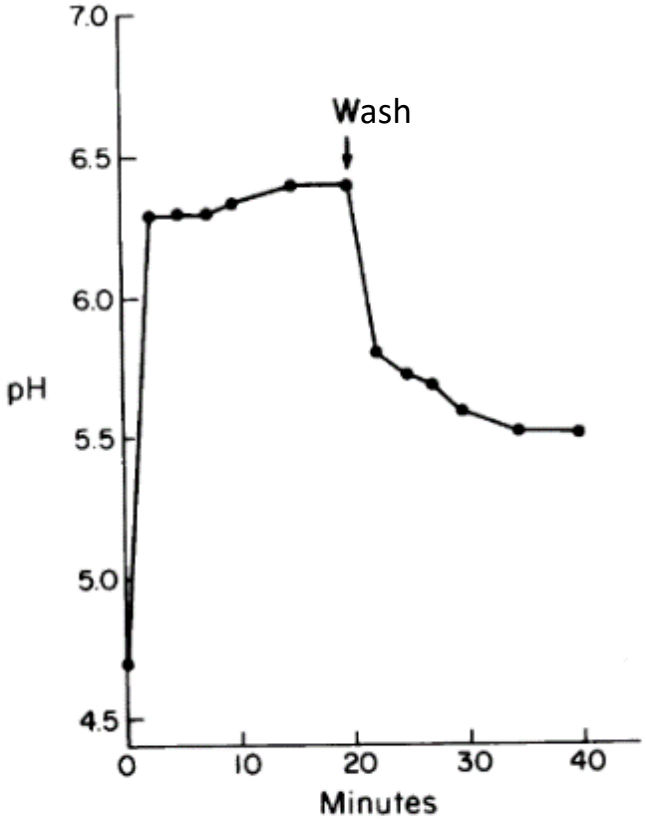


Coxiella Burnetii: synergie ATB-agents alcalinisants lysosomotropes

- NH4Cl: chlorure d'ammonium

- Agent alcalinisant lysosomotrope
- Permettrait une meilleure diffusion des ATB
  et une meilleure activité (bactéricidie??)
- Car Doxycycline inactive pour des pH bas
- Et *Coxiella* survit moins bien à des pH alcalins

D'après M. Maurin
(2019) 9:18132 | https://doi.org/10.1038/s41598-019-54556-6



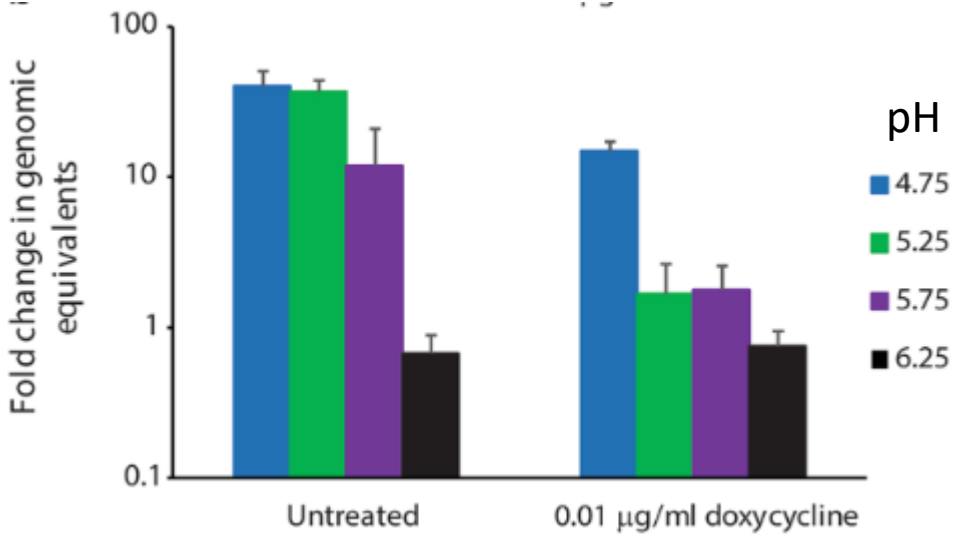
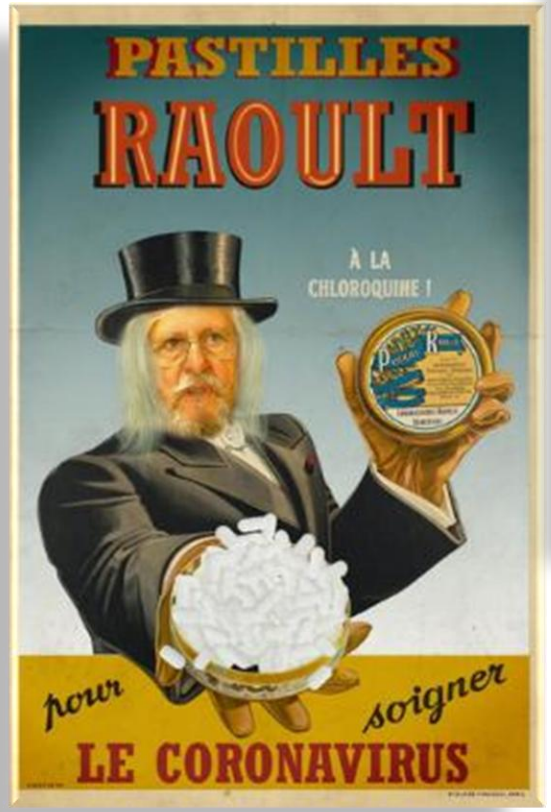


FIG. 11. Effect of chloroquine on intralysosomal pH. Procedures as in Fig. 6. The perfusion medium contained 100 µM chloroquine for the first 20 min.

(2019) 9:18132 | https://doi.org/10.1038/s41598-019-54556-6

https://doi.org/10.1073/pnas.75.7.3327

Débat



ELSEVIER

Clinical Microbiology and Infection

Volume 28, Issue 5, May 2022, Pages 637-639

Commentary

Treatment of *Coxiella burnetii* endocarditis with hydroxychloroquine. Is it evidence-based?

J.P. Stahl [1], E. Varon [2], J.P. Bru [3]

Comparison of outcome between two therapeutic regimens

	Doxycycline + ofloxacin ($n = 14$)	Doxycycline + hydroxychloroquine ($n = 21$)	p-value
Deaths, *n*	1	1	0.77
Valvular surgery, *n*	10	10	0.16
Relapses after completed treatment, *n*	7 of 15	2 of 23	0.01
Relapses after 18 mo of treatment, *n*	6 of 11	0 of 16	0.001
Mean treatment duration among cured patients (mo)	55 ± 18	31 ± 14	<0.001
Photosensibilization, *n*	14	21	NA

Treatment of Chronic Q Fever: Clinical Efficacy and Toxicity of Antibiotic Regimens

Sonja E. van Roeden,[1] Chantal P. Bleeker-Rovers,[2] Marieke J. A. de Regt,[1] Linda M. Kampschreur,[3] Andy I. M. Hoepelman,[1] Peter C. Wever,[4] and Jan Jelrik Oosterheert[1]

Table 3. Number of Events and (Subdistribution) Hazard Ratios for Primary and Secondary Outcomes

Endpoint/Treatment Strategy	Number of Events	HR	95% Confidence Interval	*P* Value
All-cause mortality[a]		HR		
Reference: TET/HCQ	30	1.00	n/a	n/a
TET/QNL	5	1.07	0.37–3.14	.90
TET/QNL/HCQ	3	2.19	0.73–6.56	.16
TET	3	0.70	0.21–2.36	.57
QNL	14	1.60	0.76–3.39	.22
First disease-related event[b]		SHR		
Reference: TET/HCQ	44	1.00	n/a	n/a
TET QNL	7	1.51	0.66–3.47	.33
TET/QNL/HCQ	2	0.94	0.22–3.96	.93
TET	7	1.27	0.53–3.06	.59
QNL	12	1.22	0.57–2.59	.61
Therapy failure[c]		SHR		
Reference: TET/HCQ	60	1.00	n/a	n/a
TET/QNL	7	0.93	0.39–2.19	.86
TET/QNL/HCQ	4	1.21	0.37–3.96	.75
TET	9	1.43	0.67–3.04	.35
QNL	15	1.26	0.68–2.35	.47

Conclusions. Treatment of chronic Q fever with TET plus QNL appears to be a safe alternative for TET plus HCQ, for example, if TET plus HCQ cannot be tolerated due to side effects. Treatment with TET plus QNL plus HCQ was not superior to treatment with TET plus HCQ, although this may be caused by confounding by indication. Treatment with TET or QNL monotherapy should be avoided; switches due to subjective, insufficient clinical response were frequently observed.

Etude rétrospective, peu de cas, traitements très hétérogènes en molécules, durées et sans puissance statistique pour conclure

- **Donc le débat n’est pas tranché:**

A ce stade, faible niveau de preuve pour HCQ+doxy dans les formes persistantes de fièvre Q

MAIS niveau de preuve concernant les alternatives tout aussi faible voire plus

- Pour conclure il faut un essai randomisé +/- contrôlé +++
- Forcement essai international vu la faible incidence de ces formes
- Difficile à mener car formes cliniques sont très hétérogènes, évolution lente
- Besoin d’études bien conduites, sans « passion » pour proposer le meilleur traitement au patient


Clinical Microbiology and Infection

Commentary

Which trial do we need? Doxycycline vs. doxycycline-hydroxychloroquine and treatment duration protocol for Q fever endovascular infections

Micol Paul

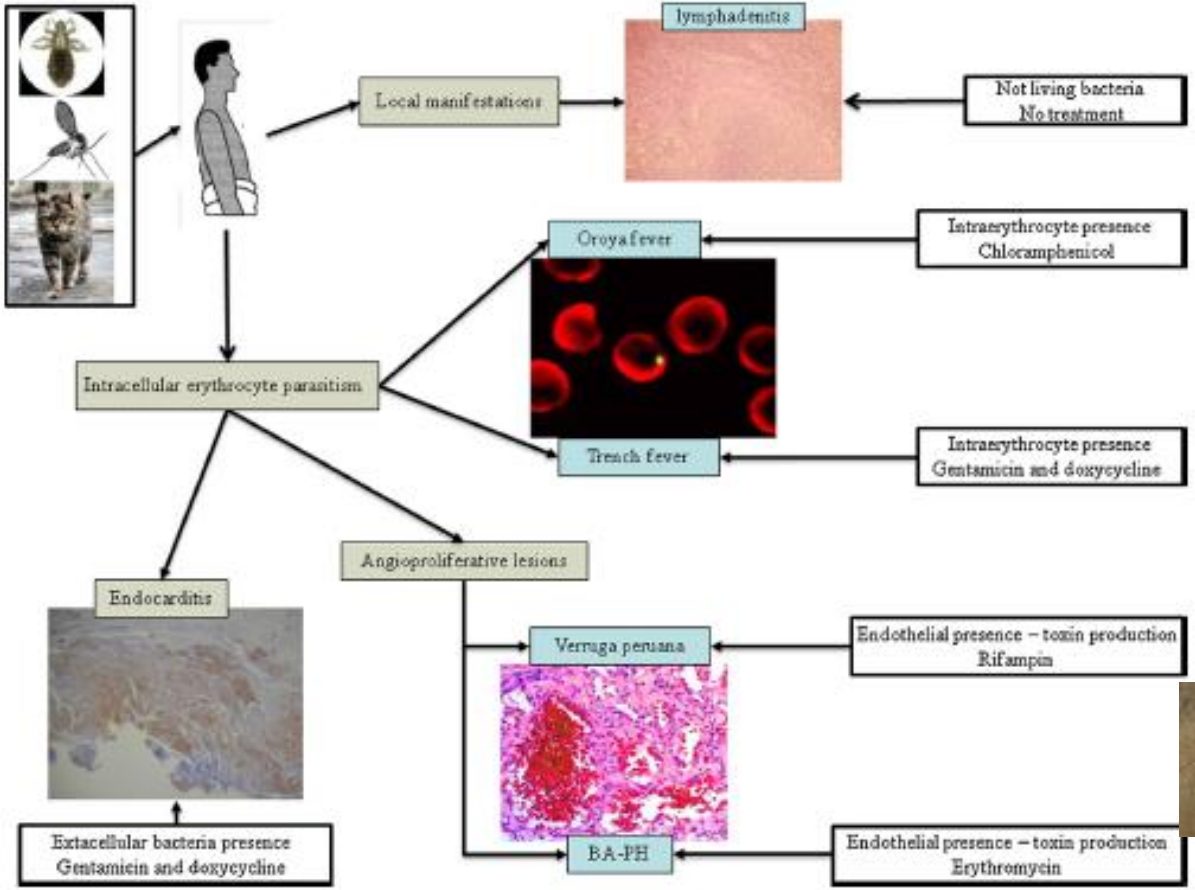

J Antimicrob Chemother 2024; 79: 1725–1747
https://doi.org/10.1093/jac/dkae145 Advance Access publication 18 June 2024

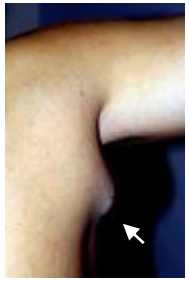
Treatment of persistent focalized Q fever: time has come for an international randomized controlled trial

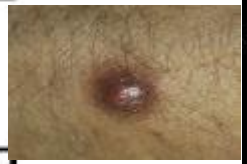
Audrey Delahaye[1]*, Carole Eldin[2], Alexandre Bleibtreu[3], Félix Djossou[4], Thomas J. Marrie[5], Nesrin Ghanem-Zoubi[6], Sonja Roeden[7] and Loïc Epelboin[1,8]

Bartonella spp.

B. henselae et B. quintana







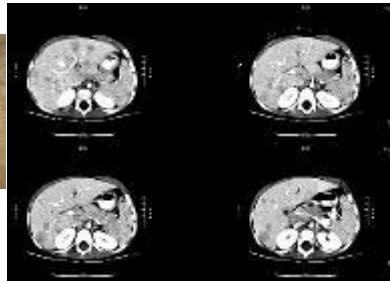
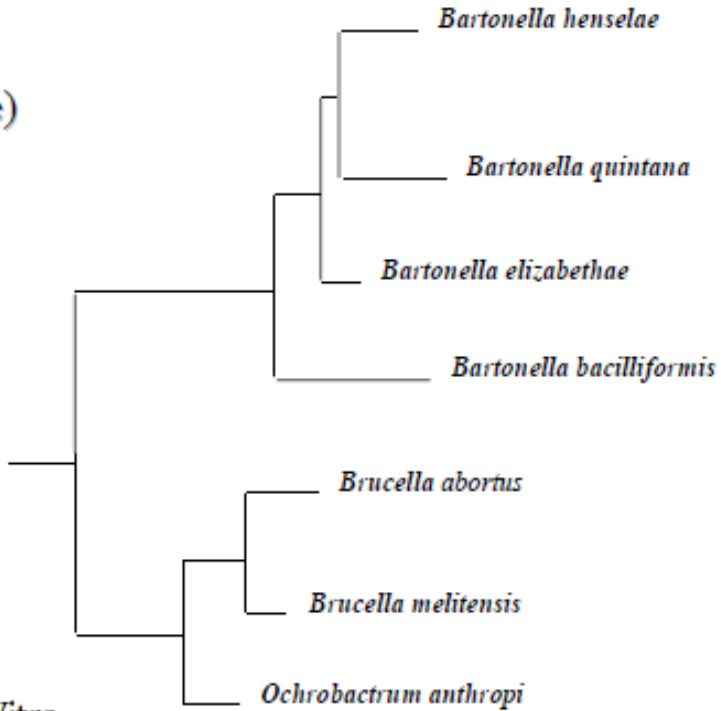


Fig. 1. Pathogenicity and treatment of *Bartonella* infections. BA, bacillary angiomatosis; PH, peliosis hepatis.

- In cellulo sensible à:
  - Aminoglycosides (bactéricide)
  - Doxycycline
  - Quinolones
  - Rifampicine
  - Erythromycine



Antiangiogenic Effect of Erythromycin: An In Vitro Model of *Bartonella quintana* Infection

Soraya Meghari,[a] Jean-Marc Rolain,[a] Georges E. Grau, Esther Platt, Lina Barrassi, Jean-Louis Mège, and Didier Raoult

Table 2
Pathogenicity and treatment of *Bartonella* spp. infections.

Pathogenicity	*Bartonella* agent	Clinical manifestation		Treatment	Duration	Reference
Local manifestations	*B. henselae*, *B. quintana*, *B. alsatica*, *B. clarridgeiae*	Lymphadenitis		No treatment		[31]
		Atypical CSD	Neuroretinitis	Doxycycline (200 mg/day) and rifampicin (600 mg/day)	4–6 weeks	[36,37]
			Hepatosplenic	Rifampicin (20 mg/kg/day) alone or with gentamicin (3 mg/kg/day)	4–6 weeks	[39]
Intracellular erythrocyte parasitism	*B. bacilliformis*	Oroya fever		Chloramphenicol (50 mg/kg/day for 3 days and then 25 mg/kg/day until completion of 14 days)	2 weeks	[7,48,67,70]
			Pregnancy	Chloramphenicol (50–100 mg/kg/day) and penicillin G (50 000–100 000 IU/kg/day)	2 weeks	[70]
	B. quintana	Trench fever		Gentamicin (3 mg/kg/day for 2 weeks) and doxycycline (200 mg/day for 4 weeks)	6 weeks	[62]
	B. henselae, *B. rochalimae*, *B. vinsonii* subsp. *arupensis*, *B. vinsonii*, *B. melophagi*	Bacteraemia		Gentamicin (3 mg/kg/day for 2 weeks) and doxycycline (200 mg/day for 4 weeks)	6 weeks	[62]
Endocarditis	*B. quintana*, *B. henselae*, other *Bartonella* spp.	Endocarditis		Gentamicin (3 mg/kg/day for 2 weeks) and doxycycline (200 mg/day for 6 weeks)	6 weeks	[81]
Angioproliferative lesions	*B. bacilliformis*, *B. ancashi*	Verruga peruana		Rifampicin (10 mg/kg/day) (maximum total daily dose 600 mg/day for children)	2–3 weeks	[27,70]
				Streptomycin (15–20 mg/kg/day)	2–3 weeks	[31,67]
	B. quintana, *B. henselae*	Bacillary angiomatosis, bacillary peliosis	Uncomplicated	Erythromycin (2 g/day) or doxycycline (200 mg/day)	3 months	[7,91,92]
			Complicated	Doxycycline (200 mg/day) with rifampicin (600 mg/day)	3 months	[7,91,92]
			Relapses	Erythromycin (2 g/day) or doxycycline (200 mg/day)	4–6 months	[35,96]

extensive lymphadenopathy, oral azithromycin at 500 mg on the first day and 250 mg on days 2 to 5

Clinical Infectious Diseases
MAJOR ARTICLE

Bartonella quintana Endocarditis: A Systematic Review of Individual Cases

Carl Boodman,[1,2,a] Nitin Gupta,[3,a] Christina A. Nelson,[4] and Johan van Griensven[2,a]

Table 4. Comparing Fatal to Non-fatal Cases of *Bartonella quintana* Endocarditis

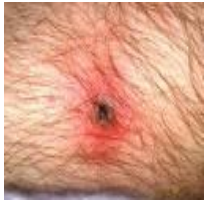
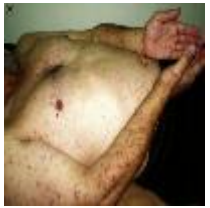
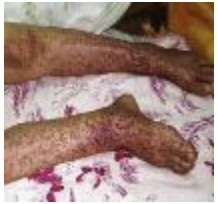
	Died			Survived				
	N	%	95% CI	N	%	95% CI	RR (95% CI)	*P* value
Age > 65	6	37.5	15.2–64.6	10	9.5	4.6–16.8	**3.9 (1.7–9.3)**	***P* = .008**
Fever	12	75.0	47.6–92.7	48	45.7	36.0–55.7	**1.6 (1.2–2.3)**	***P* = .034**
Emboli	6	37.5	15.2–64.6	37	35.2	26.2–45.2	1.1 (.5–2.1)	*P* = 1.000
Renal dysfct	6	37.5	15.2–64.7	14	13.3	7.5–21.4	**2.8 (1.3–6.3)**	***P* = .026**
Splenomegaly	2	12.5	1.5–38.4	11	10.5	5.4–18.0	1.2 (.3–4.9)	*P* = .682
Multivalvular	8	50.0	24.7–75.4	31	29.5	21.0–39.2	1.7 (1.0–3.0)	*P* = .149
Pre-valve Disease	4	25.0	7.3–52.4	42	40.0	30.6–50.0	0.6 (.3–1.5)	*P* = .284
No surgery	5	31.3	11.0–58.7	7	6.7	2.7–13.3	**4.7 (1.7–13.0)**	***P* = .010**
No gent	7	43.8	19.8–70.1	33	31.4	22.7–41.2	1.4 (.7–2.6)	*P* = .395
No doxy	9	56.3	29.9–80.3	37	35.2	26.2–45.2	1.6 (1.0–2.6)	*P* = .165
Total	**16**			**105**			…	…

Rickettsioses

Rickettsioses du groupe boutonneux

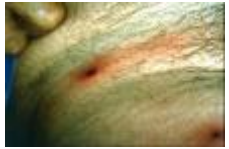
• **Fièvre boutonneuse méditerranéenne**: *R. conorii conorii*
Triade: fièvre, éruption, escarre
Ubiquitaire: méditerranée
Gravité potentielle



• **TIBOLA/ DEBONEL/SENLAT**
– *R slovaca,* possible autres pathogènes
Escarre cuir chevelu, ADP cervicales, asthenie
– *Dermacentor marginatus*
– *Europe*

• **Fièvre Africaine à tique**
– *R africae*
Escarres multiples, fébricule, rash
-Amblyomma
– Afrique du Sud



LAR: Lymphadenopathy associated rickettsiosis
R. mongolitimonae

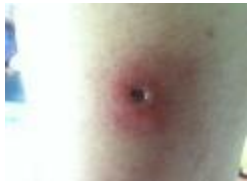


Table 2. Antibiotic treatment for major rickettsial infections, strength of recommendation and quality of evidence for adults and children.

Group	Disease	Regimen	Grade[a]	Adults	Children[b]	Pregnant women
SFG	Mediterranean spotted fever	Preferred	A I	Doxycycline 200 mg two oral doses in a single day	–	–
			A III	Doxycycline 200 mg single dose or 100 mg twice daily for 2–5 days	Doxycycline 2.2 mg/kg every 12 h for children weighing <100 lb (45 kg) or adult dosage if ≥100 lb, for 5–10 days	Josamycin 1 g every 8 h for 5 days (in severe form of Mediterranean spotted fever, single dose of doxycycline should be used)
		Alternatives	A I	Josamycin[c] 1 g every 8 h for 5 days	Josamycin 50 mg/kg every 12 h for 5 days	
			A I	–	Clarithromycin 15 mg/kg/day in 2 divided doses for 7 days	–
					Azithromycin 10 mg/kg/day in 1 dose for 3 days	–
			B II–D III	Ciprofloxacin	–	–
	Rocky Mountain spotted fever	Preferred	A III	Doxycycline 100 mg every 12 h for 5–10 days	Doxycycline 2.2 mg/kg every 12 h for children weighing <99 lb (45 kg), or adult dosage if ≥100 lb, for 5–10 days	Doxycycline 100 mg every 12 h for 5–10 days[b]
		Alternatives	A III	–	Chloramphenicol 12.5–25 mg/kg every 6 h for 5–10 days	–
	Other SFG rickettsioses	Preferred	A III	Doxycycline 200 mg single dose or 100 mg twice daily for 2–5 days	–	–
Typhus group	Murine typhus	Preferred	A III	Doxycycline 100 mg, twice daily, continued for 3 days after symptoms have resolved	Doxycycline 100–200 mg, for 3–7 days	Doxycycline (late trimester)
				Doxycycline 100–200 mg, single dose	–	–
		Alternatives	D III	Fluoroquinolones	–	Erythromycin
			D III	Chloramphenicol 60 to 75 mg/kg/day in four divided doses	Chloramphenicol	Chloramphenicol (early trimester: first and second trimesters)
	Epidemic typhus	Preferred	A II	Doxycycline 200 mg for 5 days, or 2–4 days after defervescence	–	–
			A III	Doxycycline 100–200 mg, single dose in outbreak situations	Doxycycline 100–200 mg, single dose	Doxycycline 100–200 mg, single dose
		Alternatives	D III	Chloramphenicol 60 to 75 mg/kg/day in four divided doses	–	–

[a]Strength of recommendation and quality of evidence is used as previously reported [96].
[b]Tetracycline antibiotics are generally contraindicated in children under 8 years because of the dose-dependent risk of staining of permanent teeth; however, these antibiotics are superior therapy for Rocky Mountain spotted fever and doxycycline is the drug of choice for therapy of this life-threatening disease in patients of all ages. Shorter treatment courses may be warranted in some pediatric settings; however, therapy should be continued for at least 48 h following lysis of fever and evidence of clinical improvement.
[c]Tetracycline antibiotics are generally contraindicated during pregnancy because of the risks associated with interference in the development of teeth and long bones in the fetus. However, because Rocky Mountain spotted fever is a life-threatening illness, and because of the demonstrated superiority of doxycycline as therapy for this infection, this drug represents the antibiotic of choice.
I: Evidence from ≥1 properly randomized controlled trial; II: Evidence from ≥1 well-designed clinical trial without randomization; from cohort or case–controlled analytical studies; III: ≥ Evidence from opinions of respected authorities based on clinical experience, descriptive studies or reports from expert committees; A: Good evidence; B: Moderate evidence; C: Limited evidence to support a recommendation for use; D: Limited evidence does not support a recommendation for use; SFG: Spotted fever group;
Data taken from [3,5,29,37–39,50,82,95].

Botelho-Nevers E, et al., Expert Rev Anti Infect Ther. 2012 Dec;10(12):1425-37

Analysis of risk factors for malignant Mediterranean spotted fever indicates that fluoroquinolone treatment has a deleterious effect

Elisabeth Botelho-Nevers[1,2], Clarisse Rovery[1], Hervé Richet[1] and Didier Raoult[1]*

J Antimicrob Chemother2011;66: 1821–1830

Table 5. Results of the multivariate analysis

Variable	*P* value	Odds ratio	95% Confidence interval
Tobacco use	0.037	7.008	1.120–43.867
Dehydration	0.011	11.795	1.764–78.853
Hyponatraemia	0.021	7.972	1.369–46.435
Use of corticosteroids	0.707	1.697	0.108–26.701
Doxycycline regimen	0.616	1.635	0.239–11.187
Fluoroquinolone regimen	0.031	9.767	1.234–77.291
β-Lactam regimen	0.344	2.215	0.427–11.489

The severe form of the disease was used as the dependent variable and tobacco use, dehydration, hyponatraemia, use of corticosteroids, doxycycline, fluoroquinolones and β-lactam regimens as independent variables.
Hosmer and Lemeshow test: $P=0.990$.

J Antimicrob Chemother 2012; **67**: 1677–1682
doi:10.1093/jac/dks089 Advance Access publication 30 March 2012

Journal of Antimicrobial Chemotherapy

Deleterious effect of ciprofloxacin on *Rickettsia conorii*-infected cells is linked to toxin–antitoxin module up-regulation

Elisabeth Botelho-Nevers, Sophie Edouard, Quentin Leroy and Didier Raoult*

Antibiotic treatment recommendations for facultative intracellular bacteria–related diseases

Disease	Adult	Child
Legionellosis	Levofloxacin 750mg Azithromycin 500mg 7-10 days	Erythromycin 50 mg/kg/day *or* newer macrolides
Acute brucellosis	Doxycycline 100mg bid with rifampicin (rifampin) 300mg tid or with Gentamicin 5mg/kg 6wk	**Age >8y:** doxycycline 2.5 mg/kg bid with rifampicin 20mg/kg/day or with streptomycin 1.5-2.5 mg/kg/day, 6wk **Age <8y:** cotrimoxazole (trimethoprim-sulfamethoxazole) 25-5 mg/kg/day bid with rifampicin 20 mg/kg/day or with streptomycin 1.5-2.5 mg/kg/day, 6wk
Typhoid	Azithromycin 1g then 500 mg 5-7 days *or* ceftriaxone 2g IV/IM, 7-10 days or cotrimoxazole 800-160mg bid, 14 days	Ceftriaxone 100 mg/kg/day , 7-10 days *or* cotrimoxazole 25-5 mg/kg bid, 14 days
Salmonella enteritis	Ciprofloxacin 500mg bid, 5 days *or* ceftriaxone 2g IV/IM, 5 days *or* cotrimoxazole 960mg bid, 5-7 days	Cotrimoxazole 25-5 mg/kg bid, 5-7 days if necessary
Yersiniosis	Gentamicin 3mg/kg/day in 2-3 doses, 7-14 days *or* doxycycline 100mg bid, 7-14 days *or* ciprofloxacin 500mg bid, 7-14 days	Gentamicin 3 mg/kg/day in 2-3 doses, 7-14 days
Mycoplasma hominis	Doxycycline 100mg bid, 7 days *or* clindamycin 500mg qid, 7 days *or* ciprofloxacin 500mg bid, 7 days	
Ureaplasma urealyticum	Doxycycline 100mg bid, 7 days *or* erythromycin 500mg qid, 7 days	
M. pneumoniae	Azithromycin 500mg 5 days *or* doxycycline 100mg bid 7 days	Erythromycin 50 mg/kg/day, 14-21 days *or* newer macrolides
Bacillary angiomatosis and *Bartonella* endocarditis	Gentamicin 3 mg/kg/day in 2-3 doses with erythromycin 500mg qid or with doxycycline 100mg bid or with ceftriaxone 2g IM once daily, 4-6wk	Gentamicin 3 mg/kg/day in 2-3 doses with erythromycin 50 mg/kg/day or with ceftriaxone 100 mg/kg/day IM, 4-6wk

Abbreviations: bid = twice daily; IM = intramuscular; IV = intravenous; qid = 4 times daily.

S. aureus

S. aureus: bactérie intracellulaire!

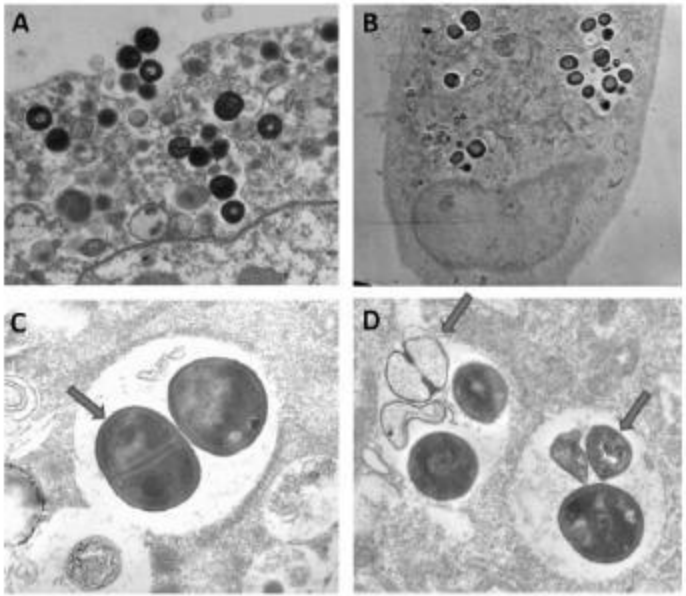
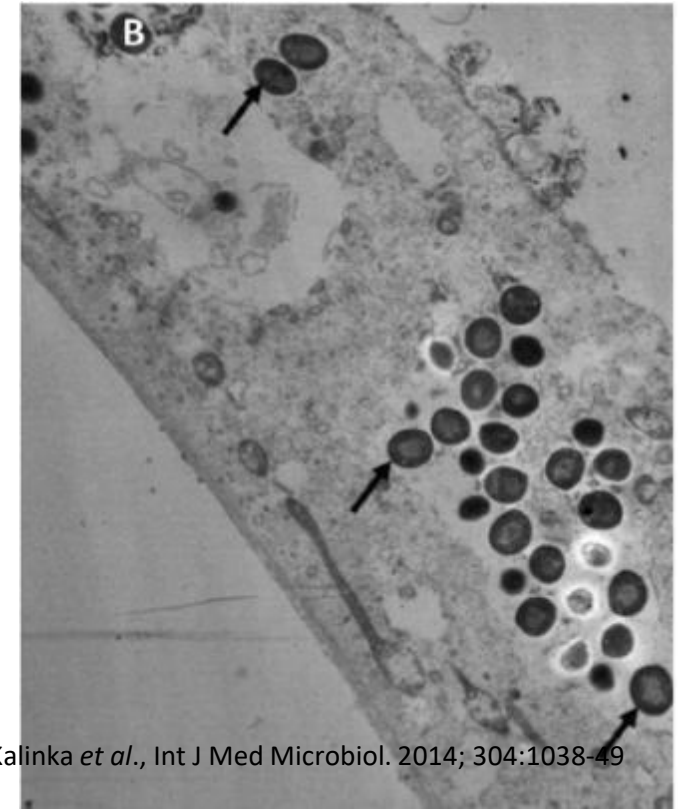


Fig. 1. Electron micrographs of different types of infected host cells. Adherence and uptake of *S. aureus* in epithelial A549 cells (A). Intracellular location of *S. aureus* after infection of primary osteoblasts (B). Dividing figure of *S. aureus* within an intracellular phagosome (C) and intracellular bacterial degradation (D) 24 h after infection of endothelial cells (HUVEC).



Kalinka *et al.*, Int J Med Microbiol. 2014; 304:1038-49

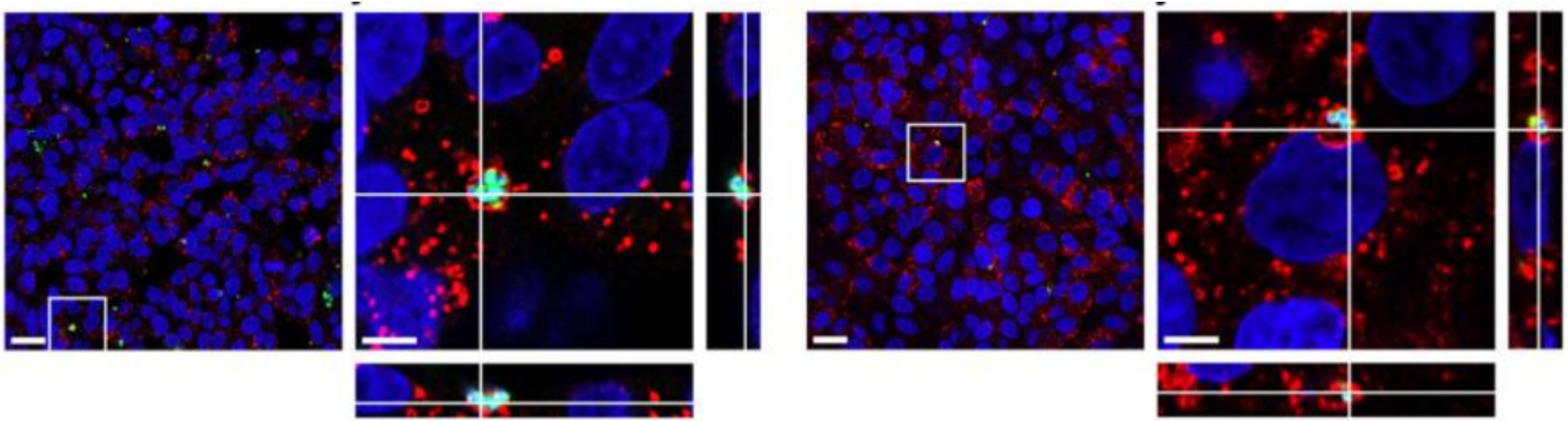
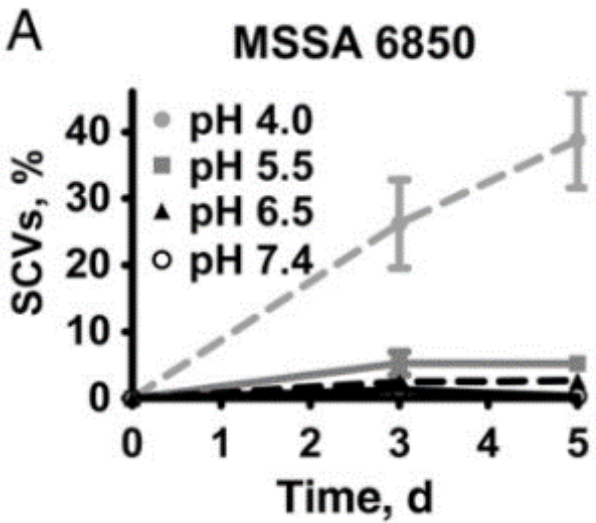


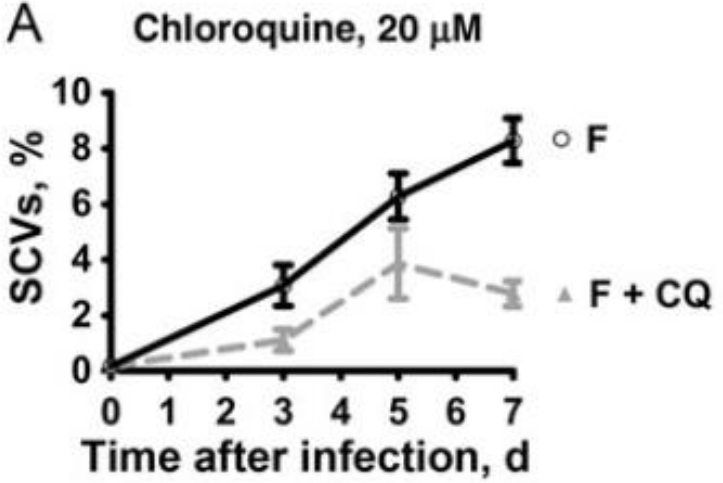
Figure 2. Intracellular persistence of *Staphylococcus aureus* within phagolysosomes. A549 cells were infected with *S. aureus* Cowan, and extracellular bacteria were killed by addition of flucloxacillin (1 mg/mL). *A*, The number and phenotype of viable intracellular persisting bacteria were determined at indicated time points. Data are mean values (±standard errors of the mean) pooled from 2 experiments performed in triplicate. *B*, The intracellular localization of persisting bacteria (CFSE; green) was analyzed by fluorescence microscopy. Lysosomes were visualized with LAMP-2 antibody (Alexa Fluor 594; red) and nuclei were stained with DAPI (blue). Scale bars 20 μm and 5 μm, respectively. Abbreviations: CFU, colony-forming units; SCV, small-colony variant.

DOI: 10.1093/infdis/jiv388

Nonstable *Staphylococcus aureus* Small-Colony Variants Are Induced by Low pH and Sensitized to Antimicrobial Therapy by Phagolysosomal Alkalinization

Nadja Leimer,[1] Carole Rachmühl,[1] Miguel Palheiros Marques,[1] Anna Sophie Bahlmann,[1] Alexandra Furrer,[1] Fritz Eichenseher,[3] Kati Seidl,[1] Ulrich Matt,[1] Martin J. Loessner,[3] Reto A. Schuepbach,[2,a] and Annelies S. Zinkernagel[1,a]





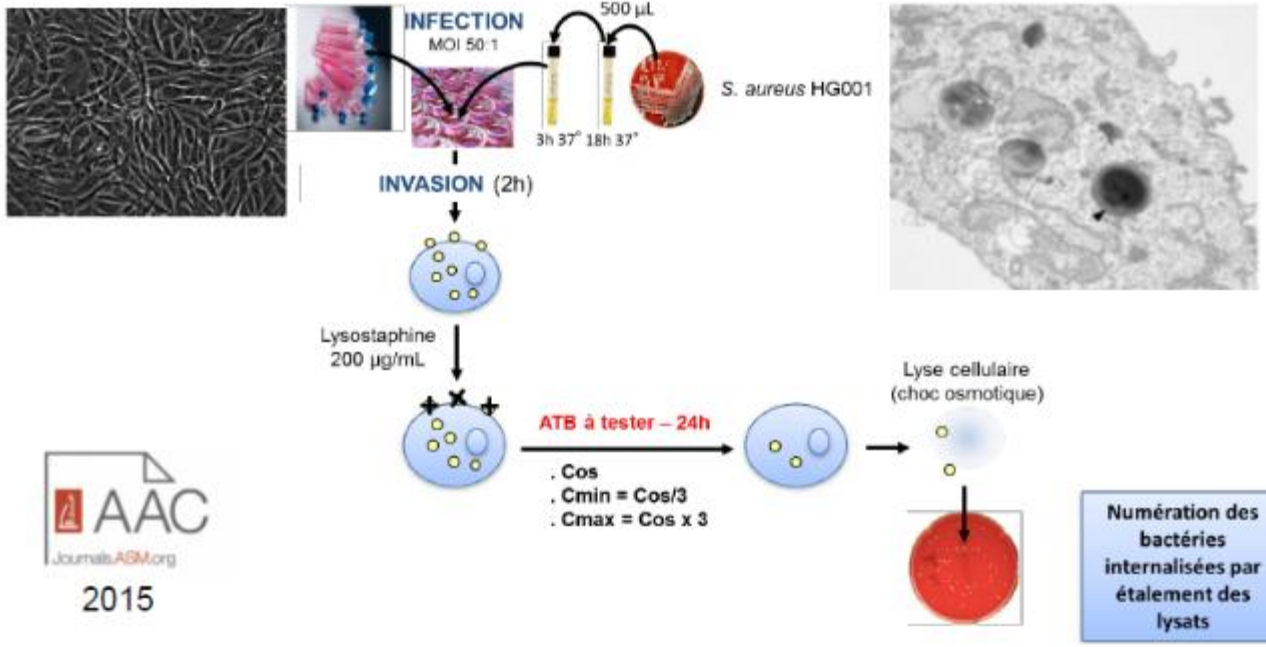
DOI: 10.1093/infdis/jiv388

Infection osseuse

Antimicrobial Activity against Intraosteoblastic *Staphylococcus aureus*

Florent Valour,[a,b] Sophie Trouillet-Assant,[b] Natacha Riffard,[b] Jason Tasse,[b] Sacha Flammier,[b] Jean-Philippe Rasigade,[b,c] Christian Chidiac,[a,b] François Vandenesch,[b,c,d] Tristan Ferry,[a,b] Frédéric Laurent,[b,c,d] on behalf of the Lyon Bone and Joint Infection Study Group

Infectious Diseases Department, Hospices Civils de Lyon, Lyon, France[a]; INSERM U1111, International Centre for Research in Infectiology, Université Claude Bernard Lyon 1, Lyon, France[b]; Laboratory of Bacteriology, Hospices Civils de Lyon, Lyon, France[c]; French National Reference Center for Staphylococci, Hospices Civils de Lyon, Lyon, France[d]



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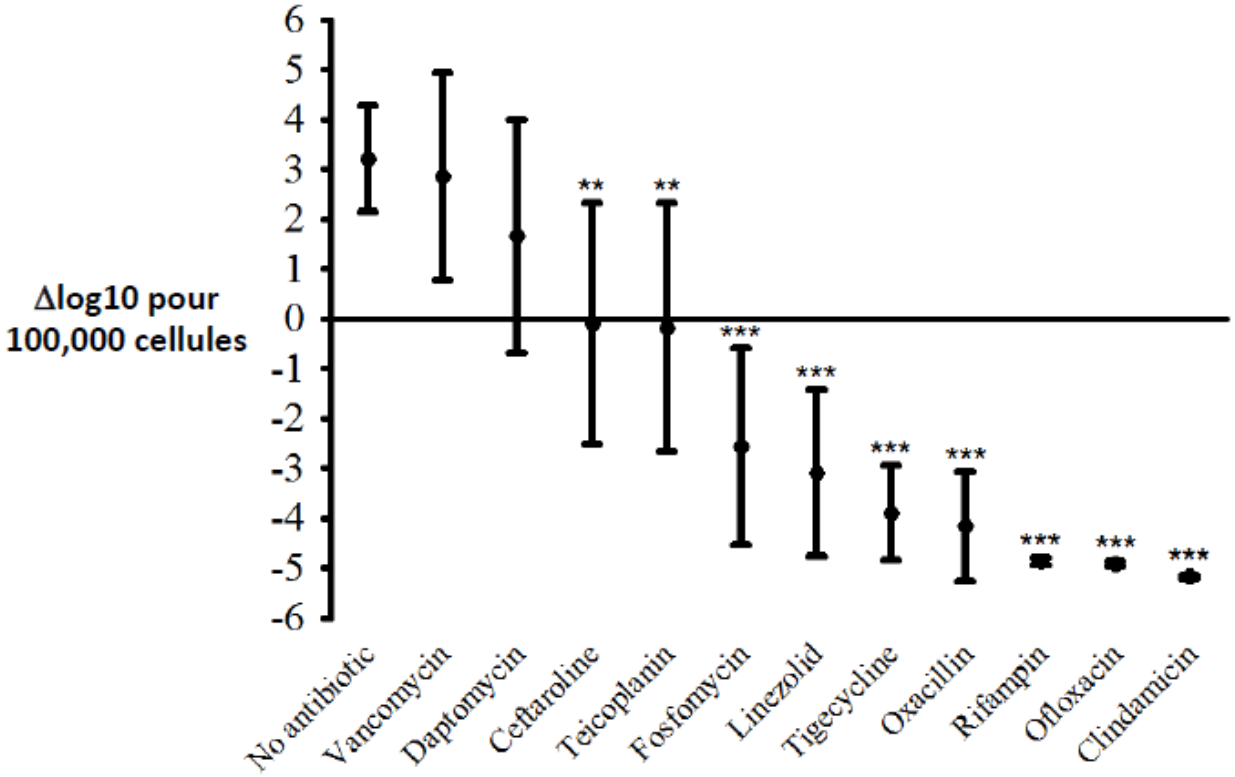
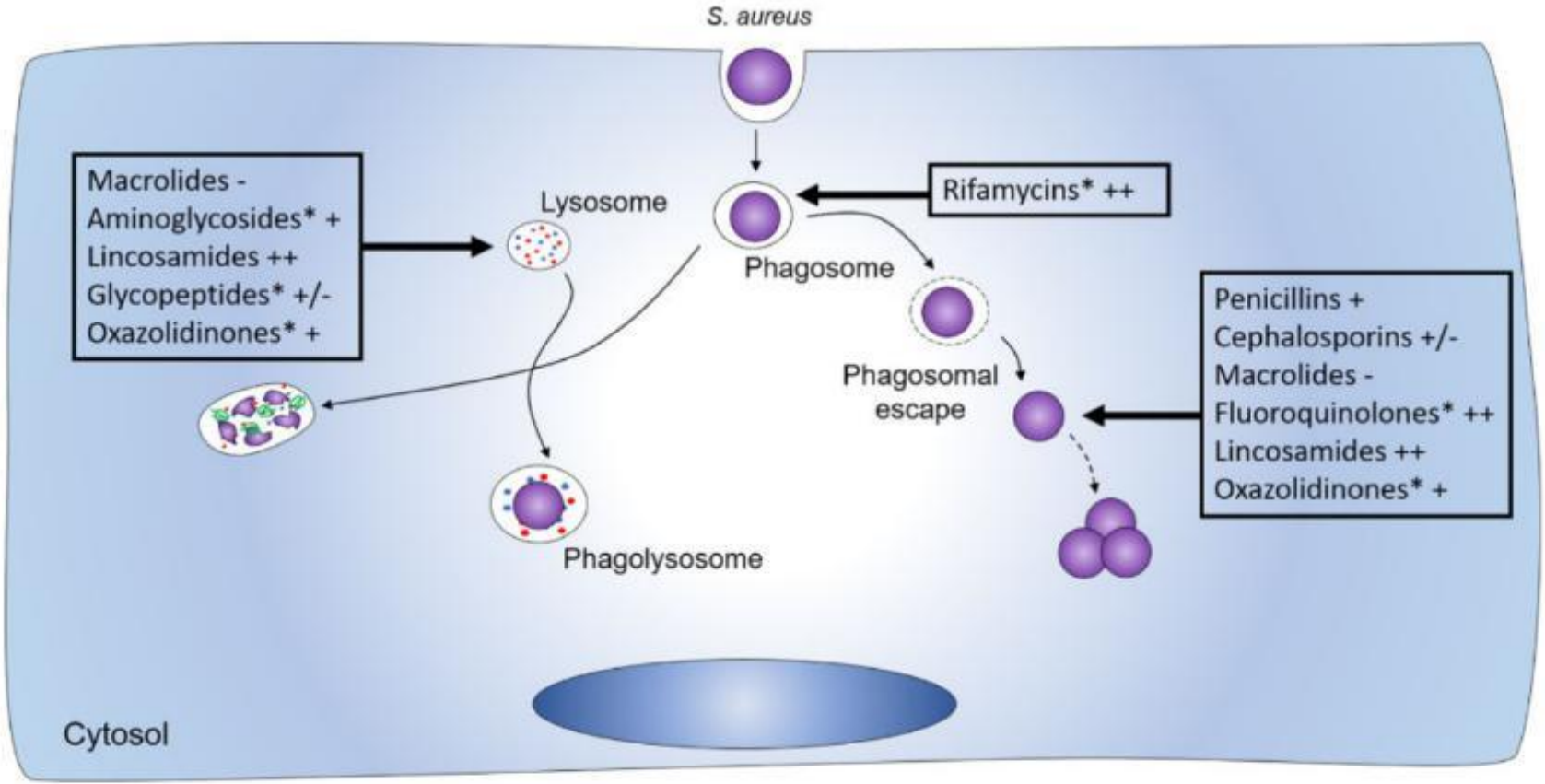
2015

Action intracellulaire à concentration osseuse

Valour et al. BJI Study Group. *Antimicrob Agents Chemother* 2015





++ Good activity
+ Moderate activity
- No to low activity
* Class keeping their activity in the chronic model

Marro FC et al., J Antimicrob Chemother 2021; 76: 3091–3102

Should we expand the indications for the DAIR (debridement, antibiotic therapy, and implant retention) procedure for *Staphylococcus aureus* prosthetic joint infections? A multicenter retrospective study

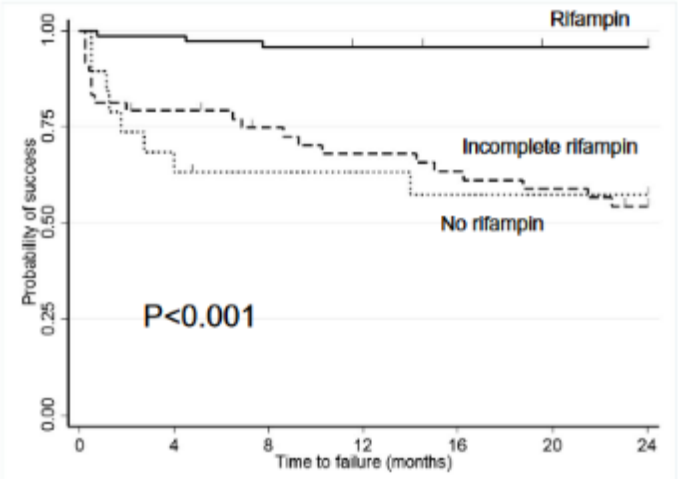
O. Lesens[1,2] · T. Ferry[3] · E. Forestier[4] · E. Botelho-Nevers[5] · P. Pavese[6] · E. Piet[7] · B. Pereira[8] · E. Montbarbon[9] · B. Boyer[10] · S. Lustig[3] · S. Descamps[11,12] · on behalf of the Auvergne-Rhône-Alpes Bone and Joint Infections Study Group

European Journal of Clinical Microbiology & Infectious Diseases (2018) 37:1949–19

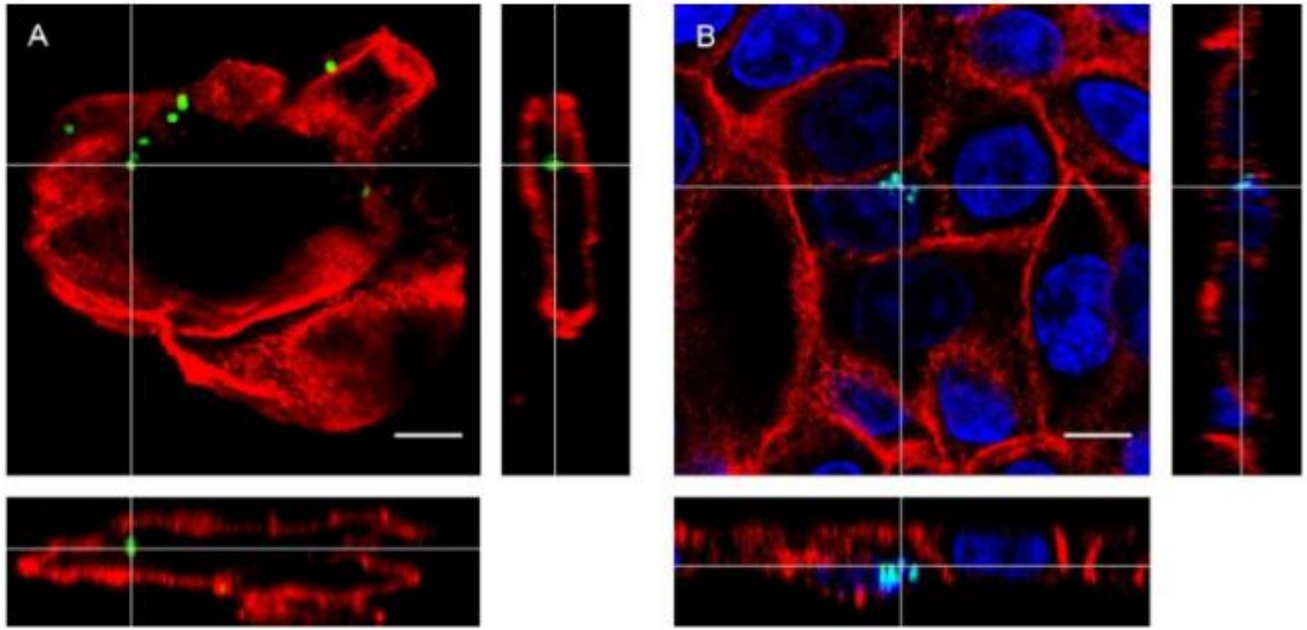
Table 3 Results of multivariate analysis (Cox model) predicting the risk of failure at 2 years of follow-up in patients with onset of infection < 3 months and the entire cohort

	Onset of infection < 3 months $n = 89$		All patients $n = 137$	
Variables	HR [95% CI]	p	HR [95% CI]	p
Incomplete rifampin regimen	0.5 [0.16–1.6]	0.248	0.5 [0.2–1.28]	0.151
Complete rifampin regimen	0.16 [0.03–0.82]	0.028	0.08 [0.018–0.36]	0.001
Treatment duration	0.76 [0.66–0.89]	0.001	0.78 [0.69–0.88]	< 0.001
Active smoking	3.29 [0.8–13.41]	0.097	3.6 [1.09–11.84]	0.036
Early acute	0.25 [0.09–0.7]	0.009	–	–

C



En situation de portage



16,1% porteurs *S. aureus* au niveau nasal avec localisation intracellulaire

Figure 5 : Visualisation de *S. aureus* intracellulaire au niveau de cellules HNECs issues d'écouvillonnage nasal (A) et de culture de HaCaT (B). Le contour cellulaire est coloré en rouge, l'acide nucléique en bleu et *S. aureus* en vert. Les échelles graphiques représentent 10 µm.

Rigaill et al., J Antimicrob Chemother. 2018 Nov 1;73(11):3044-3048.

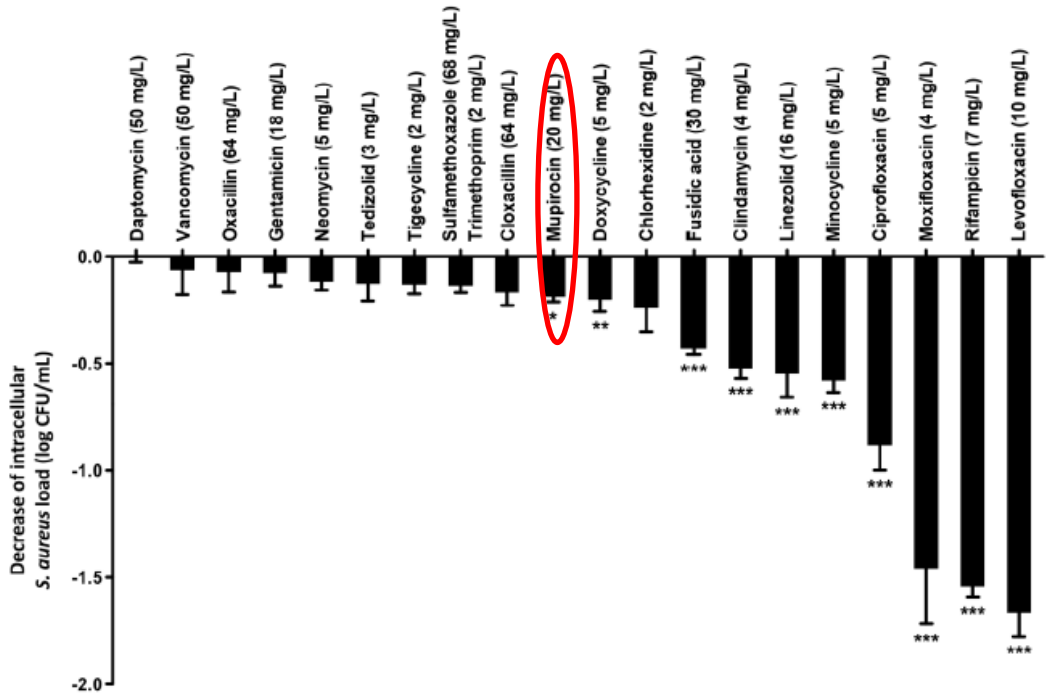
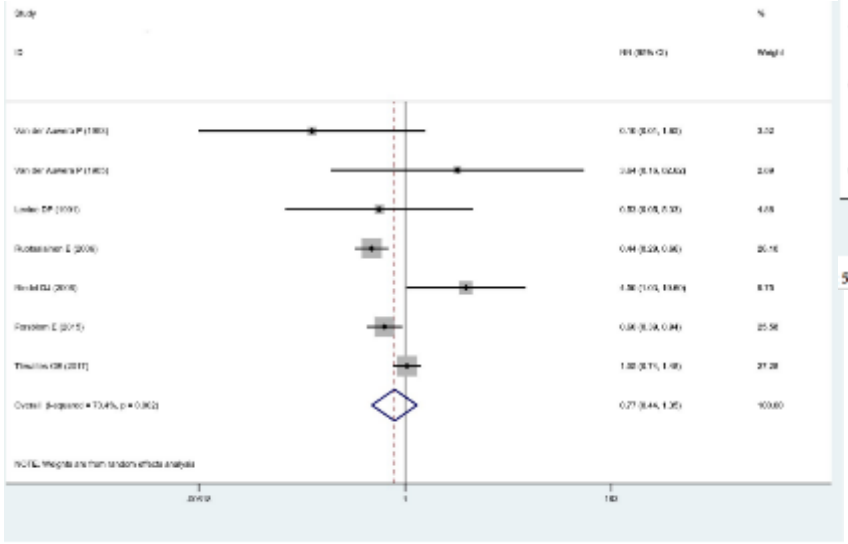


Figure 2. Impact of antimicrobial compounds on intracellular *S. aureus* load in HaCaT cells. Bars represent the mean with standard error of 6 values. The decrease of intracellular *S. aureus* load was compared to the lysostaphin control set to zero (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

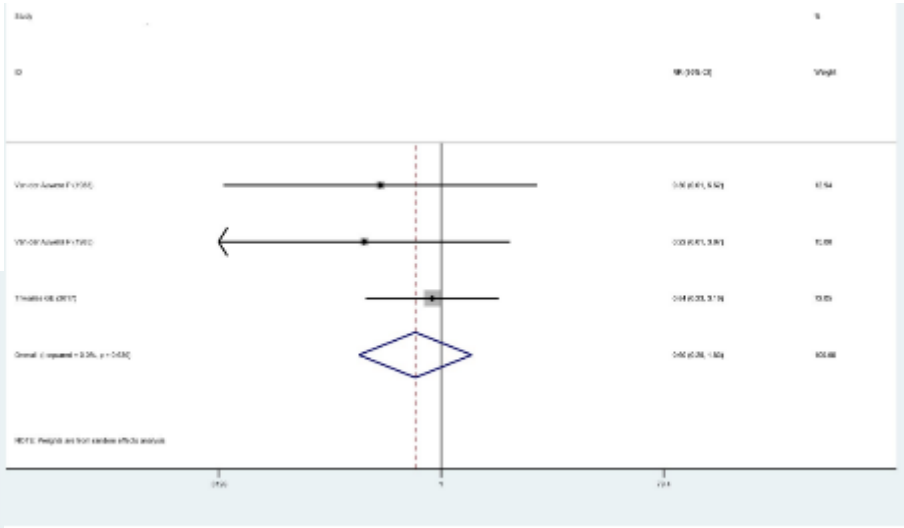
RESEARCH ARTICLE

Adjunctive rifampin for the treatment of *Staphylococcus aureus* bacteremia with deep infections: A meta-analysis

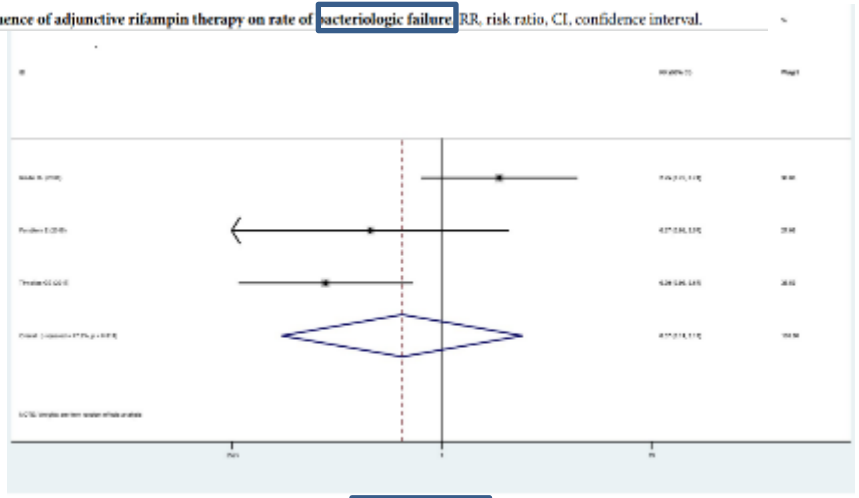
Huan Ma[1], Jie Cheng[2,3], Lengyue Peng[1], Yawen Gao[1], Guangli Zhang[1], Zhengxiu Luo[1,2,3]*



3. Forest plot: Impact of adjunctive rifampin therapy on mortality of SAB. RR, risk ratio, CI, confidence interval.



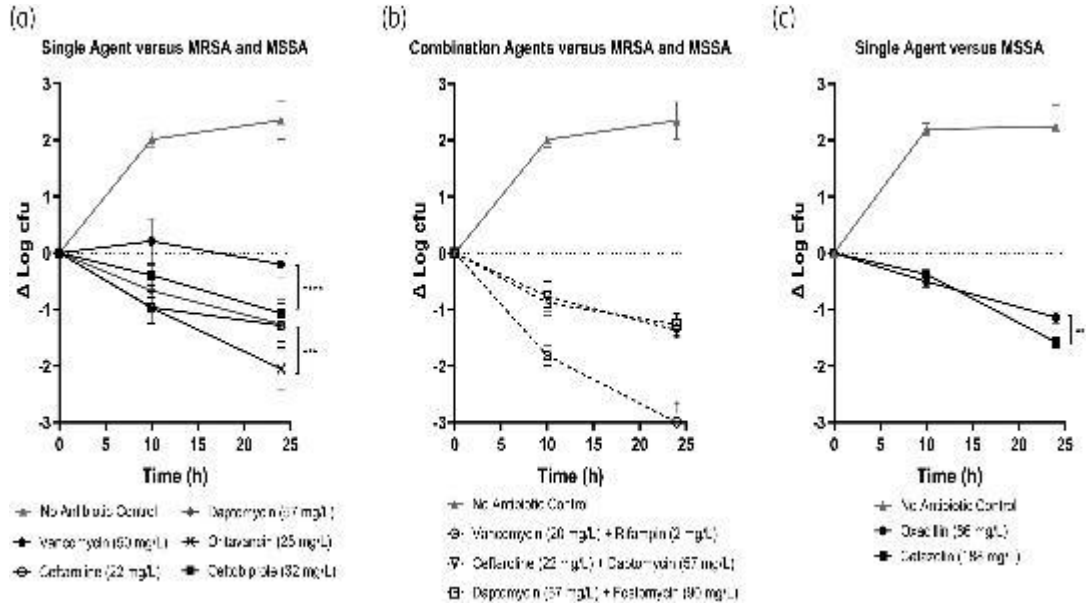
5. Forest plot: Influence of adjunctive rifampin therapy on rate of bacteriologic failure. RR, risk ratio, CI, confidence interval.



6. Forest plot: Influence of adjunctive rifampin therapy on relapse rate. RR, risk ratio, CI, confidence interval.

doi: 10.1371/journal.pone.0230383

Rifampicine et réservoir intracellulaire de SA



J Antimicrob Chemother, Volume 79, Issue 10, October 2024, Pages 2471–2478, https://doi.org/10.1093/jac/dkae241

Place de la rifampicine dans les EI sur prothèse ?

Clinical Infectious Diseases
MAJOR ARTICLE

Is Rifampin Use Associated With Better Outcome in Staphylococcal Prosthetic Valve Endocarditis? A Multicenter Retrospective Study

Audrey Le Roy,[1] Raphaël Lecomte,[2] Pierre Gouriet,[3] François Reynes,[4] Cédric Arvieux,[5] Sévrine Ansart,[6] David Boutoille,[7] Bouvet Le Borne,[1] Céline Chirouze,[8] Matthieu Lemaignen,[9] Laura Dejoie,[10] Erwan Flecher,[1] Jean-Marc Chapplain,[1] Pierre Tattevin,[1,11] and Matthieu Revest,[1,11] for the Groupe d'Epidémiologie et Recherche en Infectiologie Clinique de Centre et de l'Ouest (GERICCO)

[1]Infectious Diseases and Intensive Care Unit, Pontchaillou University Hospital, Rennes, France, [2]Department of Infectious Diseases, CCUG, MD INSERM, University Hospital, Nantes, France, [3]Infectious Diseases and Tropical Medicine, La Cavale Blanche University Hospital, Brest, France, [4]Department of Internal Medicine and pneumology, La Cavale Blanche University Hospital, Brest, France, [5]Department of Internal Medicine and Infectious Diseases, Pontchaillou University Hospital, Rennes, France, [6]Department of Bacteriology, Pontchaillou University Hospital, Rennes, France, [7]University of Rennes, Rennes, INSERM (National Institute of Health and Medical Research), UMR_S 1230, Rennes, France, and [8]CHU Nantes, 1 rue G. Veil, Pontchaillou University Hospital, Rennes, France

Table 3. Outcome of Prosthetic Valve Endocarditis due to *Staphylococcus aureus* (n = 114), or Coagulase-negative Staphylococci (n = 66) in Patients Treated With or Without Rifampin

Variable	*Staphylococcus aureus* (n = 114)				Coagulase negative staphylococci (n = 66)			
	Rifampin-based (n = 64)	**No Rifampin** (n = 50)	**Odds Ratio (95% CI)**	***P* Value**	**Rifampin-based** (n = 37)	**No Rifampin** (n = 29)	**Odds Ratio (95% CI)**	***P* Value**
Mortality								
In-hospital mortality	18 (28.1)	12 (24.0)	1.24 (.53–2.89)	.78	8 (21.6)	4 (13.8)	1.72 (.46–6.41)	.61
Six-month mortality	26 (40.6)	16 (32.0)	1.45 (.66–3.16)	.45	10 (27.0)	6 (20.7)	1.42 (.45–4.50)	.76
One-year mortality	27 (42.2)	18 (36.0)	1.30 (.61–2.78)	.63	11 (29.7)	7 (24.1)	1.33 (.44–4.01)	.82
Relapse	4 (6.3)	4 (8.0)	0.93 (.22–3.91)	.79	2 (5.4)	3 (10.3)	.49 (.08–3.18)	.78
Vitamin K antagonist imbalance	9 (39.1)	4 (22.2)	2.25 (.56–9.05)	.41	6 (50.0)	2 (22.2)	3.5 (.50–24.3)	.40
Bleeding complication	10 (15.6)	10 (20.0)	0.72 (.28–1.95)	.71	3 (8.1)	0 (0)	5.99 (.29–120.8)	.33
Length of stay, days	42.8 ± 20.1	30.7 ± 14.7	…	.0006	41.4 ± 16.1	32.4 ± 12.9	…	.02

Quantitative variables are expressed as mean ± standard deviation; qualitative variables are expressed by numbers (%).

Abbreviation: CI, confidence interval.

Merci de votre attention
Des questions?

elisabeth.botelho-nevers@univ-st-etienne.fr