Interactions of Quinupristin-Dalfopristin with Eight Other Antibiotics as Measured by Time-Kill Studies with 10 Strains of Staphylococcus aureus for Which Quinupristin-Dalfopristin Alone Was Not Bactericidal

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Quinupristin-dalfopristin (Q-D) and eight other antimicrobial agents were tested alone and in combination with Q-D in time-kill studies against 10 strains of macrolide-lincosamide-streptogramin B-resistant Staphylococcus aureus. Although Q-D is normally a bactericidal drug, it was only bacteriostatic for these isolates. Gentamicin alone was bactericidal against 7 of the 10 strains, and Q-D did not alter that killing effect. However, when vancomycin, cefepime, ceftazidime, imipenem, piperacillin-tazobactam, and ciprofloxacin were bactericidal when tested alone, the killing rates were reduced when combined with Q-D. The clinical significance of this in vitro antagonism is unknown at this time, and more studies are needed.

Quinupristin-dalfopristin (Q-D) is a new parenteral streptogramin combination that exhibits particular antibacterial potency against gram-positive pathogens, including methicillin-resistant Staphylococcus aureus (MRSA) strains (1, 3, 9, 13, 14). The two components of Q-D, quinupristin and dalfopristin, act synergistically and provide good activity against macrolide-lincosamide-streptogramin B (MLS\textsubscript{B})-susceptible and -resistant strains of S. aureus (4, 11). Although Q-D is bactericidal against most staphylococci, it is bacteriostatic against only constitutively MLS\textsubscript{B}-resistant strains (8), and Q-D therapy of experimental endocarditis due to such strains has failed (6, 7). Clindamycin resistance is a surrogate indicator of a reduced bactericidal activity of Q-D against staphylococci (8). How common is the clindamycin resistance phenotype? An international study of clinical isolates from 23 hospitals in 18 countries (2) documented clindamycin resistance among 42% of 462 MRSA isolates but only 3% of 1,003 methicillin-sensitive S. aureus (MSSA) isolates. In that large survey, 99% of MSSA strains and 95% of MRSA strains were reported to be susceptible to Q-D according to bacteriostatic determinations: Q-D should have been bactericidal against 85% of the 1,465 S. aureus isolates because they were clindamycin susceptible.

Because bactericidal activity is considered important in the treatment of certain infections, such as bacterial endocarditis, the use of Q-D in treating such infections caused by MLS\textsubscript{B}-resistant S. aureus is problematic. The present study was designed to determine if Q-D may be combined with other antimicrobial agents in order to ensure a bactericidal effect against MLS\textsubscript{B}-resistant S. aureus strains for which Q-D alone is not bactericidal. For this study, we selected 10 strains of MLS\textsubscript{B}-resistant isolates of S. aureus, including 8 MRSA and 2 MSSA strains, all of which had been previously demonstrated to have less than 99.9% killing during a 24-h exposure to 10 \( \mu \)g of Q-D/ml.

Time-kill studies were carried out following the principles defined by the National Committee for Clinical Laboratory Standards (12). The following antimicrobial agents were tested at one twofold concentration above their respective susceptible MIC breakpoints: Q-D (2.0 \( \mu g/ml \)), vancomycin (8.0 \( \mu g/ml \)), cefepime (16 \( \mu g/ml \)), ceftazidime (16 \( \mu g/ml \)), imipenem (8.0 \( \mu g/ml \)), piperacillin-tazobactam (8.0 and 4.0 \( \mu g/ml \)), ciprofloxacin (2.0 \( \mu g/ml \)), gentamicin (8.0 \( \mu g/ml \)), and rifampin (2.0 \( \mu g/ml \)). Each drug was tested alone, and each of the latter eight drugs was tested in combination with Q-D at the same concentration. One flask of inoculated cation-adjusted Mueller-Hinton broth with no antibiotic served as a control. Colony counts were performed on the control suspension at time zero and on the control as well as each antibiotic-containing suspension at 3, 6, 8, 12, and 24 h. Counts were performed in duplicate, and the average of the two values was used. For this presentation, only 24-h-colony counts are described; earlier subcultures led to similar conclusions. Bactericidal activity was defined as a \( \geq 3 \text{-log}_{10} \) reduction in colony count compared to the time zero count. Synergy or antagonism was defined as \( \geq 2 \text{-log}_{10} \) decrease or increase in colony counts with a drug combination compared to the lower of the two colony counts of the individual drugs alone.

The lack of bactericidal activity of Q-D against this set of S. aureus strains was confirmed (Table 1). During the 24-h test period, gentamicin was bactericidal to seven isolates, and this activity was not altered significantly when combined with Q-D (Fig. 1A). Vancomycin was bactericidal to five strains, and when combined with Q-D, an antagonistic response was observed with four of these strains (Table 1 and Fig. 1C). Kang and Rybak (10) have demonstrated synergy between Q-D and vancomycin in a time-kill study at much higher drug concentrations against a strain of MRSA for which vancomycin alone was bactericidal and Q-D was not.

The \( \beta \)-lactam drugs were bactericidal for the two MSSA strains and one MRSA strain, and this activity was inhibited

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FIG. 1. Representative S. aureus time-kill curves for treatment with gentamicin (8.0 μg/ml) alone and gentamicin (8 μg/ml) plus Q-D (2.0 μg/ml) (A), ciprofloxacin (2.0 μg/ml) alone and ciprofloxacin (2.0 μg/ml) plus Q-D (2.0 μg/ml) (B), vancomycin (8.0 μg/ml) alone and vancomycin (8.0 μg/ml) plus Q-D (2.0 μg/ml) (C), and imipenem (8.0 μg/ml) alone and imipenem (8 μg/ml) plus Q-D (2.0 μg/ml) (D).

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when these drugs were combined with Q-D (Fig. 1D). Ciprofloxacin showed bactericidal activity against two strains, and antagonism was also observed with these two strains when ciprofloxacin was combined with Q-D (Fig. 1B). Only one instance of synergy was observed—Q-D plus rifampin against strain SP1662, but this did not reach bactericidal levels (Table 1).

It is of interest that with the exception of gentamicin, nearly all drugs that achieved bactericidal activity when tested alone were inhibited when tested with Q-D. Since Q-D was bacteriostatic to these strains, all instances of antagonism occurred with a combination of a bacteriostatic drug with a bactericidal drug. This is consistent with other drug combinations for which antagonism has been observed (5). It must be emphasized, however, that antagonism in such in vitro tests does not necessarily translate into in vivo antagonism (5).

Vouillamoz et al. (15) tested Q-D and cefepime alone and in combination at trough and peak serum levels by time-kill studies against MLSb-resistant MRSA strains. Neither drug was bactericidal alone. At the trough levels (0.5 μg of Q-D/ml and 5.0 μg of cefepime/ml), there were ≥2-log_{10} reductions in counts at 24 h compared to the lower of the counts achieved by each drug alone. But, at peak levels, a tendency toward antagonism was observed. When experimental endocarditis caused by these strains was treated with Q-D plus cefepime, a marked reduction in vegetation bacterial counts was observed, but no reduction in counts occurred in animals treated with either drug alone (15). However, since neither drug alone was bactericidal for these organisms, it does not resolve the question of whether the in vitro antagonism observed when bactericidal drugs are combined with Q-D as a bacteriostatic agent (MLSb-resistant staphylococci) will be a problem in the clinical setting.

Under the conditions of this study, Q-D inhibited the bactericidal activity of vancomycin, ciprofloxacin, and four β-lactam antibiotics against MLSb-resistant S. aureus cells. Further studies with experimental animals would be helpful in determining whether this is strictly an in vitro phenomenon or may have clinical consequences.

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REFERENCES


5. Eliopoulos, G. M., and R. C. Moellering, Jr. 1996. Antimicrobial combina-

### TABLE 1. Effects of eight antibiotics alone and with Q-D on bacterial counts after 24-h exposure

<table>
<thead>
<tr>
<th>Strain</th>
<th>Inoculum (log₁₀ CFU/ml)</th>
<th>Treatment type</th>
<th>Q-D</th>
<th>Van</th>
<th>Cef</th>
<th>Ctz</th>
<th>Imp</th>
<th>P/T</th>
<th>Cip</th>
<th>Gen</th>
<th>Rif</th>
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<td>2.38</td>
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<td>9.00</td>
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*Van, vancomycin; Cef, cefepime; Ctz, ceftazidime; Imp, imipenem; P/T, piperacillin-tazobactam; Cip, ciprofloxacin; Gen, gentamicin; Rif, rifampin. Bold values indicate bactericidal activity, underlined values indicate antagonism, and italicized values indicate synergism (see text for definitions). NT, not tested.


