3rd International Conference Pathophysiology of Staphylococci
15 - 17 September 2016, Tübingen

Comparison of the intracellular and extracellular activities of approved and novel antistaphylococcal antibiotics using a pharmacodynamic model exploring full drug concentration-responses.

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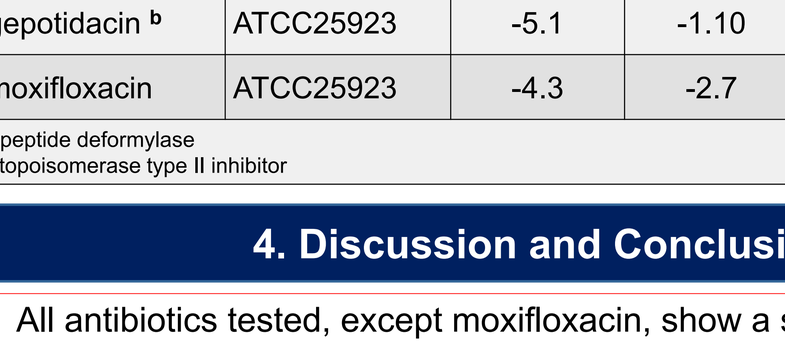
1. Background and Model

Infections caused *by Staphylococcus aureus* remain a therapeutic challenge, due, in part, to the ability of these organisms to survive in intracellular compartments of a variety of eukaryotic cells.

In this context, our laboratory has undertaken to systematically measure and compare the activities of a large array of antibiotics (approved and in development) from different pharmacological classes against the intracellular and extracellular forms of *S. aureus*, using a pharmacodynamic model allowing for a quantitative assessment of their concentration-dependent effects in these environments.

In brief, *S. aureus* isolates (susceptible to the antibiotics studied) are either (i) placed in broth (extracellular bacteria), or (ii) phagocytized by THP-1 monocytes (intracellular bacteria; non phagocytized bacteria are removed by washing and short term exposure of cells to gentamicin [100 x MIC]). Antibiotics are then added to the broth or to the monocytes culture medium for 24 h at concentrations ranging typically from 0.01 to 100 x the MIC to obtain full concentration-dependent responses and to calculate two pertinent pharmacodynamic parameters based on Hill's equation [sigmoidal function].

The figure shows a typical example of such a response for extracellular and intracellular forms of different *S. aureus* strains to an experimental antibiotic (peptide deformylase) and the parameters analyzed using different strains (compared at equipotent concentrations).



➢ **C_s (relative potency)**:
extracellular concentration resulting in no apparent bacterial growth from CFU at 0h (CFU at 0h = post-phagocytosis inoculum).

➢ **E_{max} (maximal relative efficacy)**:
CFU change (in log$_{10}$ units) at 24h from the post-phagocytosis inoculum as extrapolated for an infinitely large antibiotic concentration

See also: **In vitro Models for the Study of the Intracellular Activity of Antibiotics,** In "Bacterial Persistence", Molecular Biology Laboratory Protocols Series, J. Michiels and M. Fauvart, ed. 2016, p 147-157 Humana Press (Springer) (ebook) DOI: 10.1007/978-1-4939-2854-5

3. Pharmacodynamic parameters (from Hill's equation)

antibiotic	strain	E_{max} (Δ log$_{10}$ CFU)		C_s (multiple of MIC)	
		broth	intracellular	broth	intracellular
ceftaroline	ATCC33591	-5.3	-0.56	0.5	0.8
	multiple strains	-5.1	-0.58	0.7	3.7
daptomycin	ATCC33591	-5.1	-0.99	0.15	1.4
GSK1322322 [a]	ATCC25923	-5.5	-0.48	1.9	3.8
gepotidacin [b]	ATCC25923	-5.1	-1.10	1.3	1.1
moxifloxacin	ATCC25923	-4.3	-2.7	0.3	0.6

[a] peptide deformylase
[b] topoisomerase type II inhibitor

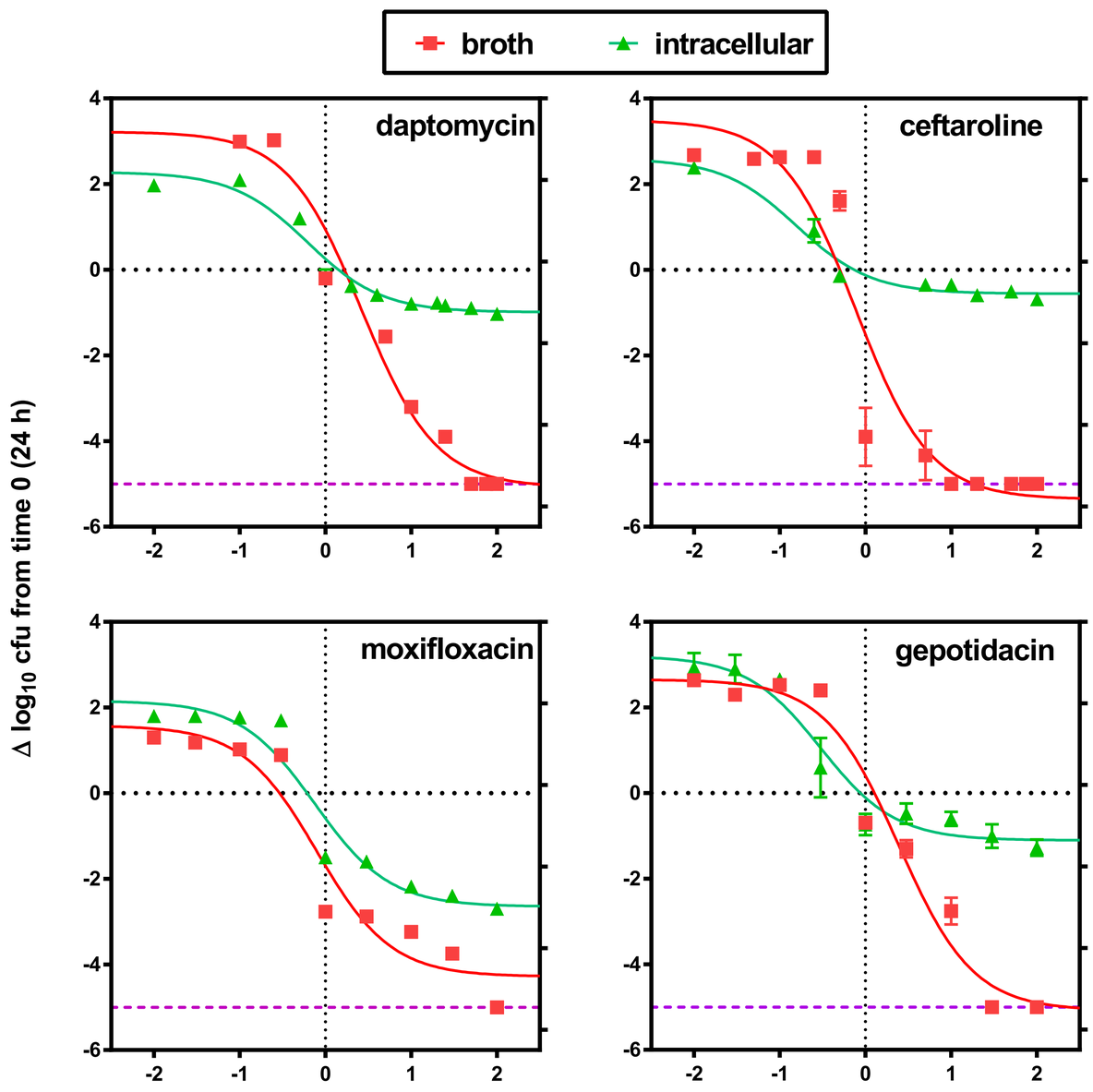
4. Discussion and Conclusions

- All antibiotics tested, except moxifloxacin, show a sharp decrease of their intracellular maximal relative efficacy (E_{max}) compared to broth, and are not bactericidal in cells (less than a 3 log$_{10}$ CFU decrease compared to the post-phagocytosis inoculum) even though all are bactericidal in broth (3 log$_{10}$ CFU or more);
- As E_{max} is the value extrapolated for an infinitely large extracellular concentration of antibiotics, this loss of efficacy cannot be due to a simple global lack of diffusion and accumulation of the antibiotics.
- Conversely, all antibiotics tested show similar relative potencies (C_s), in the range of their MIC in broth. This shows that intracellular bacteria are as susceptible as those in broth, indicating that the antibiotics tested are able to penetrate cells and reach their target (at least in part).
- We showed (not illustrated here) that intracellular *S. aureus* that remained in cells (i) are not Small Colony Variants (except in rare cases), and (ii) show an unaltered MIC when retested in broth.

➔ **Intracellular bacteria, for a low but significant proportion of the original inoculum, are probably in a state of non-responsiveness to antibiotics.**

2. Observations (equipotent concentrations [MIC normalized graphs)

1. data with ATCC 25923



Data sources: Barcia-Macay *et al*. AAC 2006;50:841-51 | Melard *et al.* JAC 2013;68: 648-58 | Peyrusson *et al*. AAC 2015;59:5747-5760 | Peyrusson *et al.* 55th ICAAC & 25th ICC 2015; Poster no. A029

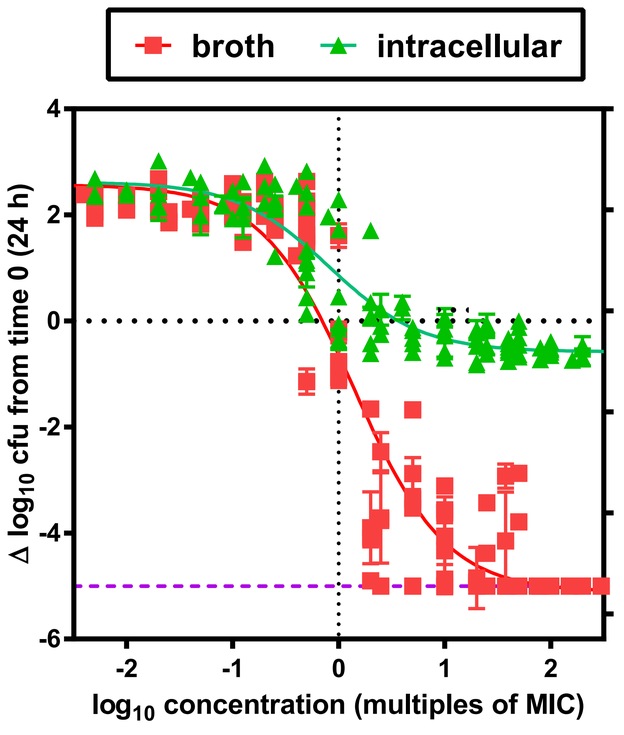
Maximal relative activity (E_{max})

- Extracellular bacteria (broth)
  For all antibiotics shown here, a marked concentration-dependent bactericidal activity was noted with a reduction of the inoculum reaching or close to the lowest detection level (-5 log$_{10}$ CFU)
- Intracellular bacteria
  For all antibiotics, activity is also concentration-dependent but the decrease of CFU is much lower (less negative E_{max}) than for activity in broth except for moxifloxacin.

Relative Potency (C_s)

The relative potencies of each antibiotic are roughly similar for extracellular (broth) and intracellular bacteria, and correspond essentially to the MIC in broth (0 on the abscissa [log scale])

2. data with multiple strains and ceftaroline (as an example)



Data sources: Melard *et al.* JAC 2013;68: 648-58

- Strains with ceftaroline MICs ranging from 0.125 to 2 mg/L and with various susceptibility/resistance phenotypes to other antistaphylococcal antibiotics

  (0.125 mg/L: ATCC 25923 [MSSA]; 0.25 mg/L: 34843 [MSSA]; 0.5 mg/L: ATCC 33591 [MRSA], SA 555 [MRSA/VISA], SA 19834 (MRSA); 1 mg/L: NRS18 [MRSA/VISA], 35165 [MRSA]; 2 mg/L: 48046 [MRSA], CM05 [MRSA/LZDR], 062-13101 A [MRSA], 062-13091 A [MRSA])
- Differences in E_{max} between broth and intracellular bacteria are observed together with C_s systematically close to MIC in broth (0 on the abscissa log scale) whatever the resistance phenotype of the strains examined.

This poster will be available after the meeting at http://www.facm.ucl.ac.be/posters.htm