Poster #219

FREQUENCY AND DISTRIBUTION OF MUTATIONS IN *mprF* IN DAPTOMYCIN NONSUSCEPTIBLE *STAPHYLOCOCCUS AUREUS* CLINICAL ISOLATES

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ABSTRACT

Daptomycin (DAP) is a lipopeptide antibiotic with potent activity against Gram positive bacteria approved for treatment of complicated skin and skin structure infections and for treatment of bacteremia and right-sided endocarditis caused by MSSA and MRSA. Resistance to DAP in clinical practice (MIC ≥2 μg/ml for *S. aureus*), has been reported but remains extremely rare. *S. aureus* strains obtained from patients that develop resistance to DAP while on therapy typically shift in MIC from 0.5 μg/ml to 2-4 μg/ml. We have observed that both laboratory-derived and clinical isolates with reduced susceptibility to DAP contain mutations in *mprF*, encoding lysylphosphatidylglycerol (LPG) synthase. We screened 22 DAP susceptible (S) and nonsusceptible (NS; MIC of 2 μg/ml) paired clinical isolates and another set of 23 paired isolates with NS strains having a DAP MIC of 4 μg/ml, for mutations in *mprF* by sequencing. We found that the majority of NS isolates (77% of DAP NS strains with MIC of 2 μg/ml and 87% of DAP NS strains with MIC of 4 μg/ml) contained point mutations in *mprF*, likely resulting in altered function of the LPG synthase. The mutants were found in both domains of the protein. The most common point mutants observed were L826F, S337L or S295L. Because MprF is involved in production of LPG, a component of the cytoplasmic membrane, we propose that the altered function of MprF in these mutants leads to a change in the overall charge of the membrane, and subsequently affects the interaction between DAP and the *S. aureus* membrane.

INTRODUCTION

- DAP (**Figure 1**) is a lipopeptide antibiotic produced by *Streptomyces roseosporus* that displays potent, rapidly bactericidal activity against Gram positive bacteria[1]
- FDA-approved for treatment of complicated skin and skin structure infections and for treatment of bacteremia and right-sided endocarditis caused by MSSA and MRSA
- Mechanism of action is not fully elucidated but involves calcium dependent membrane depolarization[2,3]
- Nonsusceptibility to DAP in clinical practice (MIC ≥2 μg/ml for *S. aureus*) has been reported but remains extremely rare[4]
- *S. aureus* strains obtained from patients that develop nonsusceptibility to DAP while on therapy typically shift in MIC from 0.5 μg/ml to 2-4 μg/ml
- Both laboratory derived and clinical isolates with reduced susceptibility to DAP were found to contain mutations in *mprF*, encoding lysylphosphatidylglycerol (LPG) synthase, and/or in the two- component regulatory system *yycFG*[5]
- MprF is a membrane embedded protein that catalyzes the conversion of phosphatidylglycerol (PG) to LPG using lys-tRNA[6] (**Figure 2**)
- Loss of MprF results in increased susceptibility to DAP and other cationic antimicrobial peptides[7,8]
- The predicted topology of MprF is shown in **Figure 3**, and suggests at least 14 membrane-spanning domains. The N terminal transmembrane portion has been shown to be involved in flipping LPG within the inner and outer leaflet of the membrane[7,9]. The C terminal cytoplasmic domain contains the lys-tRNA binding site and is needed for LPG synthesis[7].

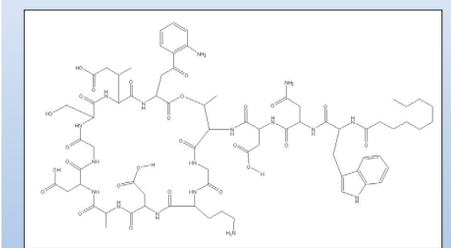

Figure 1. Structure of DAP

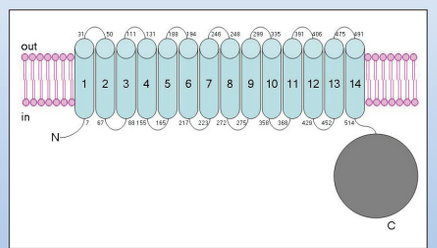

Figure 3. MprF predicted topology. The LPG flippase domain is shown in light blue and the LPG synthesis domain is shown in grey.

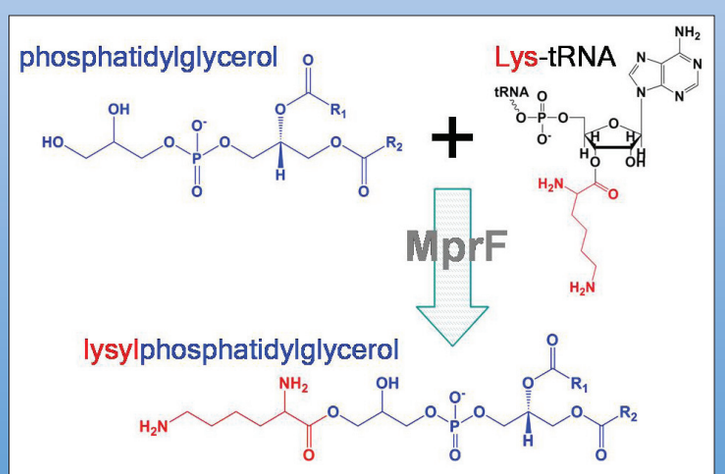

Figure 2. Reaction catalyzed by MprF

METHODS

- *S. aureus* strains were submitted by each hospital or laboratory across the US to our reference laboratory, Laboratory Specialists, Inc. (Westlake, OH), and given a unique identifier
- DAP, oxacillin and vancomycin MICs were determined by broth microdilution method using lyophilized panels obtained from TREK Diagnostics (Cleveland, OH) according to CLSI guidelines. For DAP, broth was supplemented with $CaCl_2$ to 50 μg/mL
- Isolates (DAP susceptible (S) and nonsusceptible (NS)) from each patient were obtained pre and post therapy and determined to be clonal by PFGE
- 22 paired DAP S and NS with MIC of 2 μg/ml and 23 paired DAP S and DAP NS with MIC of 4 μg/ml were selected for sequencing
- These strains represent a unique set of MRSA isolates collected across the US with the majority isolated from blood
- Sequencing was performed using previously described primers[5] at SeqWright (Houston, TX)

RESULTS

Table 1. *mprF* mutations found in *S. aureus* isolates with DAP MIC of 2 μg/ml

LSI strain number	Location of submitting laboratory or hospital	USA group	DAP MIC (μg/ml)	VAN MIC (μg/ml)	MprF allele
ARMC 388	SC	ND	0.5	1	
389			2	2	S337L
NEMC 348	MA	USA100	0.5	1	
349			2	1	none
JHH 465	MD	USA300	0.25	1	
466			2	2	none
NEMC 533	MA	USA100	0.25	2	
534			2	2	P314L
UOM 511	MD	USA100	0.25	1	
557			2	2	T345I
SMNJ 544	NJ	USA300	0.25	1	
545			2	1	none
ULH 558	KY	USA100	0.5	1	
559			2	2	S337L
ORFL 671	FL	USA100/ 800-	0.25	1	
667			2	2	L826F
SJHMC 725	MI	USA100/ 800-	0.5	2	
726			2	2	T345A
BHFL 743	FL	USA300	0.5	2	
734			2	2	S295L
WMC 745	KS	USA100	0.5	1	
746			2	2	T345I
JSU 753	NJ	USA100	0.5	1	
754			2	2	L776S
TCL 830	WA	USA100 /800	0.25	1	
831			2	2	S337L
LMMC 950	CA	USA100- var3	0.5	1	
951			2	2	L826F
OUMC 967	OK	USA100- var5	0.5	1	
968			2	2	P314L
PHIN 973	IN	USA100	0.25	1	
974*			2	1	none
PVH 1021	CO	USA100- var4	0.5	1	
1022*			2	1	L341S
NHCA 1352	CA	USA300	0.25	1	
1353			2	1	L826F
CHNH 1378	NH	USA100	0.25	1	
1379			2	1	M374R
BMC 1381	MA	USA100	0.25	1	
1389			2	2	L826F
UMMS 1472	MN	USA800	0.25	1	
1473			2	2	S337L
BRTX 1485	TX	ND	0.5	1	
1466			2	2	none

ND: not done
None: no mutation detected
*not isolated from blood

Table 2. *mprF* mutations found in *S. aureus* isolates with DAP MIC of 4 μg/ml

LSI strain number	Location of submitting laboratory or hospital	USA group	DAP MIC (μg/ml)	VAN MIC (μg/ml)	MprF allele
SMH 43	NY	ND	0.5	1	
44			4	2	L341S
RUMC 118	IL	USA500	0.5	1	
94			4	4	S295L
RUMC 117	IL	USA100	0.25	1	
95			4	2	L826F
BMC 188	MA	ND	0.5	1	
163			4	2	S337L
HSR 160	CT	USA100	0.5	2	
162			4	4	L826F
HCL 195	MI	ND	1	4	
194			4	8	Δ463-467
RWJ 215	NJ	ND	0.5	1	
216			4	4	L826F
MRMC 258	SC	ND	0.5	1	
259			4	2	L826F
DUMC 337	NC	ND	0.5	1	
339			4	4	S295L
OSF 387	IL	ND	0.5	1	
385			4	2	S295L
AMCNY 422	NY	ND	0.5	1	
403			4	1	L826F
BMC 408	MA	ND	0.5	1	
400			4	2	L337S
WBH 410	MI	ND	1	4	
411			4	4	none
CB 1840	MA	ND	1	ND	
1841			4	ND	S337L
HMC 549	PA	USA300	1	4	
550			4	8	I420N
NYU 611†	NY	USA300	0.5	2	
613			4	4	I420N
UOM 704	MD	USA300	0.5	1	
705			4	2	none
SJHMC 723	MI	USA100	1	2	
724			4	2	P314L
GSH 757	AZ	USA300	0.5	1	
759			4	2	L826F
AMCA 773	NY	USA100- var1	0.25	1	
767			4	2	L826F
MLW 779	WI	USA100- var1	0.25	1	
769			4	2	L341S
OUMC 965	OK	USA300	0.25	1	
966			4	2	T472K
CHM 1061	IN	USA600	0.5	1	
1059			4	4	none

ND: not done
None: no mutation detected
†MSSA

- Of the 22 paired clinical isolates with DAP MIC of 2 μg/ml, 77% of the NS isolates contained a mutation in *mprF* (17 of the 22 isolates tested). The most common mutant observed in these strains was either L826F or S337L.
- No significant change in VAN susceptibility was observed with these isolates.
- Of the 23 paired clinical isolates with DAP MIC of 4 μg/ml, 87% of the NS isolates contained a mutation in *mprF* (20 of the 23 isolates tested). The most common mutant observed in these strains was either L826F or S295L.
- 14 of the 23 paired isolates had no significant change in VAN susceptibility, while 26% (6 of the 23 isolates tested) shifted into the VISA (MIC = 4 - 8 μg/ml) range.
- Based on the location of the most common mutations observed in these clinical strains compared to the predicted topology of MprF, the mutations are in either the LPG flippase (S295L or S337L) or synthesis (L826F) domain.
- In 5 strains with a DAP MIC of 2 μg/ml and 3 with an MIC of 4 μg/ml a mutation in mprF was not detected. We sequenced *yycFG* in these isolates. No mutations in *yycFG* were found in these 8 strains.

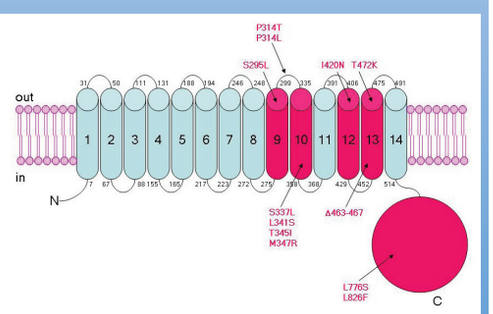

Figure 4. Location of the MprF mutants from the DAP NS isolates identified on the protein highlighted in dark pink

DISCUSSION

- Emergence of DAP NS in clinical practice is still extremely rare, with 110 confirmed DAP NS *S. aureus* isolates submitted to our central laboratory
- Estimated that >800,000 patients have received therapy since Cubicin launch in 2003
- The majority of DAP NS clinical isolates surveyed contain mutations in *mprF* , consistent with the importance this locus may play in DAP NS
- Because mutations were found in both LPG flippase and synthase domains, both domains are likely involved in DAP NS
- The DAP MIC was found to be increased in strains harboring the point mutations, consistent with a gain of function phenotype, which would result in an overall increase in positively charged LPG (either total LPG or LPG present on the outer leaflet)
- Based on the location of the most common alleles, L826F may likely affect LPG synthesis while S295L or S337L is predicted to alter LPG distribution within the membrane
- There are a few DAP NS strains that do not contain mutations on *mprF* and/or *yycFG*, suggesting that additional undefined mutations(s) exist that lead to DAP NS
- Our working hypothesis is that the altered function of MprF in these mutants leads to a change in the overall charge of the membrane and subsequently affects the interaction between DAP and the *S. aureus* membrane

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