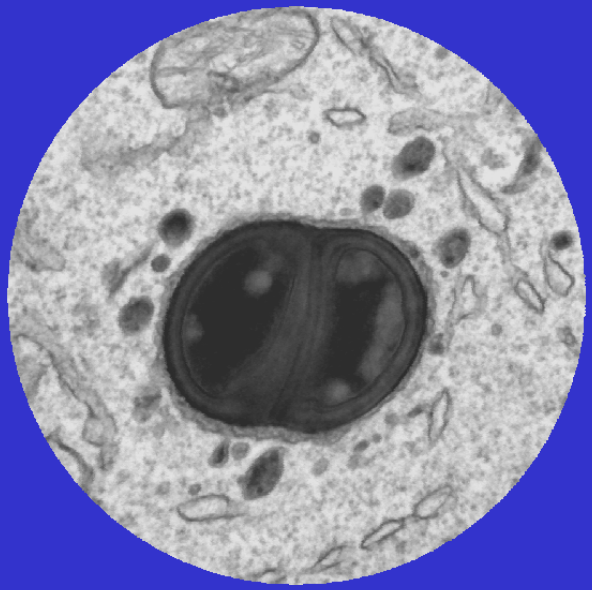
Maritza Barcia-Macay (Patterson)
Unité de Pharmacologie cellulaire et moléculaire

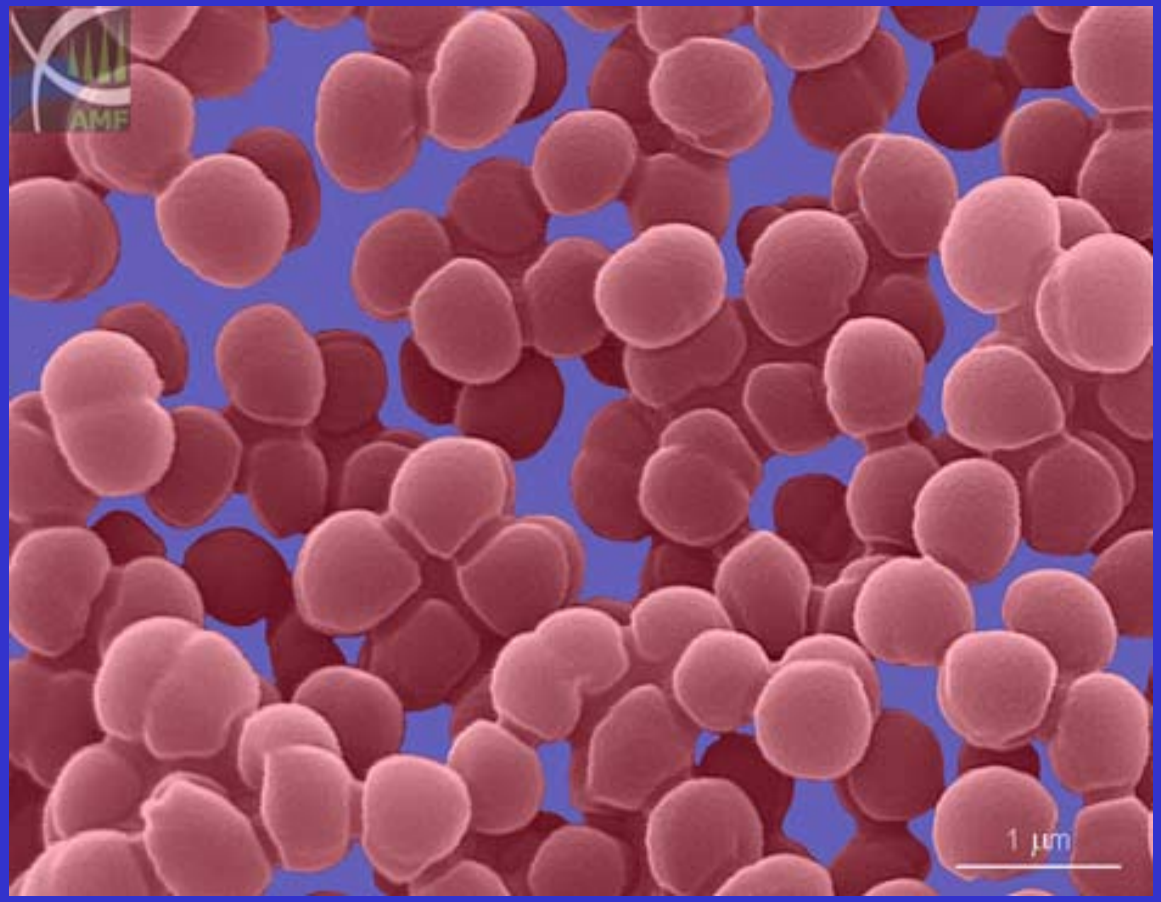
Activity of antibiotics against extracellular and intracellular forms of *Staphylococcus aureus*



Pharmacodynamic studies in vitro using a model of human THP-1 macrophages

I. INTRODUCTION

Staphylococcus aureus



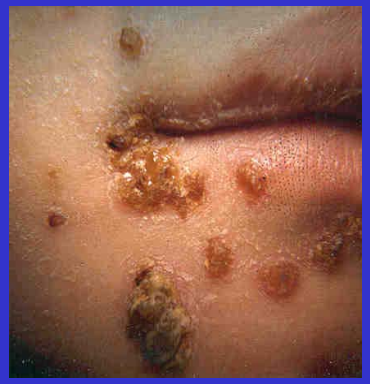
Bacteria with the greatest pathogenic potential in human infections.

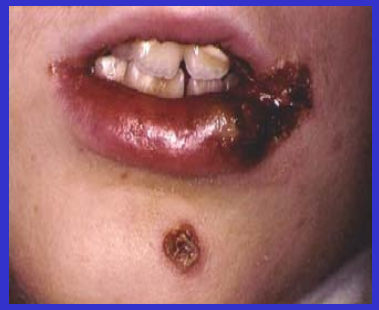
A major cause of nosocomial and community-acquired infections.

S. aureus infections

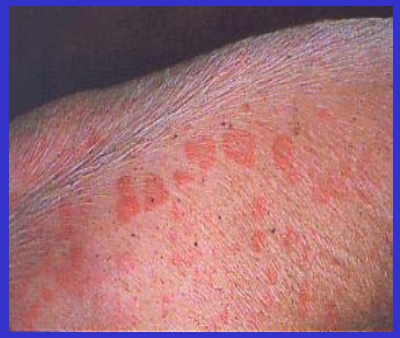
1. skin and soft tissue infections

impetigo

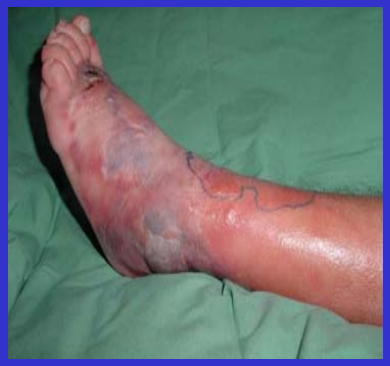




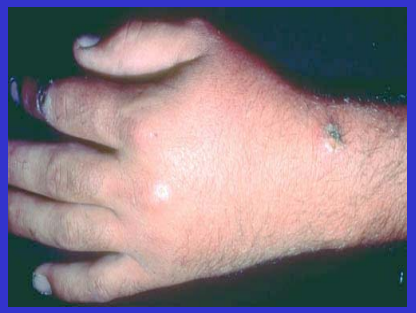
erysipelas



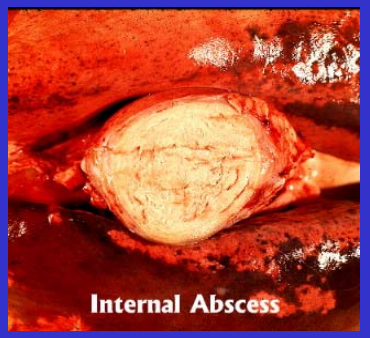
cellulitis



furuncle



abscess



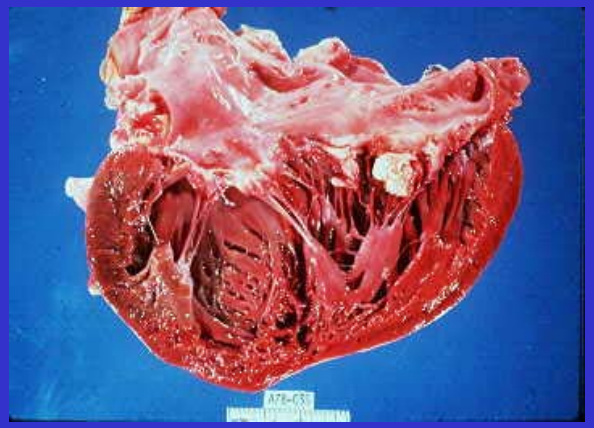
S. aureus infections

2. food-poisoning

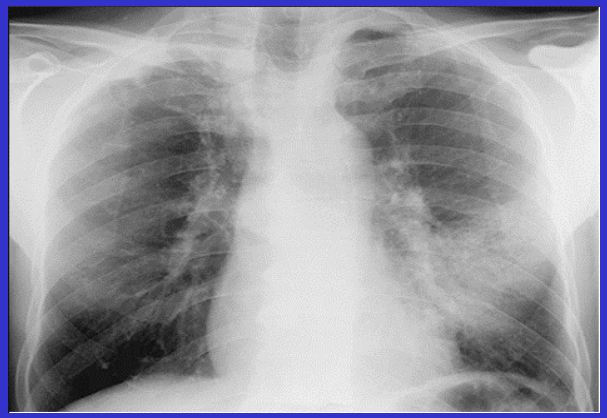
S. aureus infections

3. Deep-organ infections

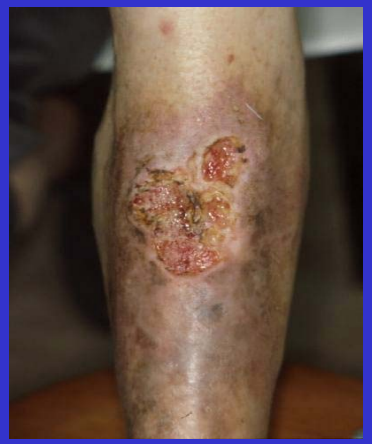
endocarditis



pneumonia



osteomyelitis



S. aureus infections

All these infections are difficult to treat :

- recurrence / persistence

  in relation with the intracellular character of *S. aureus*

  → selection of antibiotics based on pharmacokinetic / pharmacodynamics properties

- resistance to currently available antibiotics

  → need of drugs acting on multiresistant strains

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Intracellular infection by *S. aureus*

Normal process of phagocytosis

1) attachment to phagocyte membrane

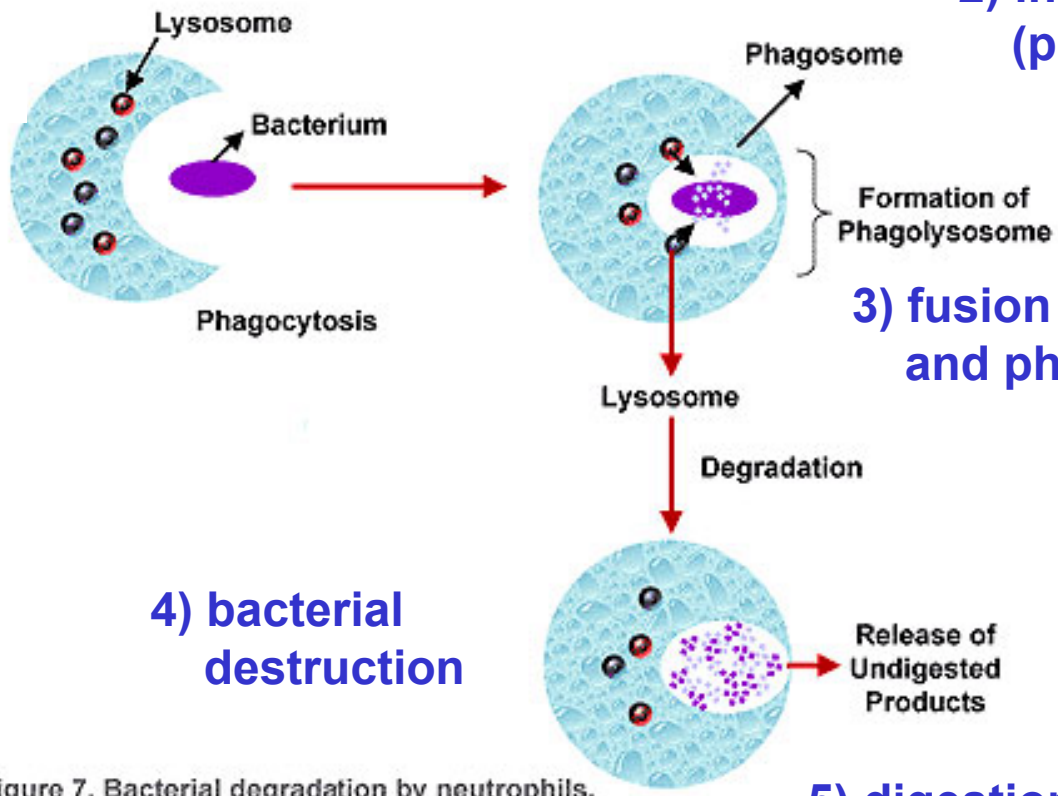


Figure 7. Bacterial degradation by neutrophils.

2) ingestion (phagosome)

3) fusion lysosome and phagosome

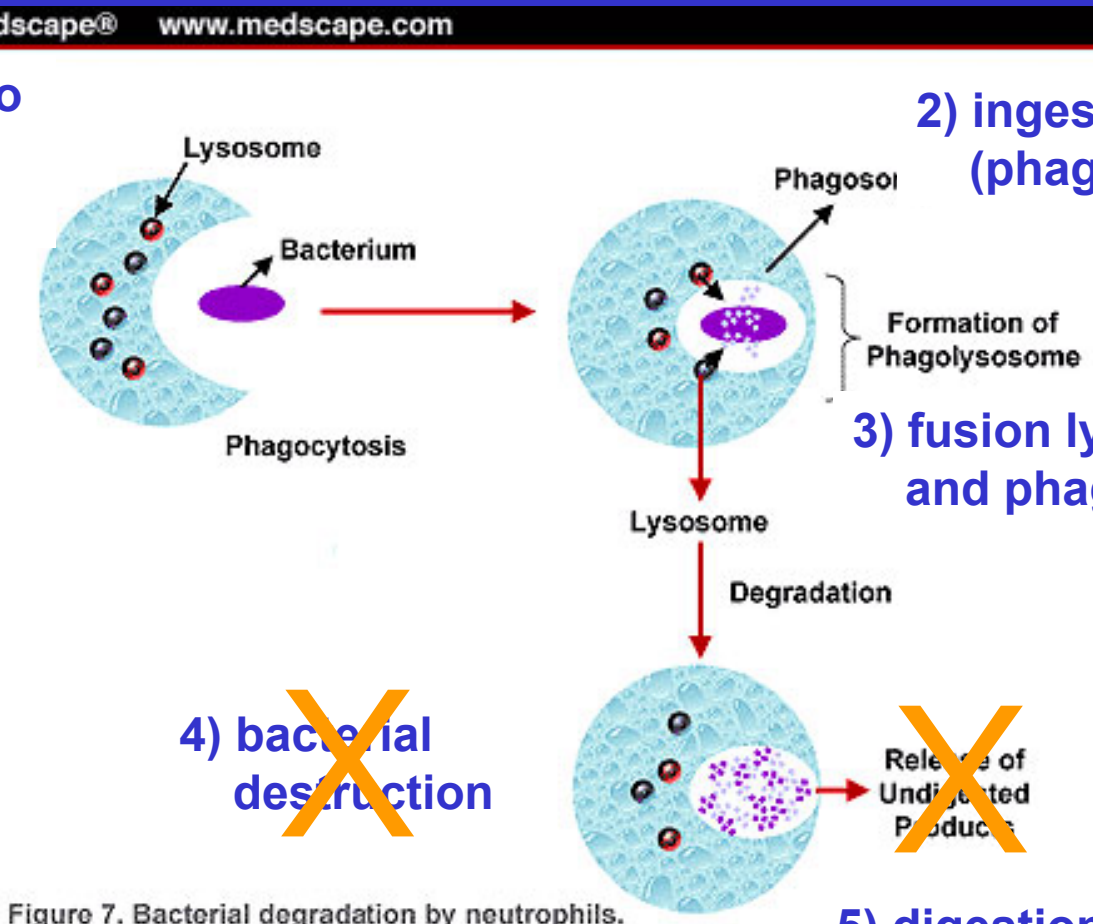
4) bacterial destruction

5) digestion and release of microbial debris

Intracellular infection by *S. aureus*

Incomplete phagocytosis of *S. aureus*

1) attachment to phagocyte membrane



2) ingestion (phagosome)

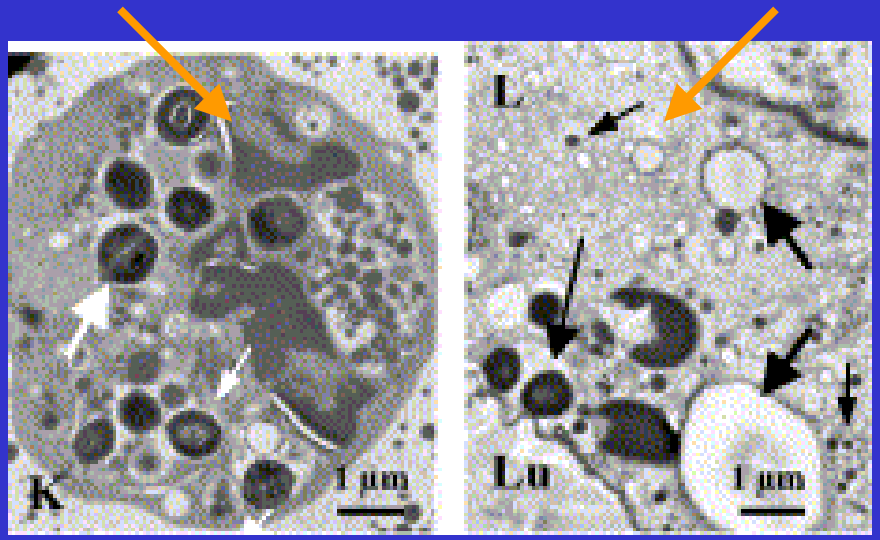
3) fusion lysosome and phagosome

4) bacterial destruction

5) digestion and release of microbial debris

Intracellular infection by *S. aureus*

Bacteria able to survive in different host cells : phagocytes and non-phagocytes



Neutrophils
Macrophages

Mammary epithelial cells
Enterocytes
Keratinocytes
Osteoblasts
Fibroblasts
Endothelial cells

Brouillette *et al*, Vet Microbiol (2004) 101:253-262; Microb Pathog. (2003) 35:159-68.

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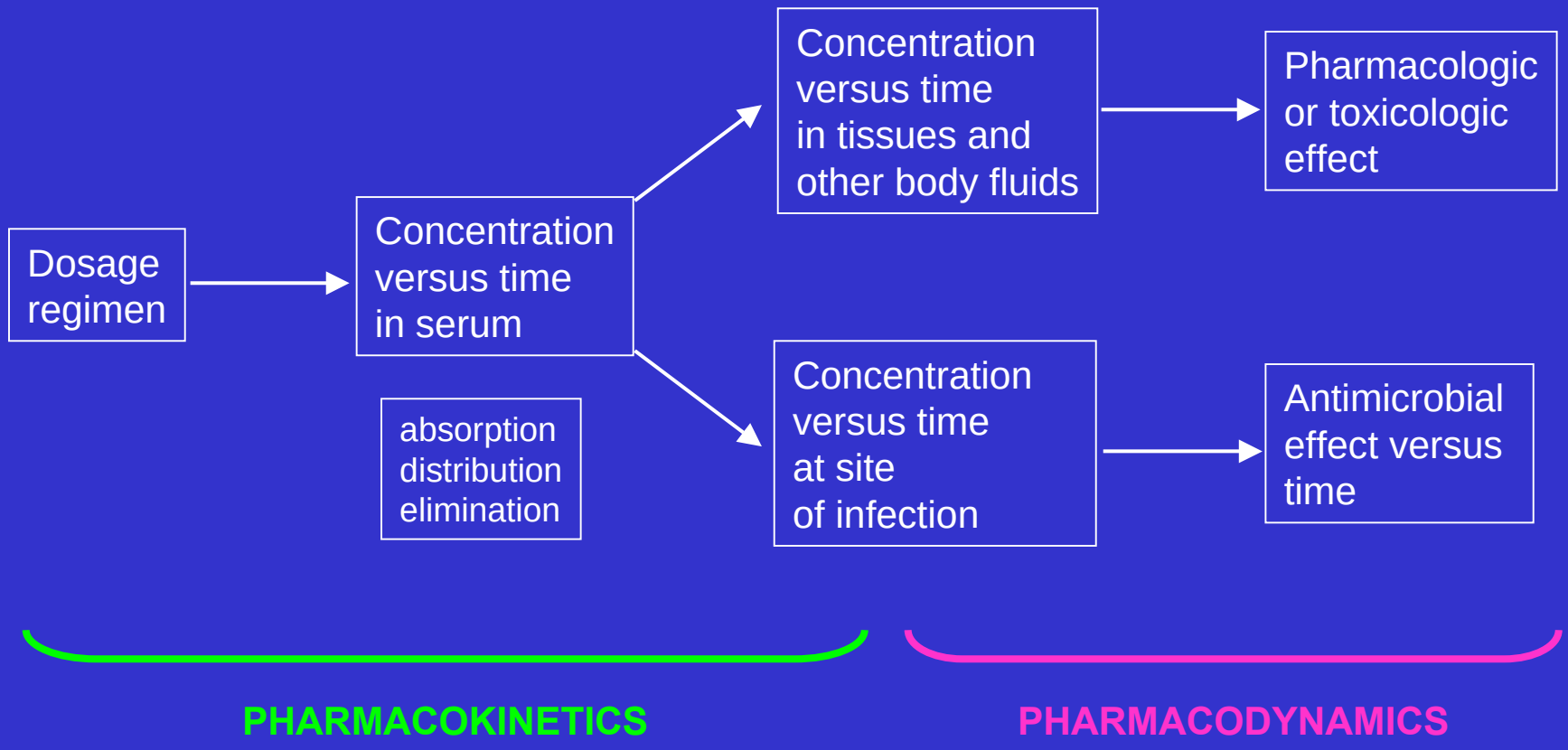
Antibiotic pharmacokinetics / pharmacodynamics

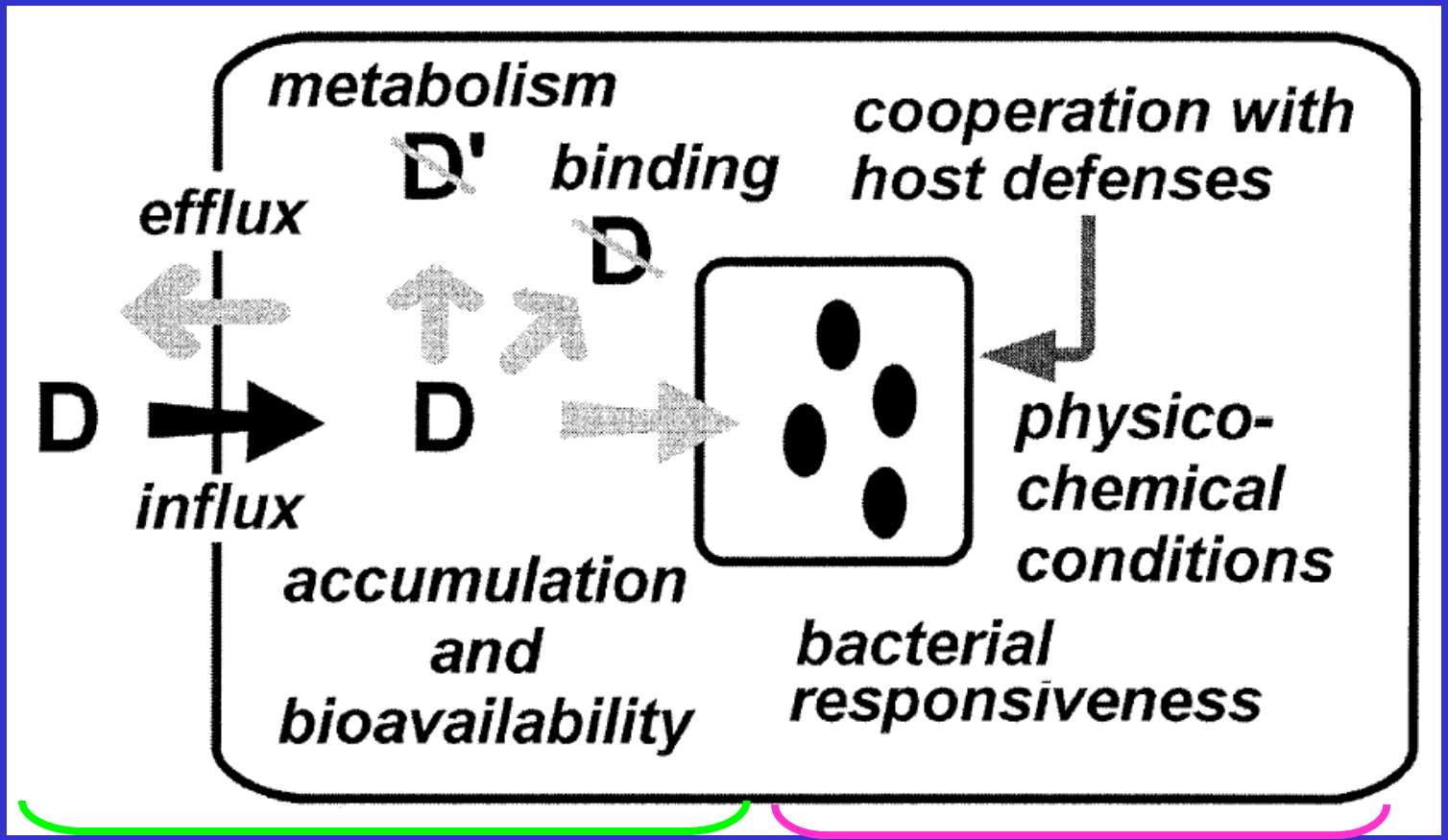
general concept



Craig CID (1998) 26:1-10

Antibiotic pharmacokinetics / pharmacodynamics

intracellularly



Carryn *et al*, Infect Dis Clin North Am. (2003) 17:615-34

S. aureus infections

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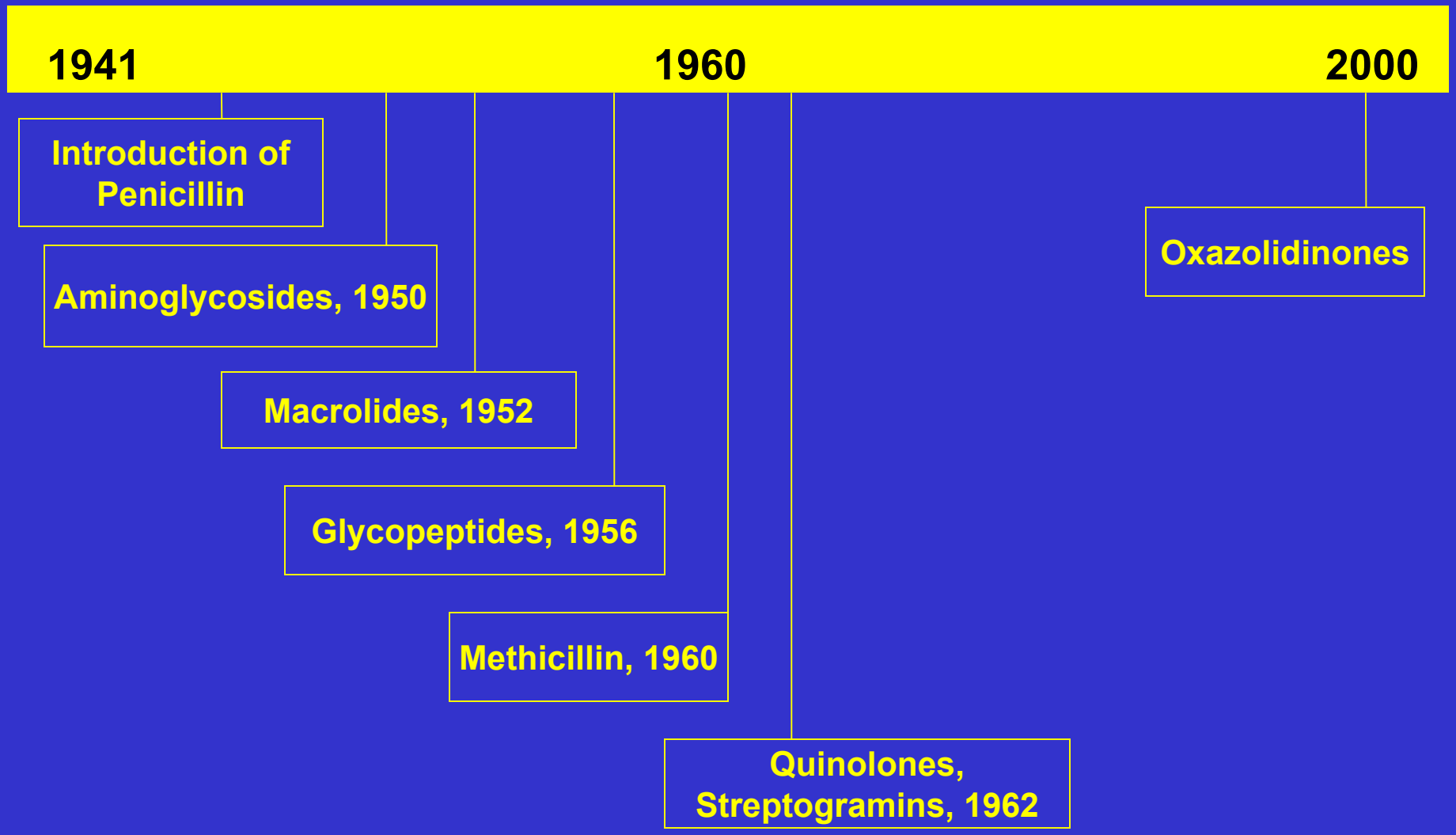
  → need of drugs acting on multiresistant strains

S. aureus resistance

The world first commercially available antibiotic appeared in 1941



S. aureus resistance

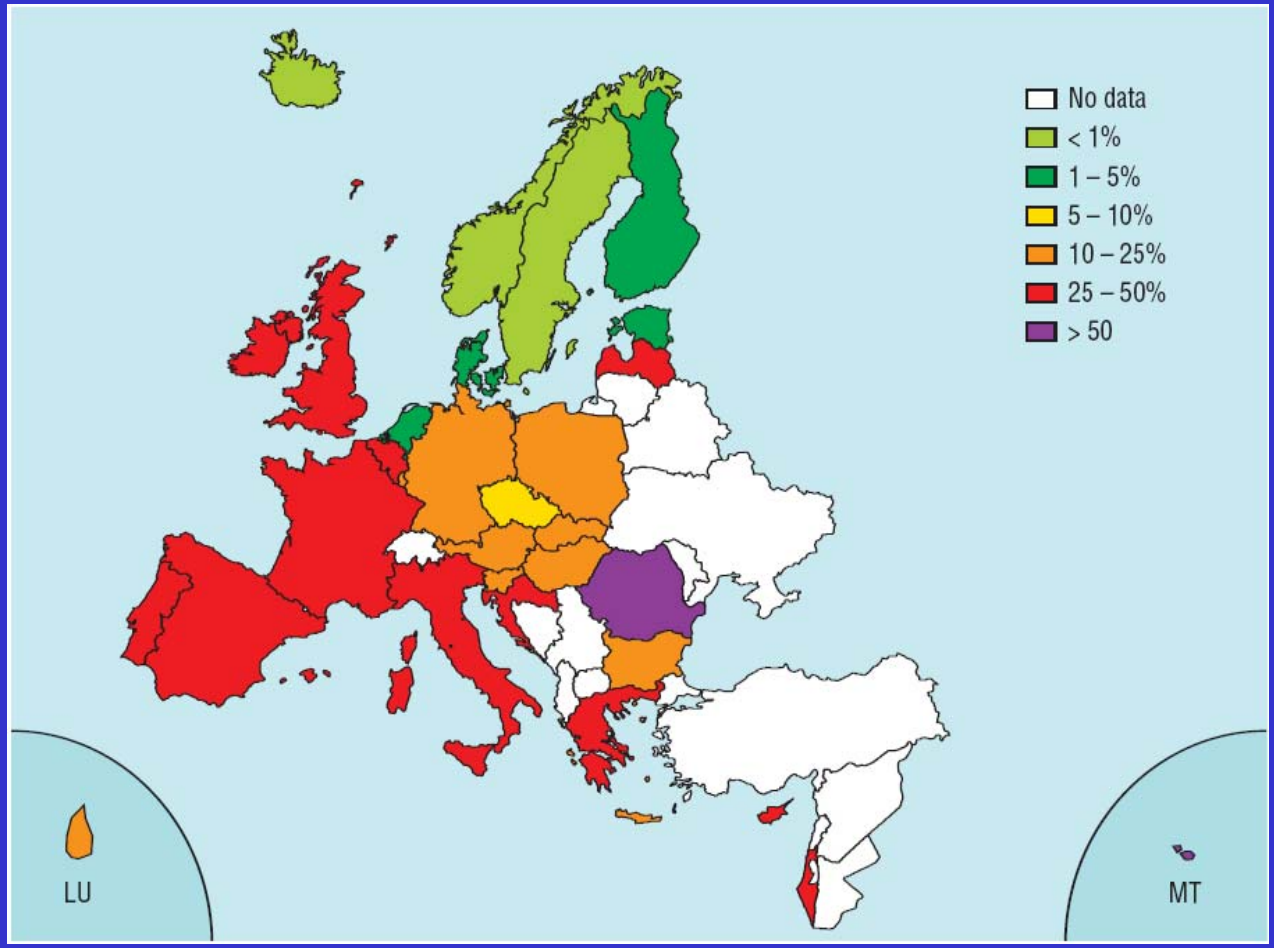
Most worrying resistance phenotypes having emerged over time

year	phenotype	first description
1960	HA-MRSA	England
1967	MDR HA-MRSA	Europe, Australia, Japan
1980	Genta-R MRSA	USA, Ireland, UK
1993	CA-MRSA	Australia
1997	VISA	Japan
2002	VRSA	USA

Grundmann *et al.*, Lancet (2006) 368:874-85

S. aureus resistance

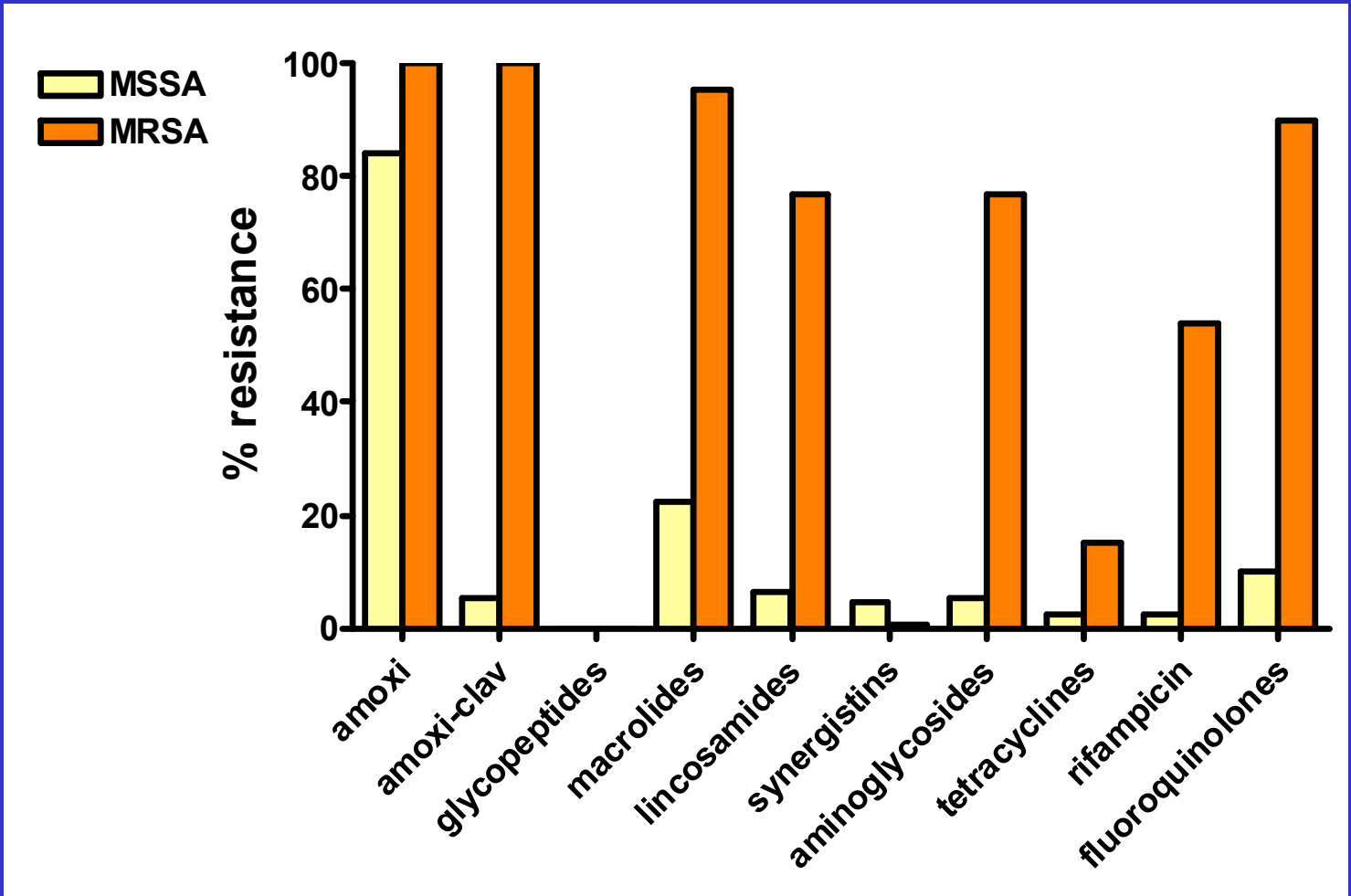
Percentage of MRSA resistance in Europe in 2004:
S. aureus proportion of invasive isolates MRSA in 2004



Data from the European antimicrobial resistant surveillance system, EARSS.

S. aureus resistance

Prevalence of resistance to other antibiotic classes



Fluit *et al.*, J Clin Microbiol (2001) 39: 3727-3732

S. aureus infections

All these infections are difficult to treat :

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  → selection of antibiotics based on pharmacokinetic / pharmacodynamics properties

- resistance to currently available antibiotics

  → need of drugs acting on multiresistant strains

Need for new antistaphylococcal agents

A lot of drugs in the pipeline …

recent and novel agents for *S. aureus*

recently brought on the Belgian market	on the market; not yet in Belgium	(late) stage of clinical development	investigational
moxifloxacin	synercid	telavancin	CS-023/PZ-601
linezolid	daptomycin	oritavancin	MX-2401
	tigecycline	dalbavancin	API-1252
		ceftobiprole	DK-619
		iclaprim	new oxazolidinones
		retapamulin	new ketolides
		WCK-771	…

What will be your choice ?

II. AIM OF THE STUDY

- recurrence / persistence

  in relation with the intracellular character of *S. aureus*

  → selection of antibiotics based on

  pharmacokinetic / pharmacodynamics properties

→ To develop an intracellular model allowing to compare the activity of antibiotics on a pharmacodynamic basis

- resistance to currently available antibiotics

  → need of drugs acting on multiresistant strains

→ To evaluate the cellular pharmacokinetics and the intracellular activity towards multiresistant strains of a new antibiotic in development

III. METHODOLOGY

Setting-up of the intracellular model

Method

1) opsonization of *S. aureus* with human serum

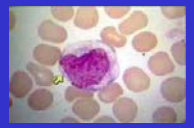
2) phagocytosis of the bacteria by THP-1 macrophages
(ratio 4 bacteria vs 1 macrophage)

3) elimination of extracellular *S. aureus*
(gentamicin 100 X MIC).
Rinse of infected macrophages (time-zero)

4) intracellularly infected macrophages,
ready to test antibiotic activity

5) maintenance of gentamicin at its MIC
during the whole incubation period for controls
to avoid extracellular contamination

Setting-up of the intracellular model



cell line ?

THP-1= many features of human monocytes/macrophages

Tsuchiya, Int. J. Cancer (1980) 26:171-176; Auwerx, Experientia (1991) 47:22-31

parameter	**characteristics**
morphological features	morphology resembling that of monocytic leukemia cells: - diameter 12-14 µm - moderate basophile cytoplasm - small azurophiles granules, few vacuoles - nuclei irregular in shape
cytochemical features	- positive for α-naphthyl butyrate esterase - negative reaction with periodic acid-Schiff and Sudan black B - diploid (46,XY) chromosome number
surface antigens and receptors	CD4, CD30, Factor X receptor, Factor Xa receptor, FcRI, FcRII, GM-CSF-receptor, HDL receptor, LDL receptor, TNF receptor, C3b receptor, LFA-1 receptor, Fibronectin receptor, Leu M1, Leu M2, Leu M3, HLA-DR antigens, scavengers receptors
secreted proteins	- hormones, cytokines: TNF-α, IL-1, IL-1b, CSF-1, M-CSF, erythrocyte differentiation factor, PDGF-1 and –2, thymosin B4, killer T cell activating factor, monocyte chemotactic factor - enzymes: lipoprotein lipase, lyzozyme - binding proteins: apoprotein E
functional features	- phagocytosis - production of lyzozyme - capacity to restore the lympocyte T mitogenic responsiveness

Setting-up of the intracellular model

bacterial strain ?

ATCC 25923

- clinical isolate from 1976
- fully susceptible
- widely used as a standard for microbiological testing of antibiotics

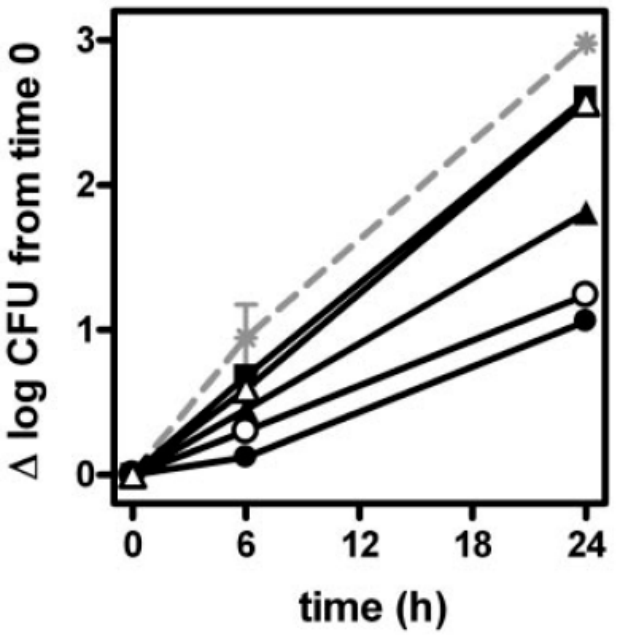
useful to compare all antibiotics towards a single strain, but may differ in virulence with current strains …

Setting-up of the intracellular model

antibiotics ?

- Beta-lactams: first choice for susceptible strains
- Aminoglycosides: highly bactericidal extracellularly
- Rifampicin: considered as first choice for intracellular infections
- Macrolides, quinolones: high cellular accumulation
- Glycopeptides: alternative for MRSA
- Linezolid: recently introduced, active on MRSA

S. aureus intracellular model



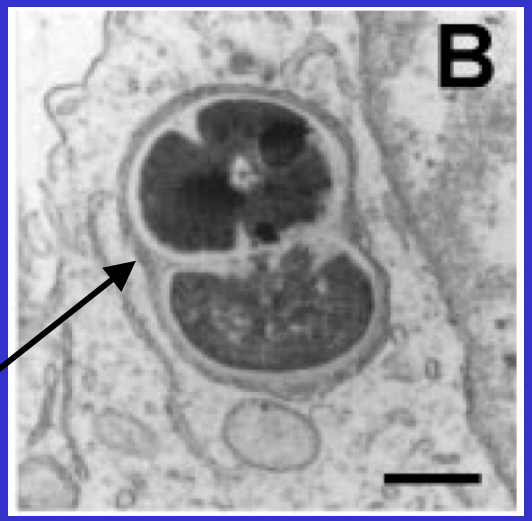
extracell. [GEN] *	extracell. contamin.**
0	17.2 ± 1.9
0.001	16.0 ± 1.0
0.01	0.013 ± 0.001
0.1	0.0026 ± 0.0003
1	< 0.001

* x MIC

** % of total bacteria in culture

gentamicin prevents extracellular contamination

Bacteria multiply in phagolysosomes where pH is acidic



membrane bond vacuole

General protocol

Assessment of antibiotic extracellular and intracellular activity

EXTRACELLULAR

10^6 CFU/ml

INTRACELLULAR

4 bacteria/cell

10^6 CFU/mg protein

Incubation over 24 h with antibiotics

Colony counting (CFU/ml)

Collection and lysis of cells (sonication)

CFU/protein content
Antibiotic concentration
(microbiological, radiochemical, fluorimetric assays)

IV. RESULTS

First goal of this thesis

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2006, p. 841–851 Vol. 50, No. 3
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.3.841–851.2006

Pharmacodynamic Evaluation of the Intracellular Activities of Antibiotics against *Staphylococcus aureus* in a Model of THP-1 Macrophages

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Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, B-1200 Brussels, Belgium

Received 17 August 2005/Returned for modification 30 October 2005/Accepted 8 December 2005

Human macrophages

Fully susceptible strain

Antibiotics accumulate to variable levels in THP-1 macrophages

TABLE 2. Cellular accumulation factor of antibiotics in THP-1 cells after 24 h of incubation at a fixed extracellular concentration

Antibiotic	Cellular accumulation	Extracellular concn (mg/liter)
Azithromycin	37.8 ± 1.3	5
Telithromycin	27.9 ± 1.3	2
Gentamicin	4.4 ± 0.1	250
Linezolid	0.5 ± 0.0	250
Penicillin V	1.2 ± 0.1	150
Nafcillin	2.6 ± 0.1	400
Ampicillin	1.0 ± 0.1	150
Oxacillin	4.0 ± 0.1	250
Teicoplanin	7.4 ± 0.2	150
Vancomycin	6.3 ± 0.1	100
Oritavancin	148.0 ± 12.0	25
Rifampin	17.6 ± 0.9	50
Ciprofloxacin	5.1 ± 0.1	4.3
Levofloxacin	7.0 ± 0.6	4
Garenoxacin	9.1 ± 0.3	4
Moxifloxacin	7.6 ± 0.3	4

High level:

- macrolides
- oritavancin
- rifampin

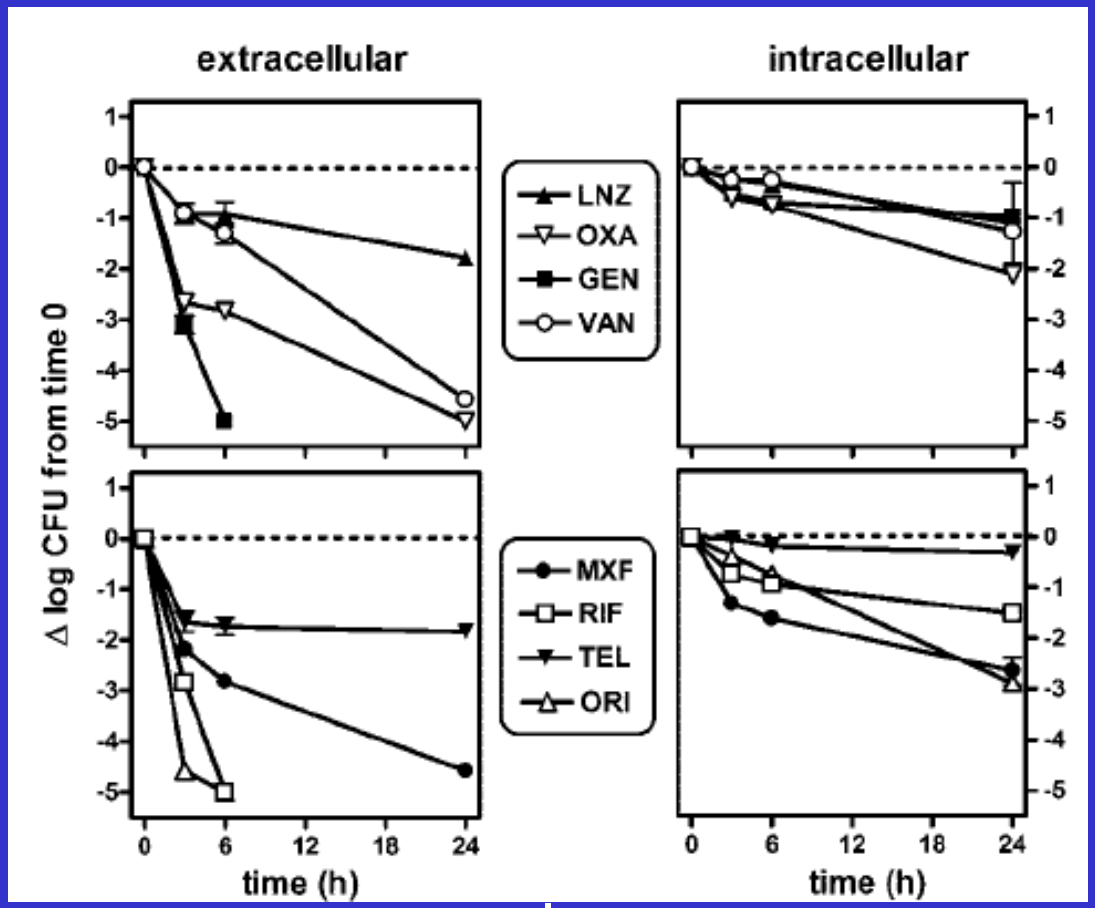
moderate level:

- aminoglycoside
- old glycopeptides
- quinolones

low level:

- linezolid
- β-lactams

influence of time on activity



low to moderate accumulation

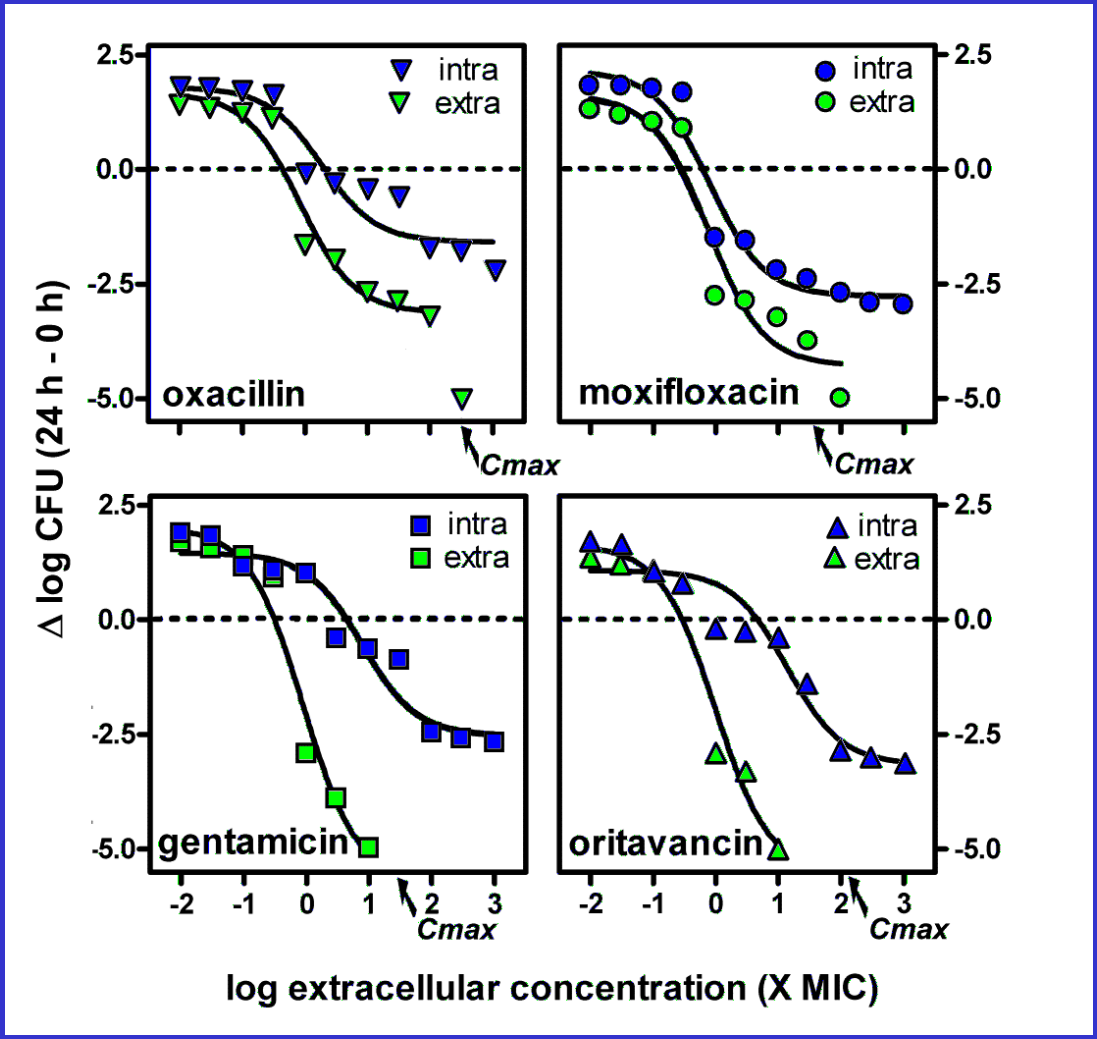
moderate to high accumulation

GEN, RIF, ORI
quickly cidal

OXA, MXF, ORI
slowly cidal

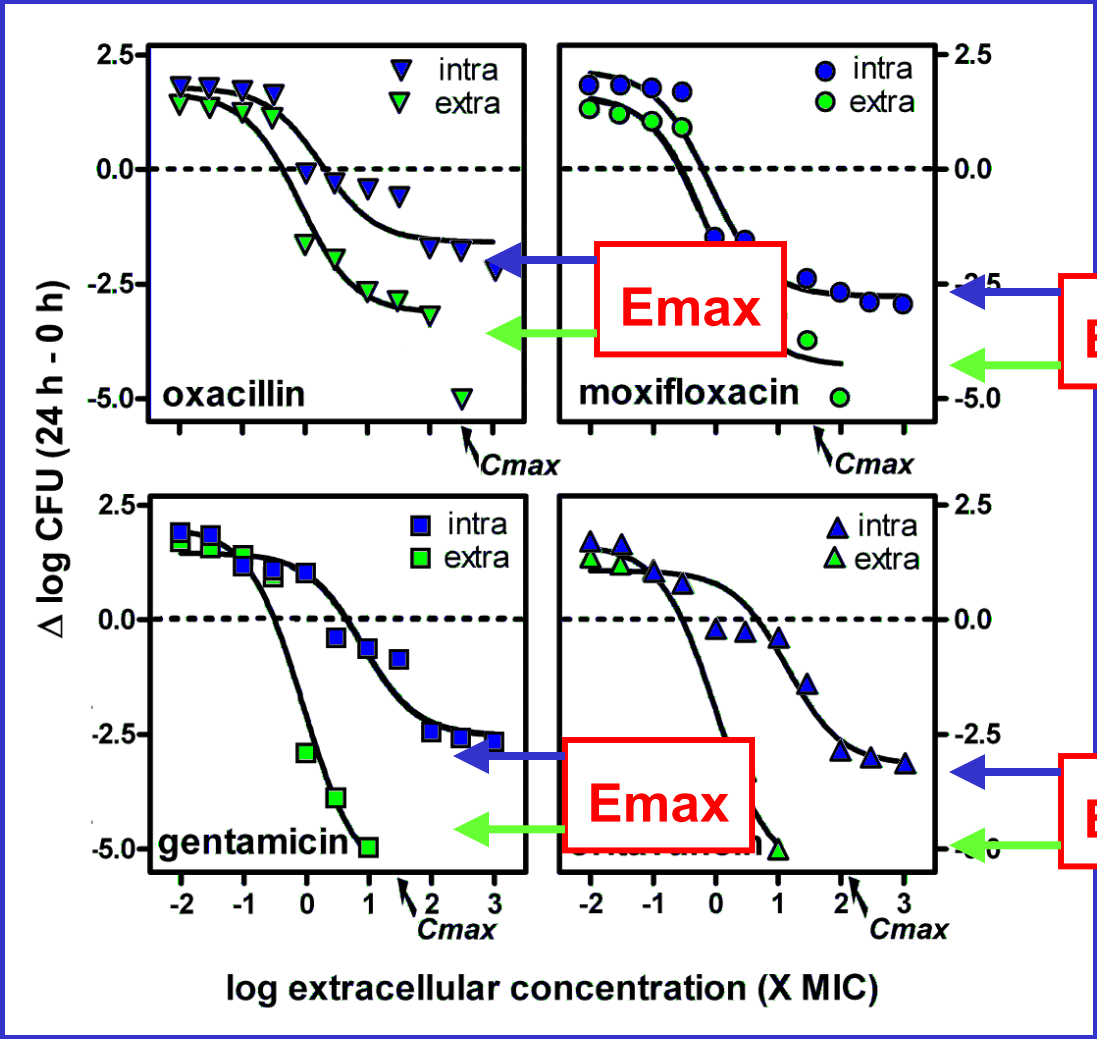
- **in all cases, intracellular activity << extracellular activity**
- **no direct relation between accumulation and intracellular activity**

concentration-effect relationships



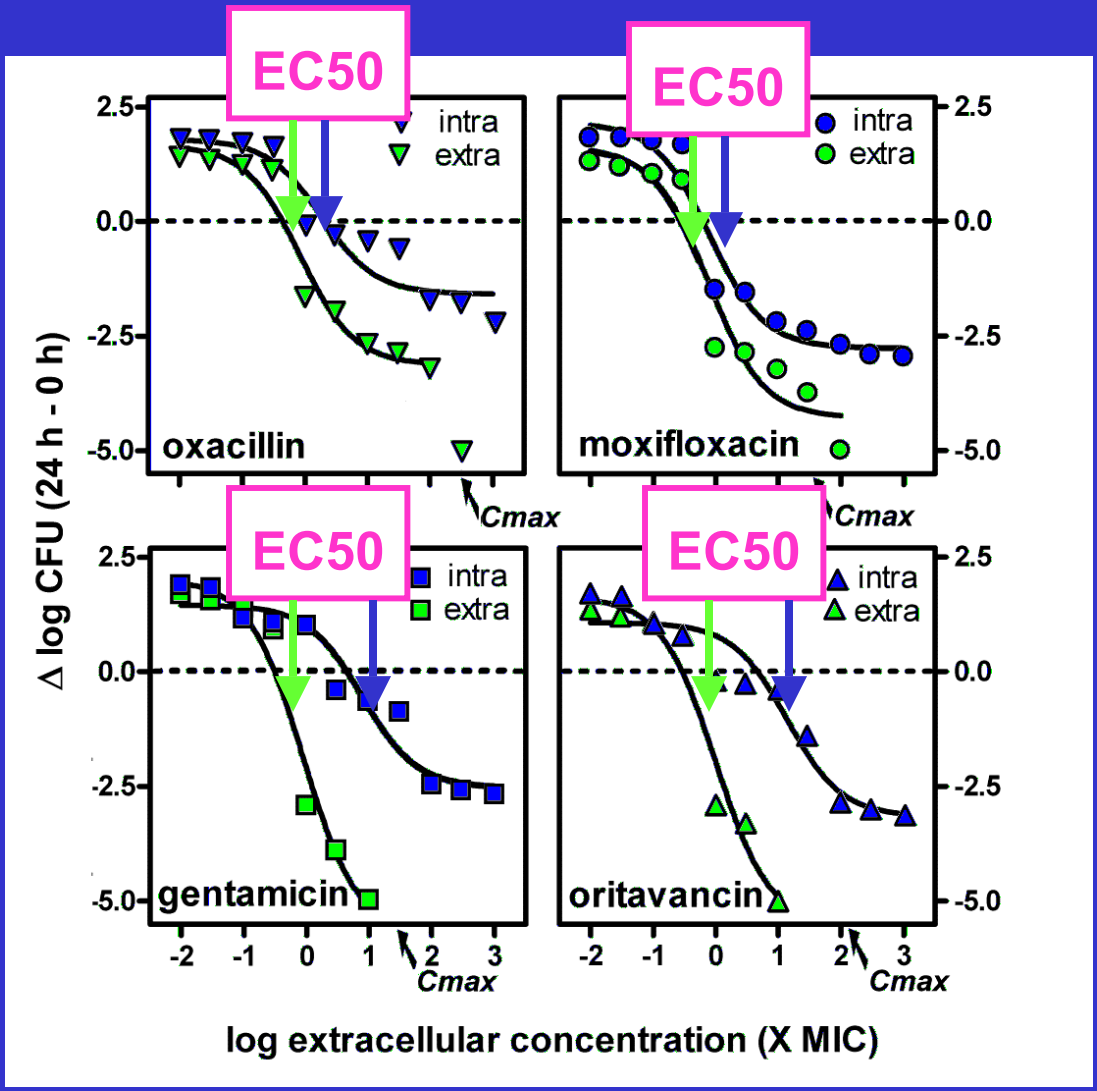
- sigmoidal relationships

concentration-effect relationships



- sigmoidal relationships
- Emax intra << Emax extra

concentration-effect relationships



- sigmoidal relationships
- Emax intra << Emax extra

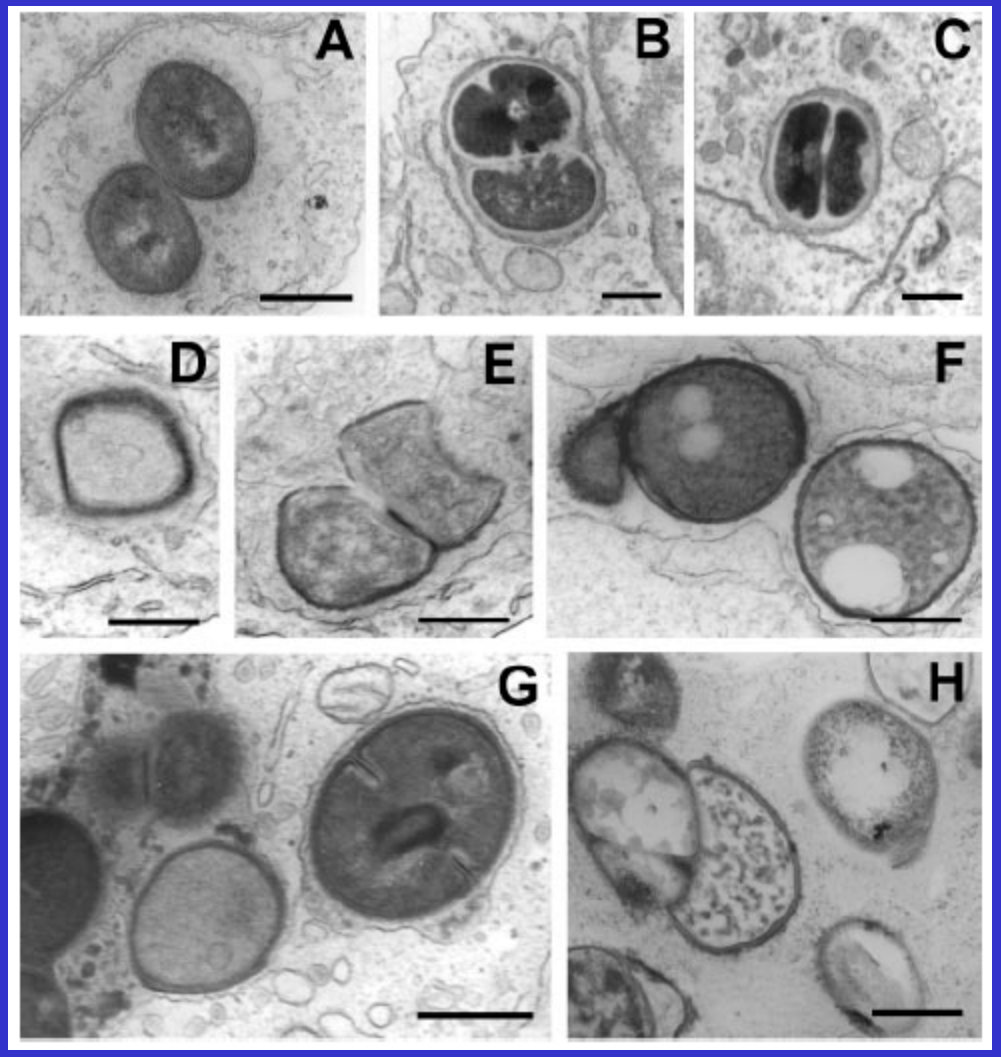
- EC_{50} extra = EC_{50} intra for OXA and MXF
- EC_{50} extra < EC_{50} intra for GEN and ORI

intracellular killing is visible !

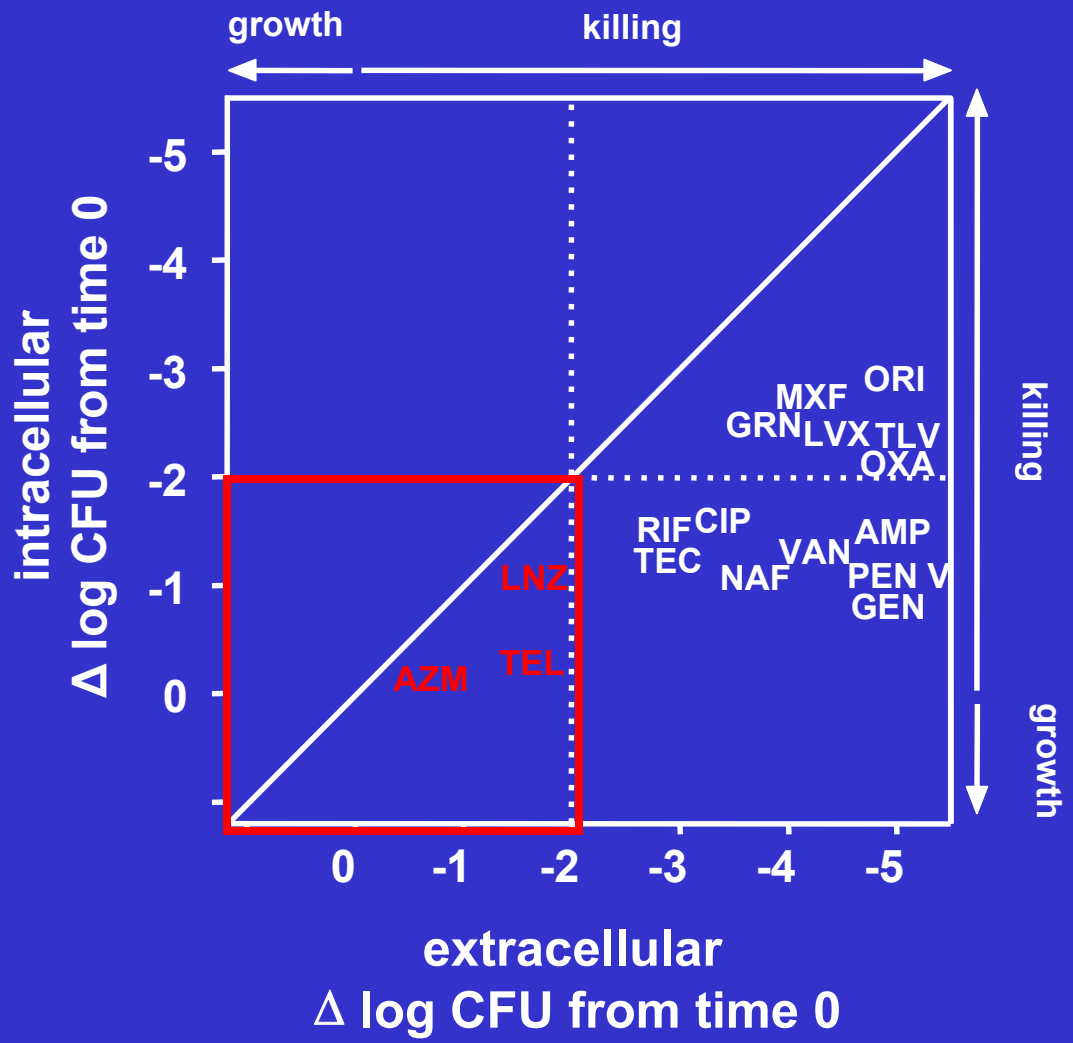
control

OXA

ORI

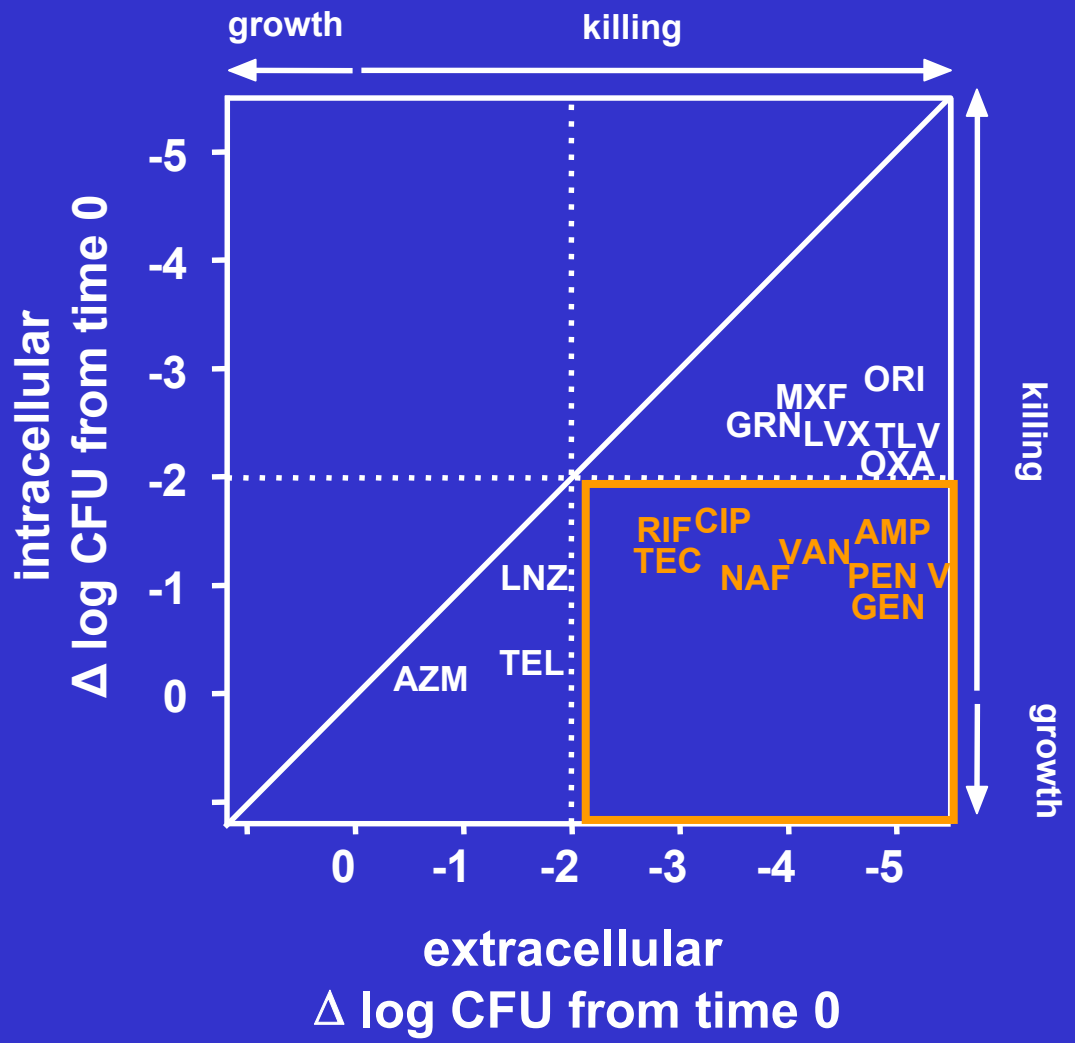


extracellular versus intracellular activity



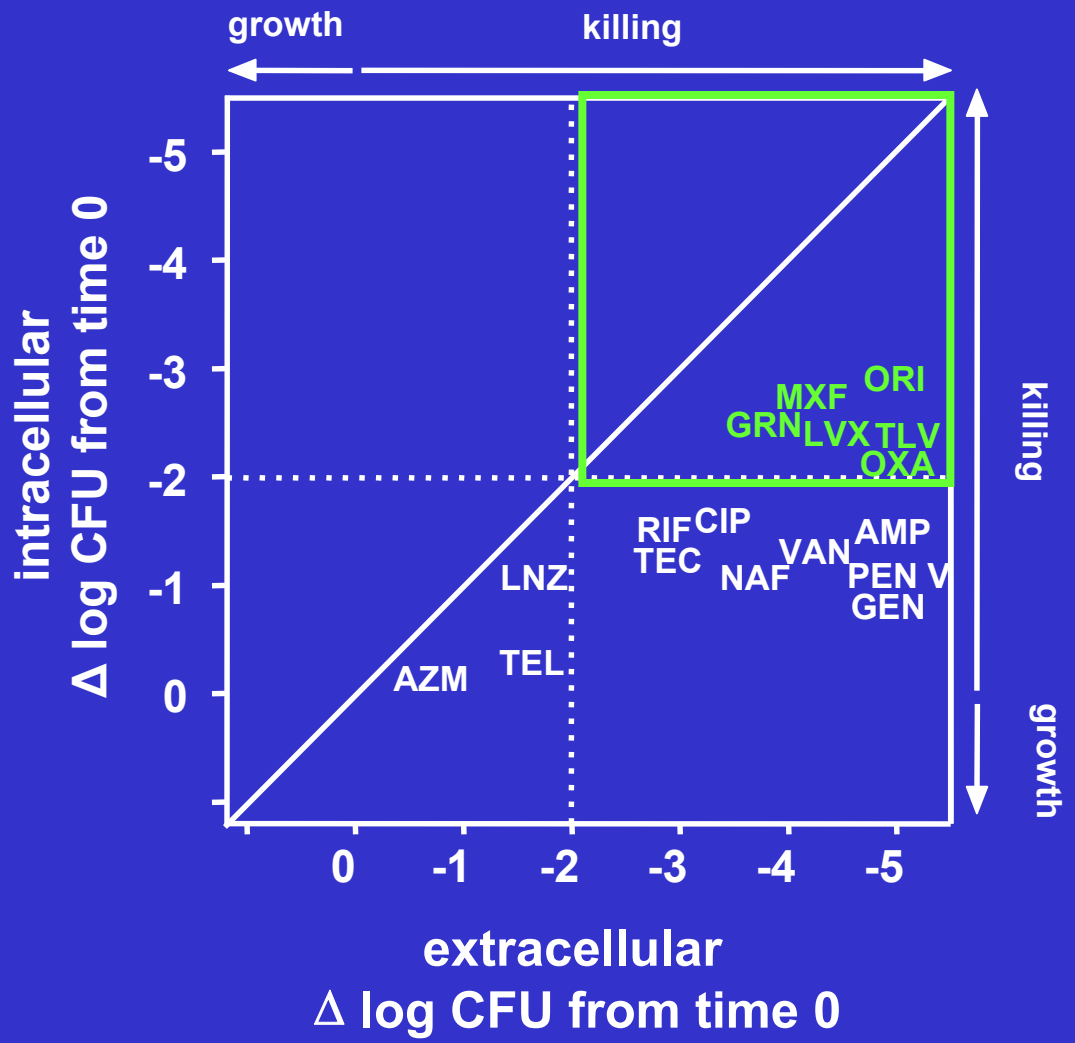
Poorly active against extra and intra *S. aureus*

extracellular versus intracellular activity



Active against extra *S. aureus*

extracellular versus intracellular activity



Best choice for activity against extra and intra *S. aureus*

conclusion

model of infection of human macrophages by *S. aureus* over 24 h allowing for the study of

- influence of time and concentration on antibiotic activity
- relation between activity and accumulation

intracellular activity << extracellular activity

- no correlation with level of accumulation
- impairing effect of acidic pH on some antibiotics

optimizing antibiotic efficacy

- choice of the drug (active extra and intracellularly)
- optimization of exposure (time and concentration)

Second goal of this thesis

Journal of Antimicrobial Chemotherapy (2006) **58**, 1177–1184
doi:10.1093/jac/dkl424
Advance Access publication 24 October 2006

JAC

Evaluation of the extracellular and intracellular activities (human THP-1 macrophages) of telavancin versus vancomycin against methicillin-susceptible, methicillin-resistant, vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus*

Maritza Barcia-Macay, Sandrine Lemaire, Marie-Paule Mingeot-Leclercq, Paul M. Tulkens and Françoise Van Bambeke*

Unité de Pharmacologie cellulaire et moléculaire, Université catholique de Louvain, B-1200 Brussels, Belgium

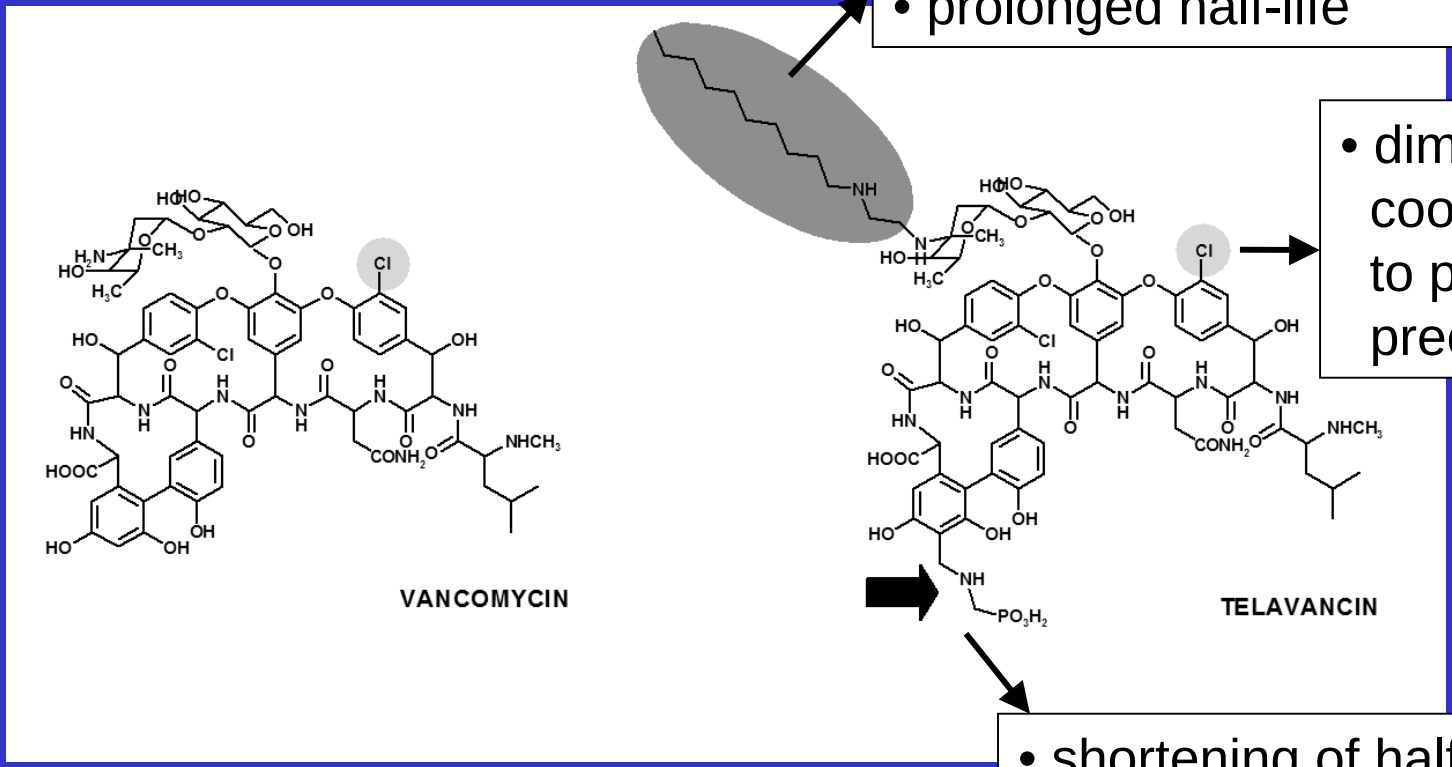
Received 26 July 2006; returned 29 August 2006; revised 15 September 2006; accepted 25 September 2006

New drug in development

Different phenotypes of resistance

Telavancin, a new glycopeptide

Hemi-synthetic derivative of vancomycin,
with new mode of action and new pharmacokinetic profile



- permeabilization of bacterial membrane
- prolonged half-life

- dimerization and cooperative binding to peptidoglycan precursors

- shortening of half-life

MIC and MBC against *S. aureus* with different resistance phenotypes

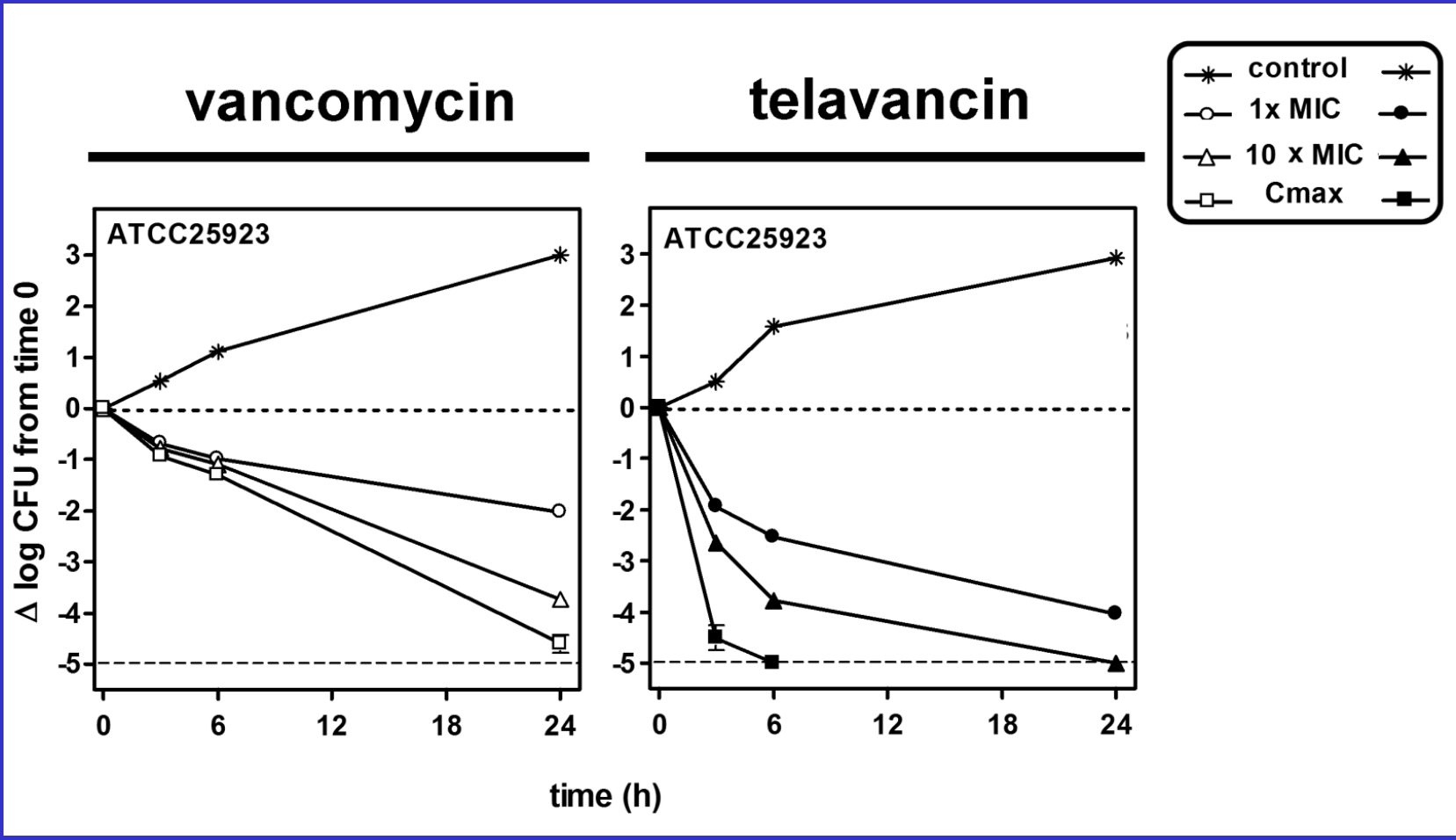
MIC and MBC of vancomycin and telavancin against the *S. aureus* strains used.

		vancomycin		telavancin	
phenotype	**strain**	**MIC**	**MBC**	**MIC**	**MBC**
MSSA	ATCC25923	1	1	0.5	0.5
	ATCC29213	1	1	0.5	0.5
MRSA	ATCC33591	2	4	0.5	1
	ATCC43300	2	2	0.5	0.5
VISA	NRS23	4	4	0.5	0.5
	NRS52	4	4	0.5	0.5
VRSA	VRS1	>128	>256	4	8
	VRS2	16	64	2	8

- more active than VAN against VISA and VRSA
- bactericidal against all strains

Influence of time on EXTRACELLULAR activity

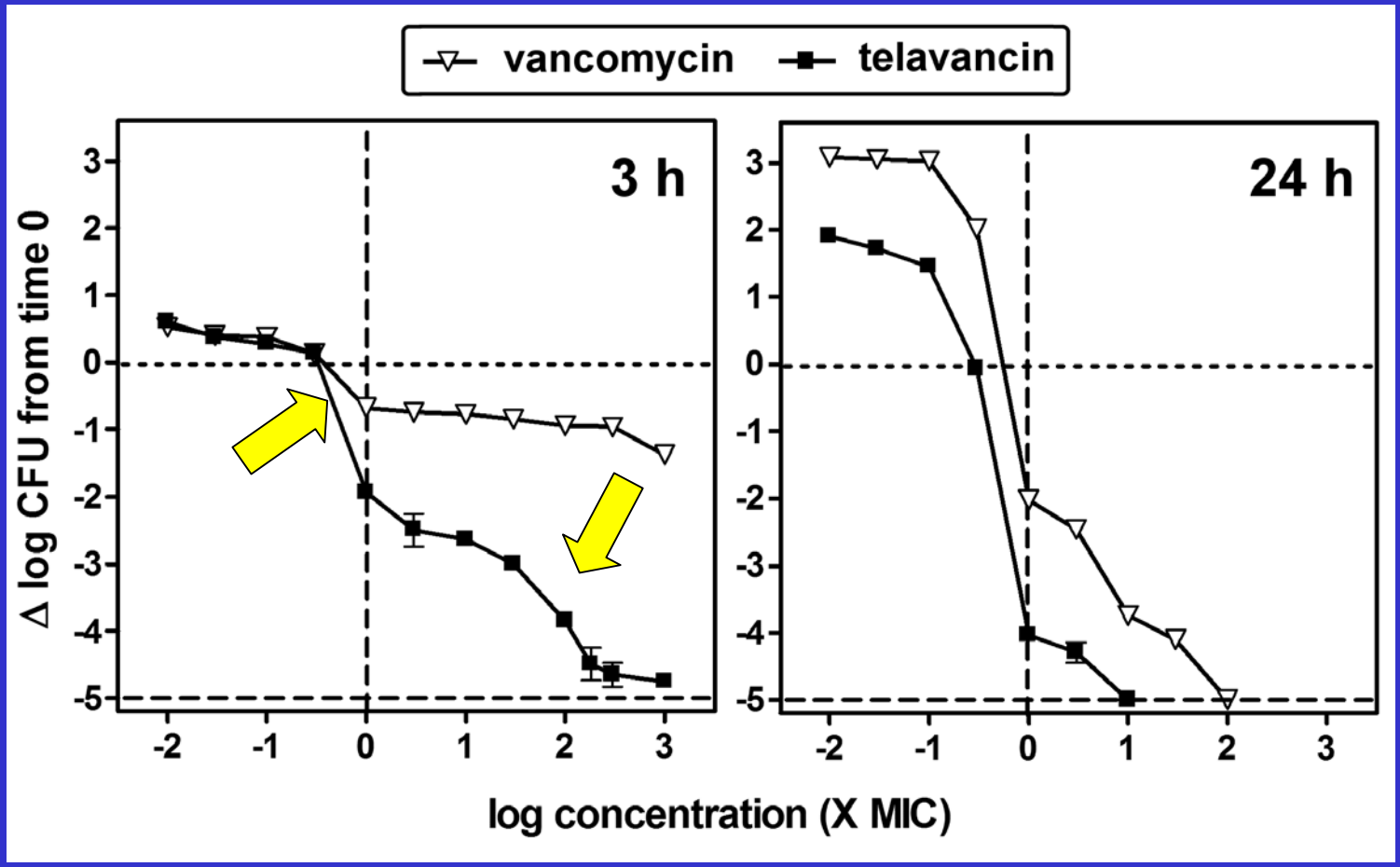
VAN vs TLV – MSSA ATCC 25923



Telavancin is more rapidly cidal than vancomycin

Influence of concentration on EXTRACELLULAR activity

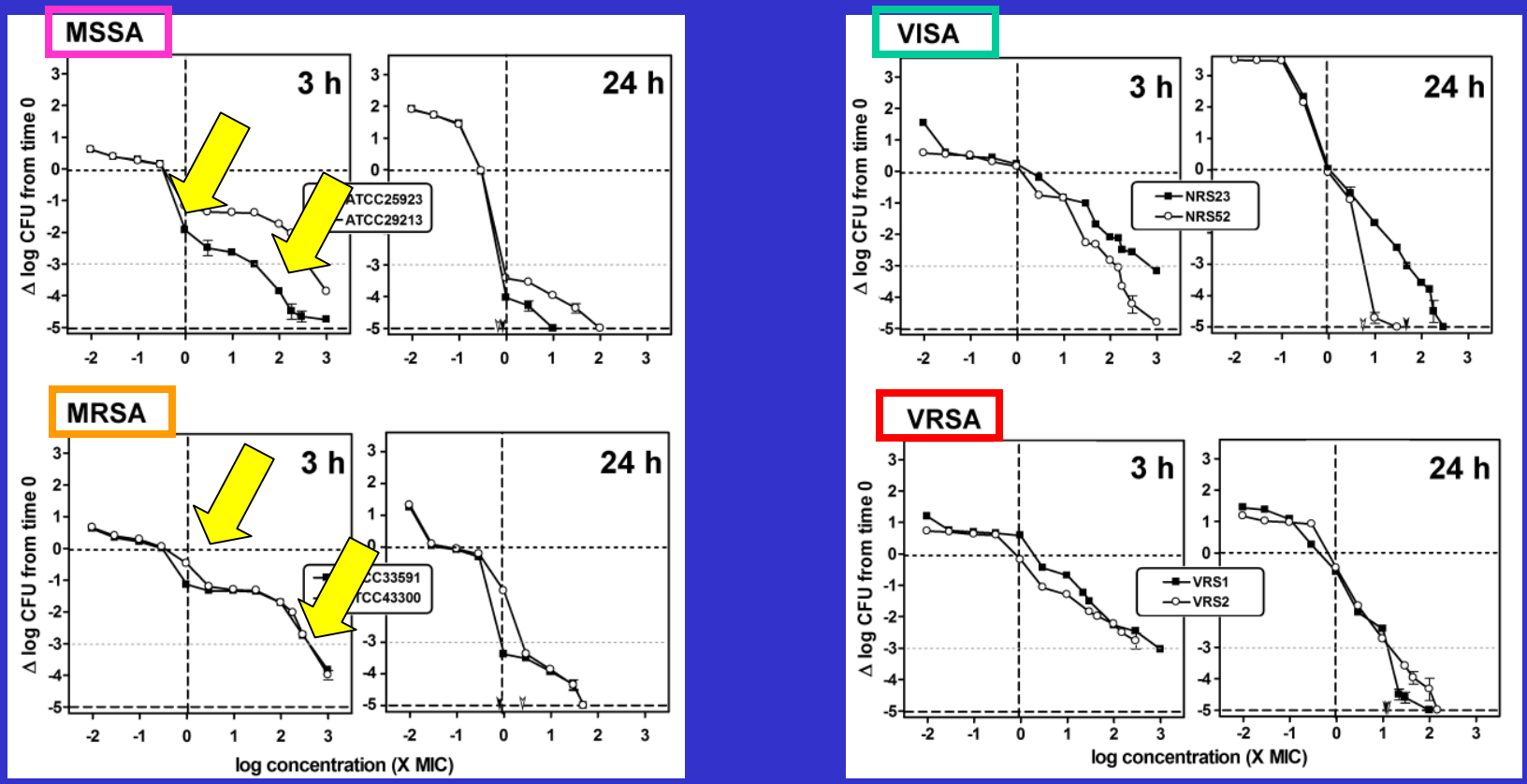
VAN vs TLV – MSSA ATCC 25923



- at 3 h, TEL shows bimodal conc-dependent effects
- at 24 h, both drugs are bactericidal at high concentrations

EXTRACELLULAR activity of telavancin: comparison of different strains

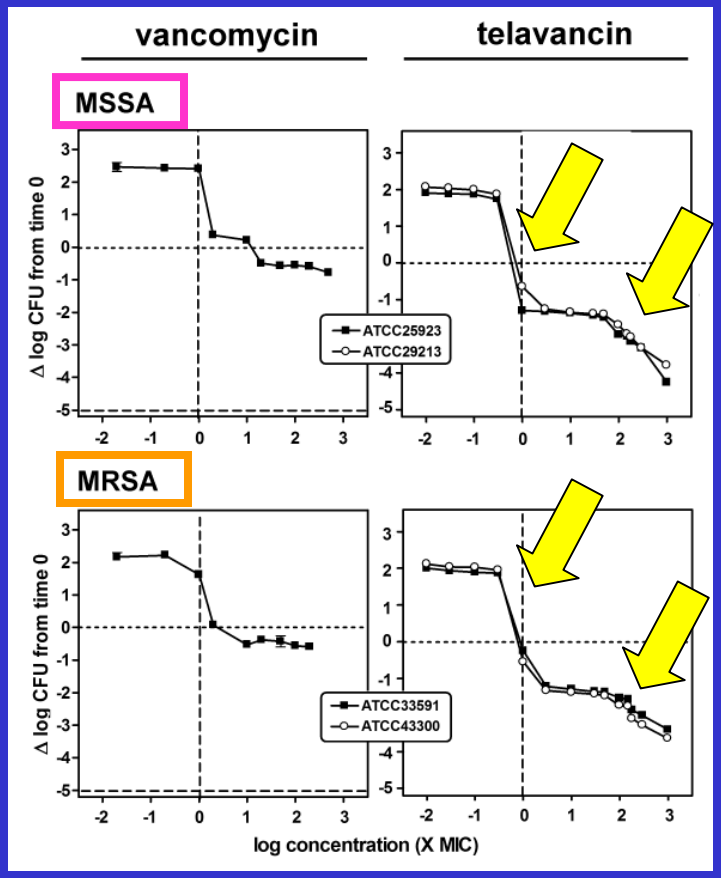
TLV versus MSSA, MRSA, VISA, VRSA

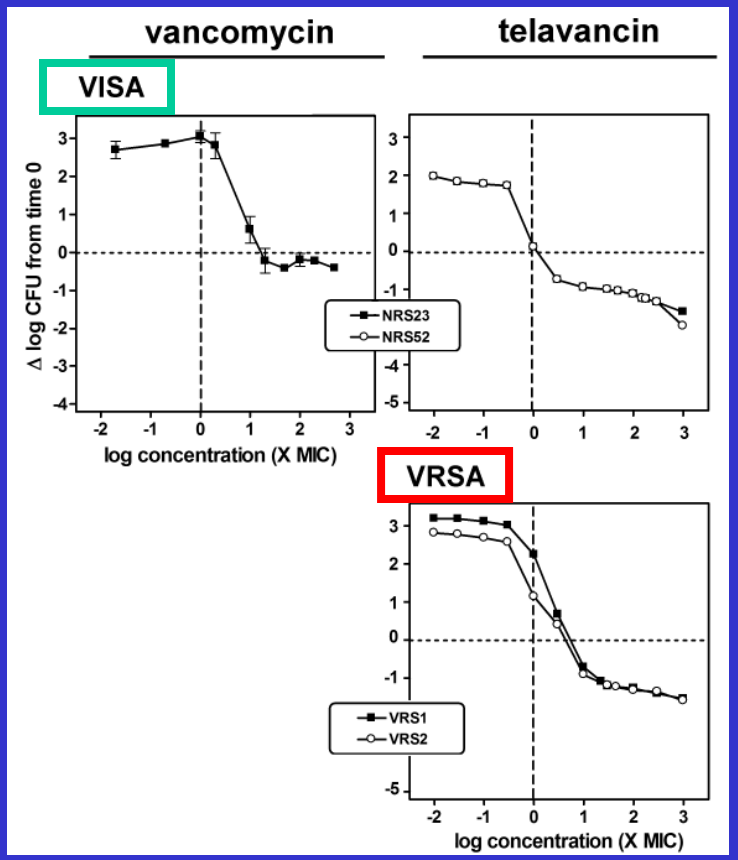


at 3 h, TEL shows bimodal conc-dependent effects towards MSSA and MRSA

INTRACELLULAR activity of vancomycin and telavancin towards different strains

VAN and TLV versus MSSA, MRSA, VISA, VRSA





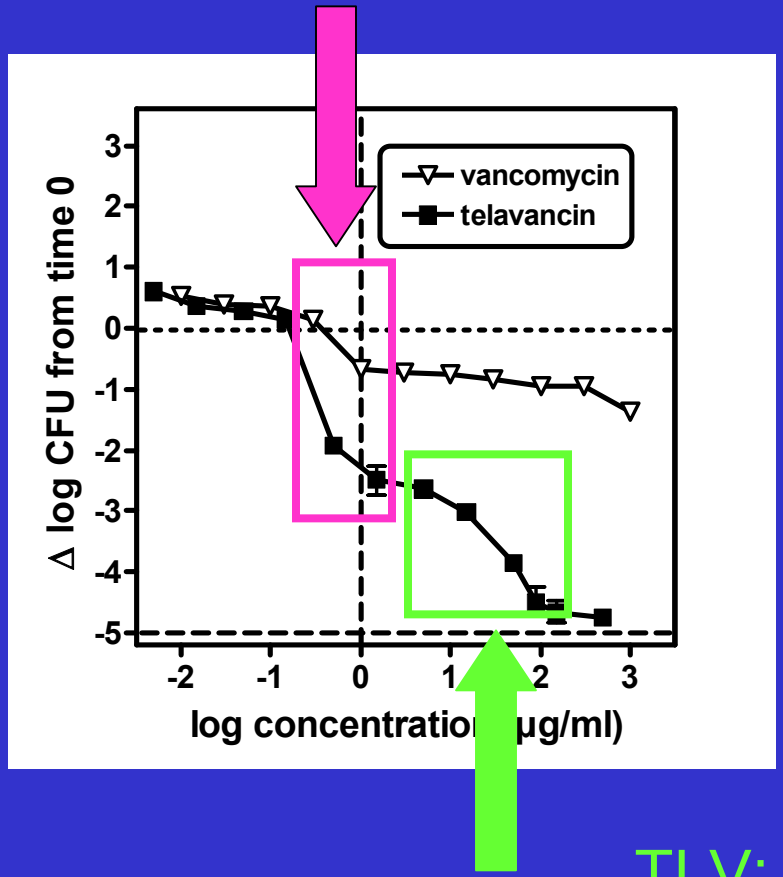
TEL shows bimodal conc-dependent effects towards MSSA / MRSA

VAN is only static intracellulalry

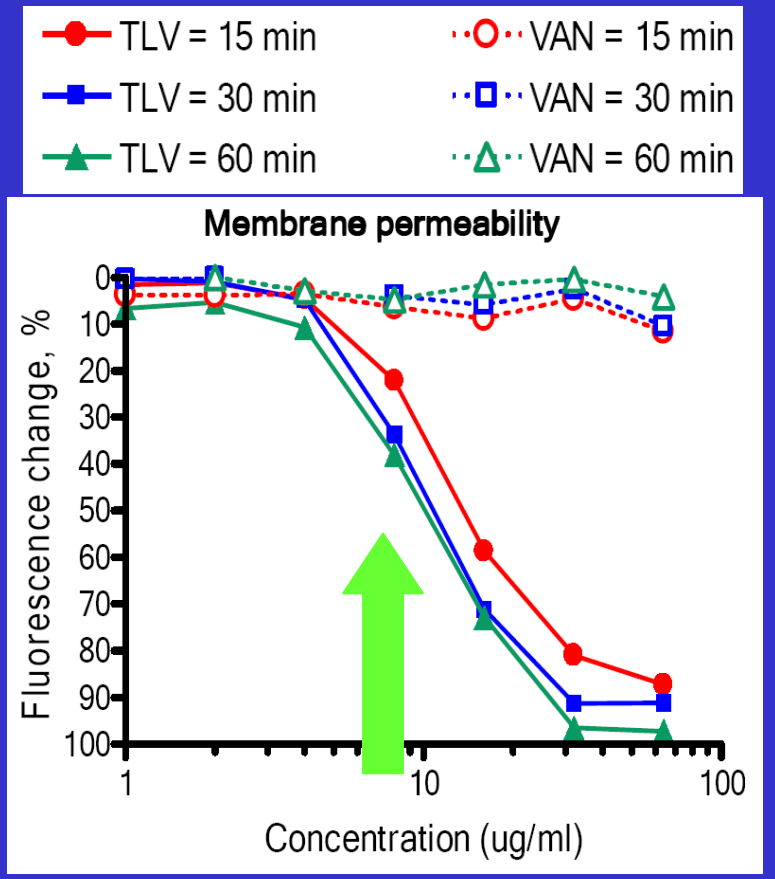
Why bimodal effects for telavancin ?

VAN and TLV: inhibition of peptidoglycan synthesis

In MSSA and MRSA, telavancin can exert multiple modes of action



TLV: membrane permeabilization



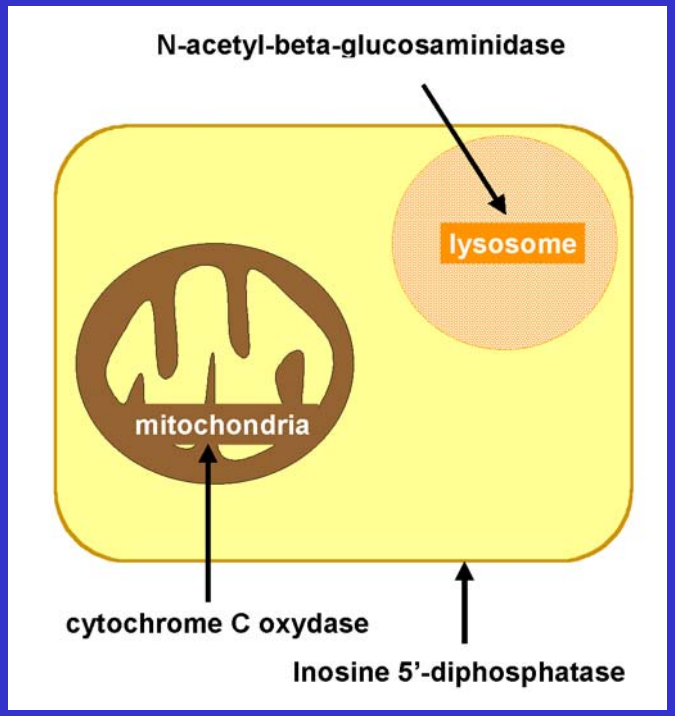
Higgins *et al.*, AAC (2004) 49:1127-34

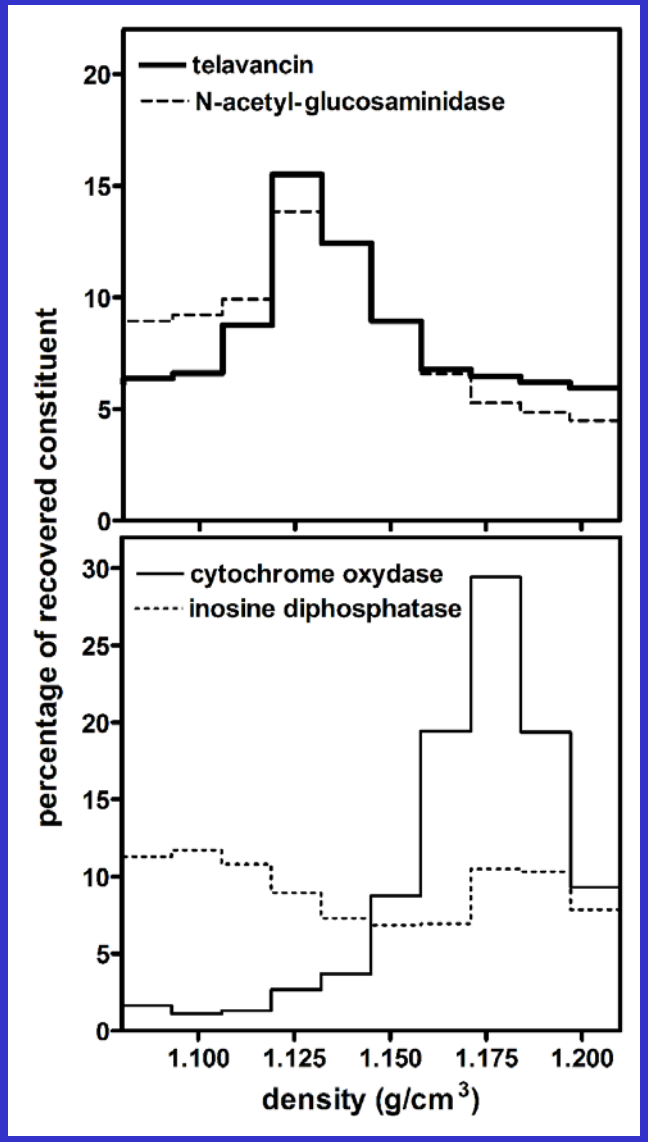
Telavancin
cellular pharmacokinetic data
rationalizing its intracellular activity

(studies with J774 macrophages)

subcellular distribution of telavancin

Same distribution
as a lysosomal enzyme





High concentration in the compartment where *S. aureus* sojourns !

conclusion

model of infection of human macrophages by *S. aureus* over 24 h applicable to multiresistant strains

vancomycin

- slowly bactericidal extracellularly (MSSA and MRSA)
- poorly or not active on VISA and VRSA
- static intracellularly

telavancin

- bactericidal extra- and intracellularly, including against resistant strains
- bimodal effect against MSSA and MRSA could be related to multiple modes of action
- high accumulation in the infected compartment

V. GENERAL CONCLUSION:

can we do better ?

Limitations of the model and perspectives for future work

Constant concentrations
(pharmacokinetic variations not taken into account):

- develop dynamic models

Protein binding
(free fraction is active and able to accumulate)

- develop in vivo models

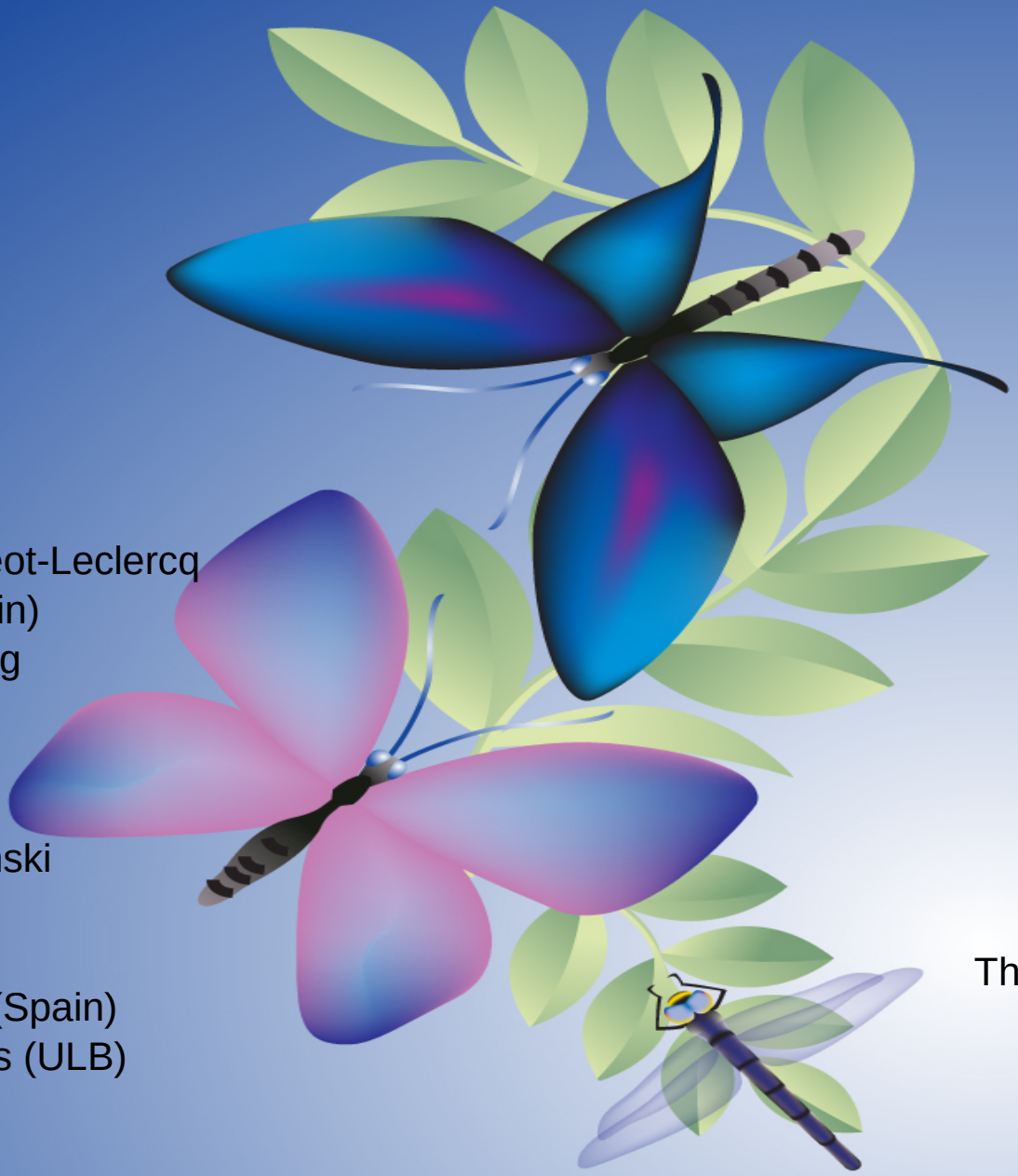
Phagocytic cells
(*S. aureus* also infects non phagocytic cells
where its fate may be different)

- develop models of infection in non-phagocytic cells

Testing of antibiotics alone
(combinations often used in the clinics to cope with resistance)

- testing of drug combinations

THANK YOU !



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