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ARTICLE

A positive interaction between inhibitors of protein synthesis and cefepime in the fight against methicillin-resistant *Staphylococcus aureus*

B. Guignard · J. Vouillamoz · M. Giddey · P. Moreillon

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Abstract Quinupristin–dalfopristin (Q-D) synergizes with cefepime for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA). Here, we studied whether the synergism was restricted to MRSA and if it extended to non-beta-lactam cell wall inhibitors or to other inhibitors of protein synthesis. Three MRSA and two methicillin-susceptible *S. aureus* (MSSA) strains were tested, including an isogenic pair of *mecA*$^{-}$/*mecA*$^{+}$ *S. aureus* Newman. The drug interactions were determined by fractional inhibitory concentration (FIC) indices and population analysis profiles. The antibacterial drugs that we used included beta-lactam (cefepime) and non-beta-lactam cell wall inhibitors (D-cycloserine, fosfomycin, vancomycin, teicoplanin), inhibitors of protein synthesis (Q-D, erythromycin, chloramphenicol, tetracycline, linezolid, fusidic acid), and polynucleotide inhibitors (cotrimoxazole, ciprofloxacin). The addition of each protein inhibitor to cefepime was synergistic (FIC ≤ 0.5) or additive (FIC>0.5 but < 1) against MRSA, but mostly indifferent against MSSA (FIC ≥ 1 but ≤4). This segregation was not observed after adding cotrimoxazole or ciprofloxacin to cefepime. Population analysis profiles were performed on plates in the presence of increasing concentrations of the cell wall inhibitors plus 0.25×minimum inhibitory concentration (MIC) of Q-D. Cefepime combined with Q-D was synergistic against MRSA, but D-cycloserine and glycopeptides were not. Thus, the synergism was specific to beta-lactam antibiotics. Moreover, the synergism was not lost against *fem* mutants, indicating that it acted at another level. The restriction of the beneficial effect to MRSA suggests that the functionality of penicillin-binding protein 2A (PBP2A) was affected, either directly or indirectly. Further studies are necessary in order to provide a mechanism for this positive interaction.

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B. Guignard · J. Vouillamoz · M. Giddey · P. Moreillon
Department of Fundamental Microbiology, Biophore Building, University of Lausanne, 1015 Lausanne, Switzerland

B. Guignard (✉)
Division of Pharmacy, University Hospitals of Geneva, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva 14, Switzerland
e-mail: Bertrand.Guignard@hcuge.ch

Introduction

Staphylococcus aureus is a major pathogen that causes both hospital-acquired and community-acquired infections. It is also mastermind in developing resistance to antibacterial agents [1]. It colonizes up to 20 % of the uninfected population [2] and, thus, is frequently exposed to the antibiotics used to treat infections caused by unrelated pathogens. Therefore, it has a great chance of acquiring resistance to any new antibacterial, even if the drug was originally targeted against other bacteria.

Methicillin-resistant *S. aureus* (MRSA) is a paradigm of this scenario. The loss of activity of beta-lactams against penicillin-binding protein 2A (PBP2A)-positive strains pushed the medical community to use numerous alternative antibacterials; however, MRSA became resistant to all of them [1]. Therefore, unless a number of new molecules with no cross-resistance are made available for the treatment of MRSA, staphylococci will develop resistance against each consecutive new compound and add the resistance mechanism to its existing multiresistance panoply [3]. As vancomycin is still the preferred treatment used against MRSA, a

continuous selective pressure in the clinical environment has led to the emergence of vancomycin-intermediate *S. aureus* (VISA) strains. These strains have a decreased susceptibility to glycopeptides and are associated with vancomycin therapeutic failure [4]. New agents such as linezolid, tigecycline, and daptomycin are now available for the treatment of MRSA infections, but linezolid and tigecycline are only bacteriostatic against *S. aureus* and are not approved for invasive and difficult-to-treat infections such as endocarditis. Moreover, resistance to these drugs is emerging [5, 6].

Producing entirely new molecules is a profound scientific challenge [7]. Thus, it is also useful to explore the unexpected features of existing drugs. For instance, understanding the mechanism of PBP2A-mediated resistance to beta-lactams [1, 8] has set the rationale for the development of beta-lactams with improved anti-PBP2A affinity, such as ceftobiprole medocaril and ceftaroline fosamil [9, 10]. Alternatively, drug combinations can be useful by acting in synergy or because their resistance mechanisms may be mutually exclusive, as is the case for methicillin and lysostaphin in MRSA [11]. The advantages of combining existing drugs are that their intrinsic toxicity is usually known and the compounds are available for use. On the other hand, a disadvantage is that the effects of drug combinations are accomplished through complex interactions that are often incompletely understood.

Recently, we and others have reported a synergism between the streptogramin quinupristin–dalfopristin (Q-D) and beta-lactams in the treatment of MRSA both in vitro and in rats with experimental endocarditis [12–14]. The combination of a low dose of Q-D with cefepime successfully cured MRSA endocarditis in the animals, despite both being ineffective on their own [12]. In the present study, we tested in vitro combinations of several classes of antibiotics with cefepime, a beta-lactamase-resistant cephalosporin widely used in the hospital setting. We show that the previously observed positive interaction between Q-D and beta-lactams is restricted to MRSA, does not translate to methicillin-susceptible *S. aureus* (MSSA), and extends to other inhibitors of protein synthesis but not to mechanistically unrelated compounds.

Materials and methods

Microorganisms and growth conditions

The test organisms are described in Table 1. They included the isogenic pair of MSSA and MRSA *S. aureus* Newman *mecA*$^-$ and *mecA*$^+$ strains [15], one multiresistant clinical isolate of MSSA (strain P7142) [16], the homogeneously methicillin-resistant MRSA COL strain [17], and the multiresistant clinical isolate MRSA P8 [18]. In certain experiments, we also used three isogenic strains, including the parent strain (MRSA BB270) [19] and two mutants lacking the *femB* and *femAB* loci (MRSA BB815 and AS145, respectively) [20, 21], which are implicated in the synthesis of the pentaglycine cross-bridge (Table 1). The bacteria were routinely grown at 35 °C in either tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) or tryptic soy agar (TSA; Difco). Cation-supplemented Mueller–Hinton broth (Difco) was used for the antibiotic-susceptibility tests that were performed in liquid medium. All of the media were supplemented with 2 % NaCl to increase the level of expression of beta-lactam resistance genes by MRSA. The stocks were kept at −70 °C in TSB supplemented with 10 % (vol/vol) glycerol.

Antibiotics and chemicals

Cefepime was provided by Bristol-Myers Squibb AG (Baar, Switzerland) and Q-D was provided by Aventis Pharma AG (Zürich, Switzerland). All of the other drugs and chemicals were commercially available products.

Susceptibility testing and antibiotic interactions

The minimum inhibitory concentrations (MICs) were determined by a standard broth macrodilution method [22], with a final inoculum of 10^5 to 10^6 colony-forming units (CFU)/ml. The antibiotic interactions were assessed by the checkerboard method in 96-well microtiter plates (Dynatech Microtiter, Chantilly, VA), as previously described [23]. The

Table 1 Strains used in this study

Strain	Origin	PBP2A	Main resistance profile	Reference
P7142	Parent	−	MSSA, $CMLS_B^r$, Cm^r, Tc^r	[16]
COL	Parent	+	MRSA, Tc^r	[17]
P8	Parent	+	MRSA, $CMLS_B^r$, Cm^r, Tc^r, Gm^r	[18]
Newman	Parent	−	MSSA	[15]
Newman *mecA*$^+$	Newman *mecA*	+	MRSA	[15]
BB270	Parent	+	MRSA	[19]
BB815	BB270 Δ*femB*	+	MSSA, Em^r	[20]
AS145	BB270 Δ*femAB*	+	MSSA, Tc^r	[21]

wells were filled with 100 μl of media containing twofold serial dilutions of each of the test antibiotics and inoculated with 10^5 CFU/ml of bacteria (final concentration) from a logarithmic-phase culture; the plates were then incubated for 24 h at 35 °C before visible bacterial growth was determined. The fractional inhibitory concentration (FIC) indices were interpreted as follows: ≤0.5 for drug synergism, >0.5 but <1 for addition, ≥1 but ≤4 for indifference, and >4 for antagonism. For each antibiotic combination, the experiment was performed in triplicate, and the lower of the FIC index values is presented.

Population analysis profiles

The phenotypic expression of cefepime resistance was determined by spreading a large bacterial inoculum (≥10^9 CFU), as well as the appropriate dilutions, onto NaCl-supplemented agar plates containing twofold serial dilutions of the drug [24]. In certain experiments, the plates were supplemented with a constant subinhibitory concentration (0.25×MIC) of a partner drug [12]. The numbers of colonies growing on the plates were enumerated after 48 h of incubation at 35 °C. The results are presented by plotting the numbers of colonies growing on the plates against the cefepime concentration of the plates. The expression of resistance to non-beta-lactam cell wall inhibitors (fosfomycin, D-cycloserine, vancomycin, teicoplanin) was determined in a similar fashion.

Results

Antibiotic susceptibility and FIC indices

The drug susceptibilities of the test organisms are presented in Table 2.

The positive or negative interactions between various antibiotics were first determined by FIC indices. The drugs included the beta-lactam cefepime, inhibitors of protein synthesis, and inhibitors of nucleic acid synthesis and assembly. Figure 1a depicts the results of combining cefepime with the protein inhibitors. Most of these combinations interacted positively against MRSA, as demonstrated by the presence of synergism (FIC ≤ 0.5) in 14/18 (78 %) of the cases and addition (FIC>0.5 but < 1) in 4/18 (22 %) of the cases. In contrast, these combinations were only additive (FIC>0.5 but < 1) or indifferent (FIC ≥ 1 but ≤ 4) against the two MSSA isolates. This MRSA–MSSA dichotomy was clearly apparent in the *mecA*$^-$ and *mecA*$^+$ versions of *S. aureus* Newman (Fig. 1a), suggesting that the presence of PBP2A was involved.

To test whether this *mecA*-related difference was also a property of other drug classes, the FIC experiments were repeated with cefepime in combination with the two inhibitors of nucleic acid synthesis and assembly, cotrimoxazole and ciprofloxacin. Figure 1b indicates that these combinations were not more active against MRSA than MSSA. Hence, the sensitization of MRSA to beta-lactams (in this case, cefepime) [12] was not a conserved feature between all of the drug classes.

Table 2 Minimum inhibitory concentrations (MICs) of several antibiotics for the five isolates used to test the different antibiotic combinations

Antibiotics	MICs in mg/L				
	Newman *mecA*$^-$	Newman *mecA*$^+$	P7142	COL	P8
Cell wall inhibitors					
Cefepime	2	256	2	1,024	32
D-cycloserine	32	16	64	64	32
Fosfomycin	8	8	8	32	16
Vancomycin	2	2	1	2	1
Teicoplanin	1	2	0.5	1	0.25
Protein inhibitors					
Q-D	0.5	0.5	0.25	0.25	0.125
Erythromycin	0.25	0.25	1,024	0.5	1,024
Chloramphenicol	8	4	64	8	16
Tetracycline	0.5	0.5	256	128	32
Linezolid	2	1	4	2	2
Fusidic acid	0.12	0.03	0.25	0.125	0.25
Nucleic acids inhibitors					
Ciprofloxacin	0.25	0.125	0.25	0.125	0.25
Cotrimoxazole	2	2	0.25	1	1

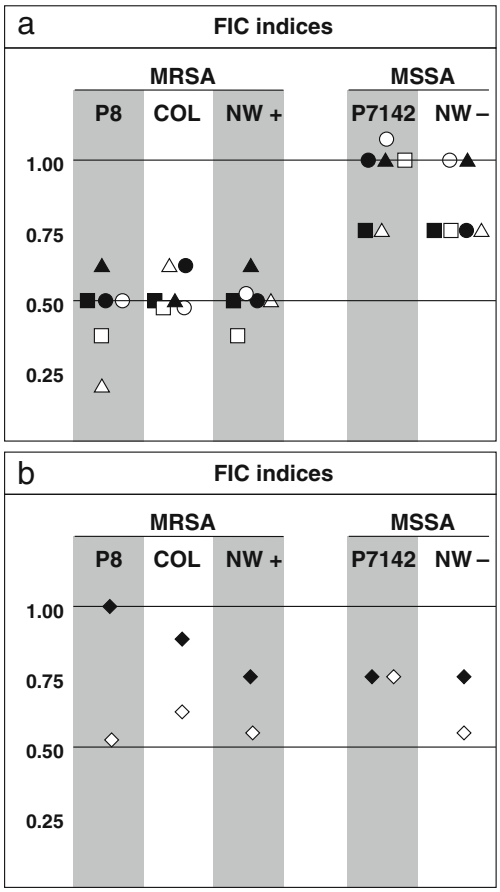

Fig. 1 Fractional inhibitory concentration (FIC) indices for cefepime combined with other drugs, including **a** the inhibitors of protein synthesis quinupristin–dalfopristin (*black squares*), erythromycin (*white squares*), chloramphenicol (*black triangles*), tetracycline (*white triangles*), linezolid (*black circles*), and fusidic acid (*white circles*) and **b** the inhibitors of nucleic acid synthesis and assembly ciprofloxacin (*black diamonds*) and cotrimoxazole (*white diamonds*). The tests were run against the three methicillin-resistant *Staphylococcus aureus* (MRSA) and two methicillin-susceptible *S. aureus* (MSSA) strains. The *horizontal line* at 0.5 indicates the limit for synergism (FIC index ≤ 0.5). The *horizontal lines* between 0.5 and 1 delineate the area of addition (FIC index > 0.5 but < 1). The area above 1 indicates indifference (FIC index ≥ 1 but ≤ 4). No antagonism between cefepime and any of the other drugs tested was observed

Population analysis profiles I: cefepime plus protein inhibitors

The FIC indices are relative values that provide information on the drug interactions independently of the bacterial resistance phenotype (e.g., $CMLS_B^r$, Cm^r, Tc^r, and Gm^r for MSSA 7142 and MRSA P8, as observed in Table 1). To more quantitatively assess the changes in MICs due to the partner compound, the drug combination experiments were repeated in population analysis profiles. A series of agar plates was prepared with increasing concentrations of the drug to be tested plus a fixed subinhibitory concentration (0.25×MIC) of the partner compound. The *mecA*$^-$ and *mecA*$^+$ *S. aureus* Newman strains were used as the model organisms.

In the absence of an accompanying drug, the *mecA*$^+$ *S. aureus* Newman strain grew on plates containing up to 500 mg/L cefepime (Fig. 2a). In the presence of 0.25× MIC of Q-D or erythromycin, the MIC of cefepime decreased by 10×and 5×, respectively, in the majority of the cell population (99.9999 %). This positive effect is in accordance with the FIC indices depicted in Fig. 1a and is supported by previous reports indicating that these drugs could affect beta-lactam resistance in MRSA [12, 13]. In comparison, the positive interaction was much less marked in the *mecA*$^-$ *S. aureus* Newman strain (Fig. 2b), a result that is also compatible with the FIC indices presented in Fig. 1a. Thus, clinically achievable concentrations of Q-D and erythromycin (0.125 and 0.062 mg/L, respectively) could affect methicillin resistance in MRSA, but they barely affected MSSA beta-lactam susceptibility.

When non-MLS_B protein inhibitors were used (e.g., chloramphenicol, tetracycline, and linezolid), a similar—yet less marked—positive effect was observed against the *mecA*$^+$ *S. aureus* Newman strain (Fig. 2c), whereas the effect was, again, virtually non-existent against the *mecA*$^-$ *S. aureus* Newman strain (Fig. 2d). In contrast, the DNA inhibitor ciprofloxacin did not alter the MIC of cefepime for either of the organisms (Fig. 2e, f).

The experiments were repeated to test the opposite setting, i.e., whether the subinhibitory concentrations of cefepime could affect the susceptibility of the bacteria to inhibitors of protein synthesis. Cefepime was added to the plates at a fixed subinhibitory concentration of 0.25×MIC (125 and 0.5 mg/L for the *mecA*$^+$ and *mecA*$^-$ *S. aureus* Newman strains, respectively), whereas the inhibitors of protein synthesis were added at increasing concentrations. No beneficial or detrimental effects were observed with either of the organisms (data not presented). Hence, minor alterations of protein synthesis caused by low concentrations of protein inhibitors could enhance the effect of the beta-lactams, whereas marginal alterations of PBP function by subinhibitory concentrations of the beta-lactams could not enhance the effect of the protein synthesis inhibitors.

Population analysis profiles II: non-beta-lactam cell wall inhibitors plus protein inhibitors

Because the inhibitors of protein synthesis, especially the MLS_B type, could decrease beta-lactam resistance in MRSA, the question arose as to whether this effect was restricted to beta-lactams or if non-beta-lactam inhibitors of cell wall synthesis were also affected. Population analysis profiles were repeated using the following: (i) D-cycloserine, as a drug acting in the early synthesis of muropeptide precursors and before PBP-mediated transpeptidation (Fig. 3), (ii) vancomycin and

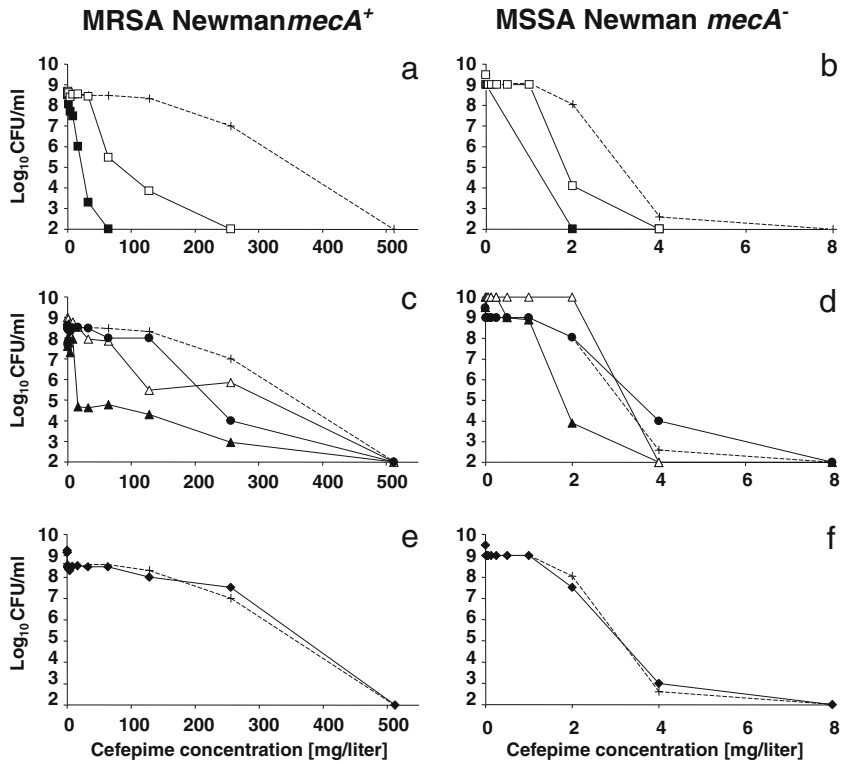

Fig. 2 Population analysis profiles for the isogenic strains of MRSA Newman *mecA*$^+$ (**a**, **c**, **e**) and MSSA Newman *mecA*$^-$ (**b**, **d**, **f**) plated on increasing concentrations of cefepime in combination, or not, with a subinhibitory concentration (0.25×MIC) of various partner drugs. Large numbers of bacteria [$\geq 10^9$ colony-forming units (CFU)] and the appropriate dilutions were plated, and the colonies were enumerated after 24 h of incubation at 35 °C and plotted against the concentration of antibiotic. The details are as follows: cefepime alone (+) or cefepime combined with a constant subinhibitory concentration of quinupristin–dalfopristin (0.125 mg/L, *black squares*), erythromycin (0.062 mg/L, *white squares*), chloramphenicol (2 mg/L, *black triangles*), tetracycline (0.125 mg/L, *white triangles*), linezolid (0.25 mg/L, *black circles*), or ciprofloxacin (0.062 mg/L, *black diamonds*)

teicoplanin, as drugs acting after precursor maturation and blocking the latest stages of cell wall assembly, and (iii) fosfomycin, as a dual inhibitor of early precursor synthesis and PBP2A expression [25] (Fig. 3).

Agar plates containing increasing concentrations of these compounds were supplemented, or not, with 0.25×MIC of Q-D and inoculated with the *mecA*$^+$ and *mecA*$^-$ *S. aureus* Newman strains as above (Fig. 4). Q-D marginally affected the MIC of D-cycloserine for both organisms (Fig. 4a, b), suggesting that the beneficial effect of Q-D with the cell wall inhibitors must take place later in cell wall synthesis. At the other extreme of cell wall synthesis, Q-D had no effect in combination with vancomycin and teicoplanin on the two bacteria (Fig. 4c–f), suggesting that the beneficial interaction did not involve blockage of the precursor at the latest steps in cell wall assembly. Finally, Q-D interacted positively with the dually active fosfomycin, but this interaction was much more marked against the *mecA*$^+$ (>10×decrease in the MIC of fosfomycin) than against the *mecA*$^-$ (2×decrease in the MIC of fosfomycin) *S. aureus* Newman strain (Fig. 4g, h). Given that the D-cycloserine results indicated that the early steps of precursor synthesis were not involved, and because fosfomycin affects both early precursor assembly and the expression of PBP2, 2A, and 4 [25] (Fig. 3), the anti-MRSA effect of Q-D plus fosfomycin most likely operated at the level of PBP functionality.

Effect of Q-D on the *fem* mutants

One critical feature of the function of PBP2A is its requirement for pentaglycine-decorated stem peptides in the muropeptide precursors (Fig. 3) [26]. The *femA* and *femB* genes ensure that the pentaglycine side chains are added in a stepwise manner [21]. To test whether the positive effect of the protein inhibitors could operate by altering the pentaglycine decorations, the Q-D and cefepime FIC experiments were repeated against the two *fem* mutants *femB* BB815 and *femAB* AS145, which carry triglycine and monoglycine decorations instead of pentaglycine, respectively (Table 1), and their MRSA parent BB270 [19–21]. The FIC indices were unaltered by the *fem* mutations (0.6, 0.6, and 0.75 for the two mutants and the parent, respectively), indicating that the positive effect of the drug combination against the *mecA*$^+$ staphylococci was an addition, and not a synonym, of the *fem* mutations.

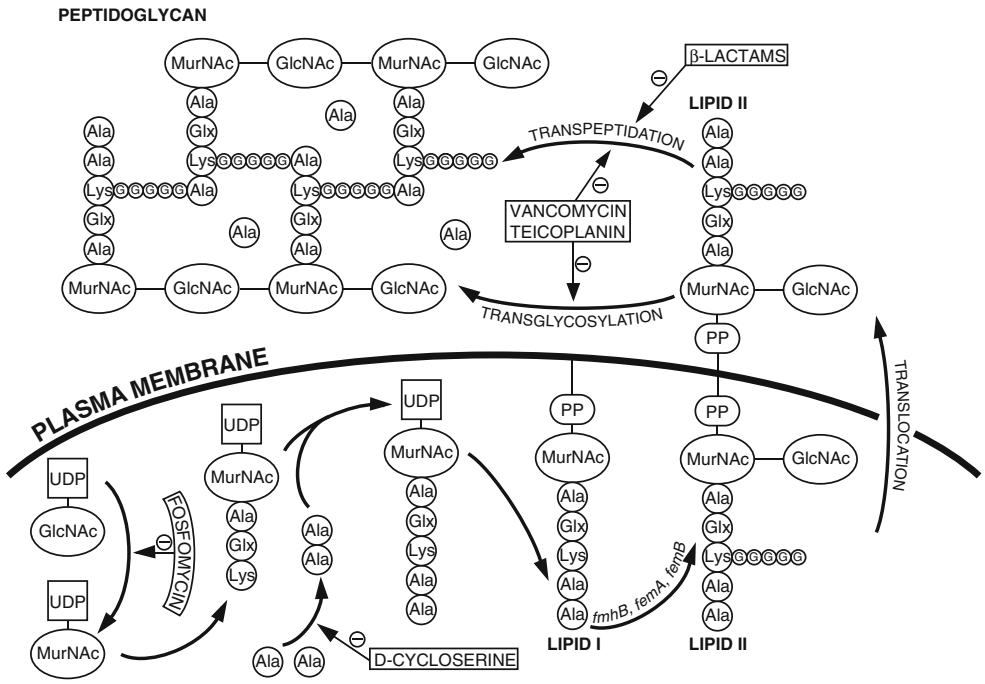

Fig. 3 Principal steps of peptidoglycan assembly in *S.aureus* and locations of inhibition by some cell wall inhibitors. Cell wall precursors are synthesized in the cytoplasm from uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc) and transformed into uridine diphosphate *N*-acetylmuramic acid (UDP-MurNAc). This is followed by the successive addition of one L-alanine (Ala), one D-isoglutamine or isoglutamate (Glx), one L-lysine (Lys), and the dipeptide D-Ala-D-Ala. The MurNAc pentapeptide is then linked to the plasma membrane to form lipid I. A cytoplasmic transglycosylase adds a GlcNAc to the MurNac moiety and five glycine (G) residues to the ε-NH_2 terminal of L-lysine, by the products of the *fmhB*, *femA*, and *femB* genes, to complete the formation of lipid II. After membrane translocation, the precursors are processed by the membrane penicillin-binding proteins (PBPs) through the transglycosylation and transpeptidation steps. The steps affected by beta-lactams, glycopeptides (vancomycin or teicoplanin), D-cycloserine, and fosfomycin are indicated within the figure. Fosfomycin was also reported to decrease the expression of PBP2, 2A, and 4 (not depicted in the figure) [25]

Discussion

These results highlight some salient features of the previously described synergism between Q-D and beta-lactam against *S. aureus* [12–14]. First, the positive interaction between the two drug classes was restricted to MRSA and was much less potent in MSSA. This was clearly apparent in the *mecA*$^+$ and *mecA*$^-$ versions of *S. aureus* Newman and was also observed across the five test isolates. Second, this positive interaction extended to other inhibitors of protein synthesis, although it was less marked with the non-MLS_B compounds. Third, this positive interaction did not extend to mechanistically unrelated compounds such as DNA inhibitors and non-beta-lactam inhibitors of cell wall biosynthesis, except for fosfomycin. Together, these features help to delineate, at least partially, the mechanism of the drug interactions.

The restriction of the beneficial effect to MRSA indicates that the inhibitors of protein synthesis affected the functionality of PBP2A, either directly or indirectly. However, it does not specify the mechanism by which the positive interaction occurred. Our results obtained by combining the protein inhibitor Q-D with the non-beta-lactam cell wall inhibitors suggested that the level of the interaction was between the two extremes of the wall assembly line. At the early stage of wall assembly, the positive effect of Q-D most likely occurred after the addition of the D-ala-D-ala terminal to the muropeptide precursor, as no positive interaction was observed between Q-D and D-cycloserine. At the late stage of wall assembly, Q-D did not synergize with direct blockers of the precursors, such as glycopeptides, suggesting that the function of PBP and/or the earlier steps were involved.

One possibility was that an interaction with the maturation of muropeptide precursors was occurring, possibly at the level of adding pentaglycine decorations to the stem peptides. PBP2A requires pentaglycine-decorated precursors to be effective [26–28]. Indeed, mutations in the *fem* genes, which block the addition of glycines to the precursors at various levels, block the expression of methicillin resistance, regardless of the amount of PBP2A [17, 28, 29]. Protein inhibitors could affect the *fem* pathway by decreasing the amounts of glycine-adding enzymes, thus, formally replacing the *fem* effect. If the protein inhibitors do affect the *fem* pathway, then the Q-D plus beta-lactam synergism should be lost against the *fem* mutants, while it should persist against the parent MRSA strain. The experiments indicate that the beneficial effect of Q-

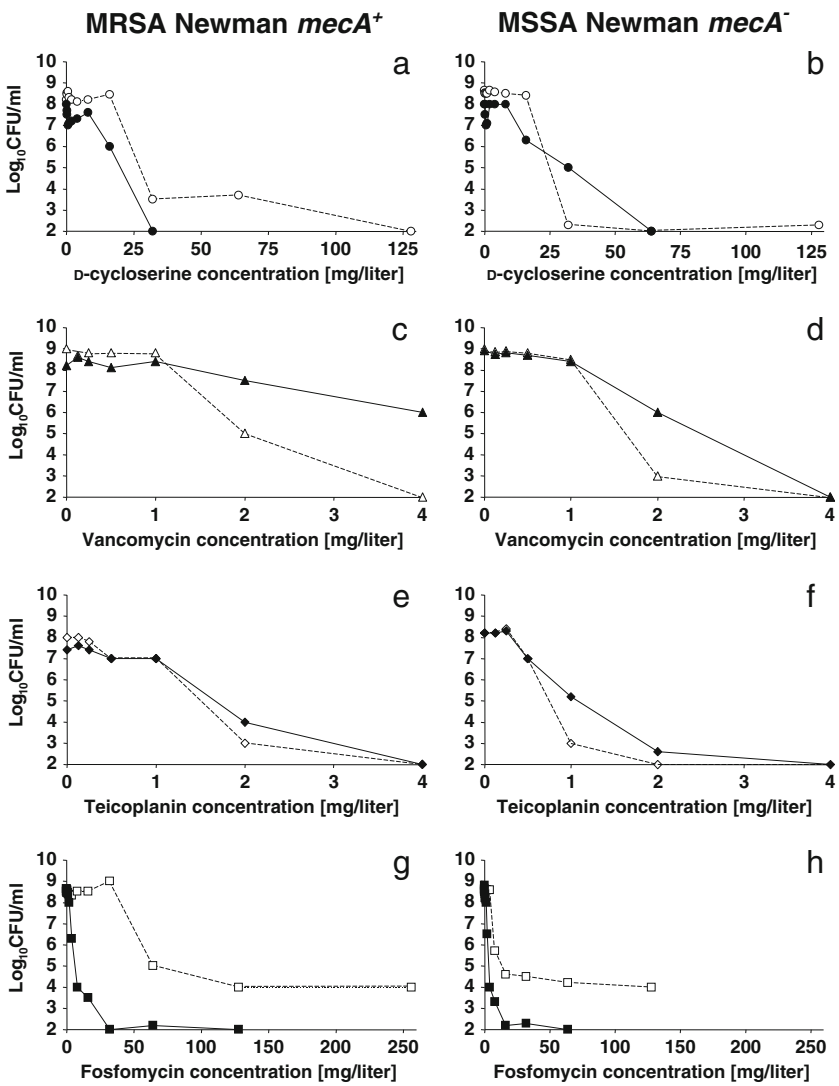

Fig. 4 Population analysis profiles of the isogenic strains of MRSA Newman *mecA*$^{+}$ (**a**, **c**, **e**, **g**) and MSSA Newman *mecA*$^{-}$ (**b**, **d**, **f**, **h**) plated on increasing concentrations of the non-beta-lactam cell wall inhibitors D-cycloserine (*white circles*), vancomycin (*white triangles*), teicoplanin (*white diamonds*), or fosfomycin (*white squares*) either alone (*open symbols* and *dashed lines*) or in combination with a constant subinhibitory concentration (0.125 mg/L) of quinupristin–dalfopristin (*closed symbols* and *continuous lines*). The details are the same as in Fig. 2

D in combination with cefepime was identical in both the parent and the *femB* and *femAB* mutant strains, as shown by the FIC indices of 0.75, 0.6, and 0.6, respectively. Therefore, the drug combination operated in addition to the *fem* mutations and not instead of them.

Q-D had a positive interaction with fosfomycin, which was consistent with recent reports of synergism between fosfomycin and other protein synthesis inhibitors, such as linezolid [30]. Fosfomycin disrupts the expression of PBP2, 2A, and 4. It is tempting to hypothesize that the expression of the PBPs could also be altered, albeit in a different way, following protein synthesis inhibition by Q-D or other protein inhibitors. This would explain the observed positive interaction with fosfomycin, by complementary alterations in the expression of the PBPs, as well as the beta-lactam susceptibility restoration. A decrease in the amount of PBP2A would be the simplest explanation for the observed restoration of cefepime susceptibility in the tested MRSA strains and the absence of this effect in the MSSA strains. However, a decrease in PBP2 activity would also provide a rationale for the MRSA-specific synergism observed. Native *S. aureus* PBP2 is a class A PBP providing transglycosylase activity for the glycan chain elongation step in the cell wall building process. Hence, it is indispensable for the functionality of the other purely transpeptidase PBPs, including PBP2A. When MRSA are exposed to beta-lactams, all of their transpeptidase sites are blocked except for the low-affinity PBP2A site and the indispensable PBP2 transglycosylase site [31]. If this transglycosylase is further inhibited by either decreasing its amount, as hypothesized herein, or by specific drugs such as moenomycin, then PBP2A can no longer function and the beta-lactams regain their antibacterial activity. In MSSA, in contrast, the beta-lactams need only to block the high-affinity native transpeptidases to block bacterial growth. This occurs at very low drug concentrations, and additional inhibition of the transglycosylase does not provide any additional effects. Thus, the dichotomy of synergism between MRSA and MSSA

could be explained by the hypotheses reported above. Further investigations, including the simultaneous titration of PBPs (mainly PBP2 and 2A) in the presence or absence of Q-D or other protein synthesis inhibitors at subinhibitory concentrations, would be required.

Several additional genes affecting methicillin resistance could also be involved. These include *glnR* (*femC*), which is responsible for glutamic acid amidation in precursor stem peptides, *glmM* (*femD*), which is responsible for UDP-*N*-acetylglucosamine biosynthesis, and *murE* (*femF*), which is responsible for lysine addition in precursor stem peptides [28]. Moreover, protein inhibitors such as chloramphenicol and tetracycline can inhibit the stringent response of *Escherichia coli* and promote the assembly of an abnormally thickened cell wall [32]. *S. aureus* can also undergo the stringent response [33], and subinhibitory concentrations of Q-D have been shown to induce cell wall thickening in this very organism [34].

Proteomic and DNA array analysis of *S. aureus* exposed to cell wall inhibitors revealed an ample alteration in gene expression through a phenomenon known as the cell-wall-stress stimulon [35, 36]. Among the altered genes, the expression of PBP2 was increased after induction of the *vraSR* genes. A possible interference of Q-D (or other protein inhibitors) with the physiological beta-lactam stress response (e.g., the VraSR system) would be another indirect rationale for the Q-D–beta-lactam effect.

Answering the numerous questions requires the additional titration of gene expression by microarrays. While such an approach is being attempted, the present observation offers further characterization of the previously observed anti-MRSA synergism between Q-D and the beta-lactams. Moreover, this precedent with MRSA may provide the rationale for testing this synergism using additional organisms that resist beta-lactams via decreased PBP affinity, including pneumococci and enterococci, as well as examining new combinations of bactericidal beta-lactams with the new anti-MRSA protein inhibitors linezolid and tigecycline.

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Conflict of interest The authors declare that they have no conflict of interest.

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