Rapid Resistance Development to Three Antistaphylococcal Therapies in Daptomycin-tolerant *Staphylococcus aureus* Bacteremia

Miller CR[1], Monk JM[2], Berti AD[1,2]

[1]Wayne State University College of Pharmacy, [2]University of CA-San Diego College of Engineering

IDWeek 2020 Advancing Science, Improving Care

Poster #910171

ABSTRACT

Background

We report a patient case of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia which over 30 days developed resistance to all three primary antistaphylococcal antibiotics. Index blood cultures displayed susceptibility to vancomycin (VAN), ceftaroline (CPT) and daptomycin (DAP). The patient was maintained on VAN/CPT with negative blood cultures by hospital day 7. The regimen was later modified to DAP±beta lactam. One month after initial presentation, during the same encounter, blood cultures were again positive for MRSA, now displaying intermediate resistance to VAN (MIC=2) and CPT (MIC=2), and resistance to DAP (MIC=4). These resistances were not stable over time but re-emerged rapidly upon changes to pharmacotherapy.

Methods

Isolates were collected from the initial bacteremia episode (①, ②), the first recurrence (③, ④) and the second recurrence (⑤). Susceptibilities were established using broth microdilution and Etest methodology. Draft whole-genome sequences were determined for each clinical isolate using hybrid assembly (Unicycler v0.4.2) of MinION and Illumina (150bp PE) reads. *In-vitro* one-compartment pharmacokinetic/pharmacodynamic modeling was performed on each isolate to determine which antibiotics or combinations would effectively eradicate cultures. Regimens examined included DAP (10mg/kg), DAP/cefazolin (CFZ) and VAN/CFZ.

Results

DAP/CFZ combination reduced viability of ①, ③ and ⑤ below limit of detection by 12 hours and maintained efficacy for 72 h. DAP, initially effective in reducing ③ cell concentrations below limit of detection, allowed regrowth by 36 h. All other modeled therapies were less effective. Interestingly, DAP took significantly longer to kill ① relative to *S. aureus* collected contemporaneously from other patients indicating antimicrobial tolerance. Comparative genomics of sequential isolates identified single nucleotide *vraT* and *mprF* polymorphisms in all relapse isolates with additional mutations in *tagH*, *agrB* and *saeR* in isolates ③, ④ and ⑤ respectively. Phenotypic assays support the functional loss of regulatory systems identified by whole genome sequencing.

PATIENT CLINICAL ENCOUNTER

October 2018

M	T	W	R	F	S	N
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	①	21
②	23	24	25	26	27	28
29	30	31				

November 2018

M	T	W	R	F	S	N
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	③	17	18
④	20	21	22	23	24	25
26	27	28	29	30		

December 2018

M	T	W	R	F	S	N
					1	⑤
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30

Antibiotic Susceptibility and Genetics

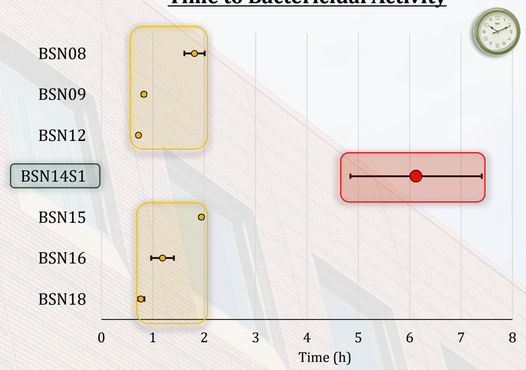
Strain Name	Collection Date	Genetics	Minimum Inhibitory Concentration			
			CPT	DAP	LZD	VAN
BSN14S1	20 Oct 2018	Wild type	0.5†	0.5	2	1, 2†
BSN14S2	22 Oct 2018	BSN14S1 (isogenic)	0.5	0.5	2	1, 2†
BSN14R1	16 Nov 2018	BSN14S1 *mprF vraT tagH*	2†	4†	≤ 1	2
BSN14R2	19 Nov 2018	BSN14S1 *mprF vraT agrB*	2	0.5	≤ 1	1, 2†
BSN14RB	02 Dec 2018	BSN14S1 *mprF vraT saeR*	0.5	2†	≤ 1	2

ISOLATE CHARACTERIZATION

1 The index isolate is Daptomycin-Tolerant

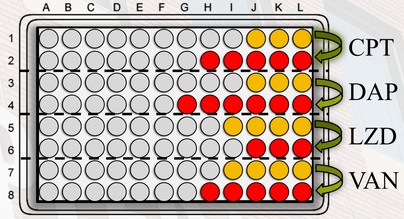
- The time to bactericidal activity is prolonged
- Antibiotic tolerance facilitates subsequent development of resistance

Time to Bactericidal Activity



2 ...following DAP/VAN/CPT selective pressure the microbe rapidly adapts to survive the insult

- Organism develops simultaneous resistances to VAN, DAP and CPT



4 ...when DAP/VAN selective pressure is removed, the microbe rapidly adapts to alleviate negative effects of vraT mutation

- DAP+CPT therapy is changed on 16 NOV to LZD+CPT
- By 19 NOV the microbe developed an *agrB* mutation
- *agr* mutation appears to antagonize *vraT* phenotypes while *vra* mutation appears to antagonize *agr* phenotypes

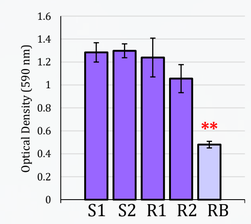
Microbe remains hemolytic despite *agr* mutation

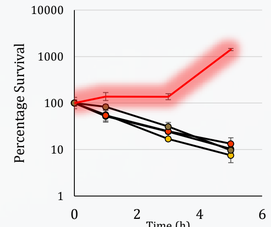
Genetics	Minimum Inhibitory Concentration		
	CPT	DAP	VAN
BSN14S1 *mprF vraT tagH*	2†	4†	2
BSN14S1 *mprF vraT agrB*	2	0.5	1, 2†

Microbe becomes DAP/VAN-susceptible despite *vra* mutation

5 ...with resumption of DAP selective pressure, the microbe again rapidly adapts to rebalance resistance and metabolic costs

- On 19 NOV therapy is changed back to DAP+CPT
- By 02 DEC the microbe reverted *agr* and developed *saeR* mutation restoring the DAP/VAN resistance phenotype and improving survival in whole blood
- However, mutation in *saeR* imparts a cost in impaired biofilm formation





CONCLUSION

- Antimicrobial tolerance can rapidly develop into antimicrobial resistance in clinical isolates.
- Bacteria adapt to changes in pharmacotherapy balancing fitness costs with survival.
- DAP/BL combination therapy may remain effective against both DAP-tolerant and DAP-resistant clinical isolates.