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Antibiotic Modulation of Kupffer Cell Eradication of Intracellular *Staphylococcus aureus* Reservoirs in Bloodstream Infection

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Introduction

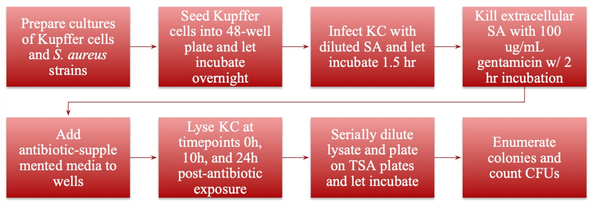
- *Staphylococcus aureus* (SA) is a major human pathogen that is the leading cause of bloodstream infections and sepsis[1]
- Kupffer cells are phagocytic tissue-resident macrophages which reside within the liver, the sentinel organ for clearing bacteria from the bloodstream
- Over 90% of *S. aureus* strains produce the virulence factor alpha hemolysin toxin (Hla) that binds to the host cell receptors and negatively affects the cells, which may explain the persistence of ~10% of SA surviving intracellularly despite receiving antibiotic treatment[2]
- As past studies have shown that antibiotic activities were altered when exposed to intracellular conditions, it may be critical to optimize antibiotic treatments against SA that reside within the low pH, intracellular environment[3,4]

Methods

Determine the minimum inhibitory concentrations of antistaphylococcal antibiotics at extracellular and intracellular pH

1. Prepare pH-adjusted CAMHB (pH 7 and 5)
2. Add serially-diluted antibiotic into pH-adjusted broth to get a range of concentration
3. Prepare bacterial culture of S. aureus strains (WT, Deletion, HH 35, HH 37) and normalize
4. Add inoculum to the microplate with pH-adjusted CAMHB and observe growth following incubation at 37°C for 16-20 hours
5. Compare and contrast minimum inhibitory concentrations (MICs)

Determine the ability of antistaphylococcal antibiotics to eradicate intracellular SA within Kupffer cells and MH-s cells



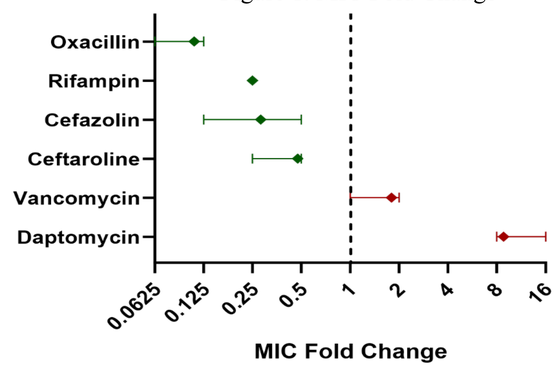
Results

Variable Antimicrobial Potency in Lysosome-mimicking Acidic pH 5

Table 1. MIC Comparison at Different pH

Strain ID	Antibiotic											
	Vancomycin		Daptomycin		Ceftaroline		Rifampin		Oxacillin		Cefazolin	
	pH 7.4	pH 5	pH 7.4	pH 5	pH 7.4	pH 5	pH 7.4	pH 5	pH 7.4	pH 5	pH 7.4	pH 5
ATCC 29213	1	2	1	8	0.25	0.125	0.0156	0.0039	0.25	0.0312	0.25	0.125
USA300	1	2	1	8	0.25	0.125	0.0156	0.0039	-	-	-	-
HH35	1	2	1	8	0.25	0.125	0.0156	0.0039	-	-	-	-
HH70	2	2	1	8	0.5	0.25	0.0156	0.0039	0.5	0.062	0.5	0.125
LAC82	1	2	2	16	0.5	0.25	0.0156	0.0039	-	-	-	-
HH131	2	2	1	8	0.5	0.25	0.0156	0.0039	-	-	-	-
HH92	1	2	1	8	0.25	0.125	0.0156	0.0039	0.5	0.0312	0.5	0.062
LAC164	1	2	1	8	0.5	0.25	0.0156	0.0039	0.5	0.062	0.25	0.062
HH37	2	4	1	16	0.25	0.125	0.0156	0.0039	-	-	-	-

Figure 1. MIC Fold Change



Differential Antibiotic Intracellular Killing of SA in Kupffer Cells[4]

Table 2. Characteristics of Strains Used for Kupffer cells

Strain ID	Patient Age, Sex	Duration of bacteremia (Days)	Outcome	Resistance type	MLST	Spa type	SCCmec type
HH35	70 F	17	Persistent, Died	MRSA	ST97	t267	IV (2B)
HH70	60 F	11	Persistent, Died	MSSA	ST72	t148	I (1B)
LAC82	56 M	17	Persistent, Survived	MRSA	ST8	t955	IV (2B)
HH131	47 M	7	Persistent, Survived	MRSA	ST8	t008	IV (2B)
HH92	66 M	7	Persistent, Survived	MSSA	ST30	t338	I (1B)
LAC164	34 F	1	Resolving, Survived	MSSA	ST188	t189	V (5C2)
HH37	66 M	1	Resolving, Survived	MRSA	ST5	t242	II (2A)

Figure 2. *S. aureus* survival inside Kupffer cells across all 7 strains

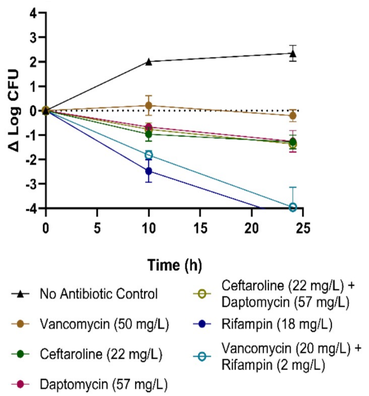
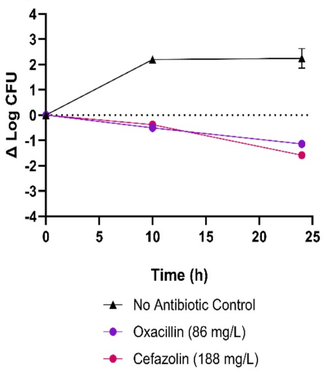
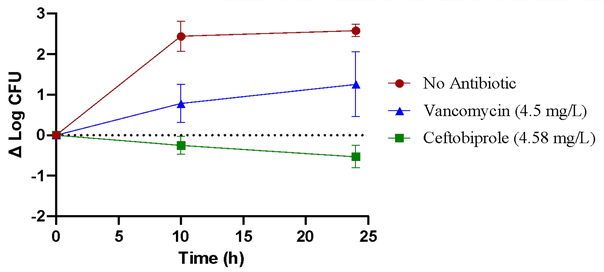


Figure 3. *S. aureus* survival inside Kupffer cells across 3 MSSA strains



Different Antibiotic Intracellular Killing of SA in MH-S cells[5]



Figures 4. *S. aureus* survival inside MH-S cells for 6 clinical pneumonia-causing SA strains at concentrations attainable in the pulmonary epithelial lining fluid for vancomycin and ceftobiprole over 24 hours

Table 3. Characteristics of Strains Used for MH-S cells

Strain ID	Patient Age, Sex	Outcome	Ventilation Status	ICU Admit	Vasopressor Used
CK013	75 M	Died	Required	Yes	Yes
NB233	82 F	Died	Required	Yes	Yes
CK206	91 M	Survived	Not required	No	No
NB177	86 M	Survived	Not required	No	No
NB178	91 F	Survived	Not required	No	No
NB211	52 M	Survived	Not required	No	No

Conclusions

- Relative increase in intracellular SA eradication seen by some of the antibiotics may be due to its increased anti-staphylococcal action in acidic environment
- Vancomycin could not elicit a log reduction in intracellular CFU levels when compared to initial intracellular CFU levels
- Ceftobiprole performed better in eradicating intracellular SA in MH-S cells when in comparison to vancomycin
- Our characterization of these antibiotics highlight the shortcomings of some first-line anti-staphylococcal antibiotics in combating intracellular reservoirs of SA, but also supports the use of others
- Clinicians may need to take into account antibiotic penetration and potency intracellularly, when selecting treatment option for *S. aureus* bacteremia

Future Directions

- Expand clinical isolates set for MH-S cell assays
- Expand antibiotic tested for MH-S cell assays, such as linezolid
- Examine and analyze differences in intracellular killing of antibiotics between SA stains that caused bloodstream infection associated with varying patient outcome
- Analyze differences in antibiotic efficacy may be elucidated between SA strains that cause varying severity of pneumonia

References

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