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Synergistic Effect of Quinolones and Oxacillin on Methicillin-Resistant *Staphylococcus* Species

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Various combinations of antistaphylococcal antimicrobial agents have been tested against 17 selected *Staphylococcus* isolates, including methicillin-susceptible and methicillin-resistant strains of *S. aureus* and coagulase-negative *Staphylococcus* species. With the checkerboard technique the following combinations were tested: oxacillin-ofloxacin, oxacillin-temafloxacin, oxacillin-fleroxacin, vancomycin-fleroxacin, gentamicin-fleroxacin, and rifampin-fleroxacin. Against methicillin-resistant staphylococci the combination oxacillin-quinolone tested at 35°C always showed a fractional inhibitory concentration (FIC) index of <0.75, which is interpreted as synergistic or additive. Equal or more synergistic effects were observed at 30°C. In contrast, when methicillin-susceptible *Staphylococcus* species were tested, the FIC for the combination oxacillin-quinolone was always 1 or 2, which is considered to be indifferent. For the other mentioned combinations the FICs were also 1 or 2. Killing kinetics showed synergistic or additive bactericidal activity for the combination oxacillin-ofloxacin against methicillin-resistant *Staphylococcus* species, killing 1.5 to 2.8 $\log_{10}$ CFU more of these per ml than did the most active drug after 24 h of incubation. This difference was not observed for methicillin-susceptible strains. In vitro evidence for the potential clinical use of quinolones in treating infections due to methicillin-resistant staphylococci in combination with a β-lactamase-resistant penicillin is provided.

Infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative *Staphylococcus* species remain a therapeutic challenge. Presently, the drug of choice is intravenous vancomycin because these strains are usually resistant to other alternative drugs. Since MRSA and methicillin-resistant coagulase-negative *Staphylococcus* species have become an epidemiological threat worldwide, the search for new drugs or drug combinations is warranted.

The advent of quinolones led to a multitude of new clinical applications (1, 4, 20). However, their role in the therapy of *S. aureus* infections has not been clearly established. Oral quinolones have been suggested as a therapy for chronic osteomyelitis or foreign body infections, which often require prolonged chemotherapy (4).

The initial aim of our investigation was to analyze the susceptibility patterns of *Staphylococcus* species to various antimicrobial agents alone and the effect of a quinolone combined with another antimicrobial agent. The strains studied were isolated from patients suffering from a typical foreign body infection (8), i.e., intravenous-device-related bacteremia. In addition, we also tested some strains of a previously documented MRSA group (7, 25). In the course of our investigation, we found that quinolones and oxacillin acted synergistically or in an additive way against methicillin-resistant *Staphylococcus* species. In view of its potential clinical importance, this observation was documented in detail.

MATERIALS AND METHODS

Strains. We selected 17 strains of staphylococci: 3 methicillin-susceptible *S. aureus*, 3 methicillin-susceptible coagulase-negative *Staphylococcus* species, and 5 methicillin-resistant coagulase-negative *Staphylococcus* species, which were isolated from blood cultures of patients with proven septicemia associated with intravenous device infection (8). Six MRSA strains were chosen from a collection described in detail in a previous work by our group (7, 25). All strains have been identified as to species as indicated (see Table 1) with an API Staph strip (API System, Montalieu-Vercieu, France).

Antimicrobial agents. Standard antibiotic solutions were freshly prepared before use. The following nonquinolone antimicrobial agents were used: oxacillin, gentamicin, vancomycin, and rifampin. The following quinolones were tested: ofloxacin, temafloxacin, and fleroxacin. The antibiotics were kindly provided by their manufacturers.

Susceptibility testing. The MICs for the staphylococcal isolates were determined by the microdilution method recommended by the National Committee for Clinical Laboratory Standards (11). Microdilution plates were inoculated with 100 μl of Mueller-Hinton broth (Oxoid Ltd., Hampshire, United Kingdom) containing the appropriate antimicrobial concentration and a final concentration of 10^5 CFU of the test organism per ml. These experiments were also performed with Mueller-Hinton broth supplemented with 2% NaCl. After an incubation of 24 h at 35°C, the plates were examined for growth.

Combination studies. With the checkerboard technique, different antimicrobial combinations were evaluated in a plate microdilution assay, with incubations at 35 or 30°C for 24 h (9). As for the susceptibility testing, Mueller-Hinton broth with 2% NaCl or without it was used. The fractional inhibitory concentration (FIC) index was the minimum concentration of each of the two antimicrobial agents that had an inhibitory effect when acting together divided by the MIC of that drug alone. The sum of the FICs of both antibiotics was the FIC index. The results were interpreted as synergism, addition, indifference, or antagonism when the FIC indices were ≤0.5, 0.5 to 0.75, 1 to 4, or >4, respectively (9).

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Bactericidal kinetic assays were performed in glass tubes containing 10 ml of Mueller Hinton broth (without NaCl) according to published recommendations (9, 12, 14, 21). We tested oxacillin at 2 μg/ml and ofloxacin at 1 μg/ml. These concentrations represent the lower recommended breakpoints (2, 12). Other oxacillin and ofloxacin concentrations were also tested when indicated. After 0, 2, 4, 6, and 24 h of incubation at 35°C in a shaking water bath, samples were plated onto Mueller-Hinton agar (Oxoid) with a spiral plater (Spiral System Inc., Cincinnati, Ohio). The agar plates were incubated for 18 to 24 h at 35°C before the viable CFU were determined with a colony counter (Spiral System Inc.). The Spiral system not only allows continuous counts from 1.3 to 5 $\log_{10}$ CFU per ml, it also reduces the errors due to antibiotic carry-over (28). To determine the carry-over effect, we plated low-inoculum suspensions (about 1 to 3 $\log_{10}$ CFU per ml) of the highly susceptible strains *S. aureus* ATCC 29523 and *S. epidermidis* F26 in the presence or absence of antimicrobial agents (oxacillin at 2 μg/ml and ofloxacin at 4 μg/ml). The subsequent colony counts differed by less than 4%. Results of the time-kill curves were interpreted as synergistic when an increase in killing at 24 h of ≥2 $\log_{10}$ CFU per ml was measured with the antimicrobial combination in comparison with the most active drug alone. An increased killing of 1 to 2 $\log_{10}$ CFU per ml was interpreted as additive, a 1 $\log_{10}$ change (increase or decrease) was interpreted as indifference, and a decreased killing of ≥2 $\log_{10}$-fold CFU per ml was interpreted as antagonism (9, 12, 14, 21).

For statistical analysis the two-tailed Fisher exact test was applied.

RESULTS

Checkerboard experiments. (i) Combination of a quinolone and oxacillin. When methicillin-resistant *Staphylococcus* species were tested, the FICs for the combination oxacillin-ofloxacin were <0.75; i.e., the effect is synergistic or additive (Table 1). The FIC results were temperature dependent. More synergism was often observed at 30°C than at 35°C. The additive effect of the combination was not dependent on the ofloxacin susceptibility of the test organism. The combinations oxacillin-temafloxacin and oxacillin-fleroxacin very similarly showed synergistic or additive activity against three methicillin-resistant strains tested.

When methicillin-susceptible *Staphylococcus* species were tested, the FICs were always 1 or 2, regardless of which quinolone was combined with oxacillin (Table 1).

When the Fischer exact test was applied between oxacillin activity (MICs of ≥4 μg/ml versus ≤0.5 μg/ml) and FIC categories (indices of ≤0.63 versus ≥0.75), the correlation was significant ($P < 0.002$).

(ii) Other combinations. We also tested the combinations vancomycin-fleroxacin, gentamicin-fleroxacin, and rifampin-fleroxacin against six strains. The FICs were always 1 or 2.

Time-kill curves. To confirm the findings of the checkerboard experiments with the quinolone-oxacillin combination, time-kill curves were established. The killing effects of oxacillin (2 μg/ml) and ofloxacin (1 μg/ml) used alone and in combination against six *Staphylococcus* strains are illustrated in Fig. 1. The two upper panels represent the results for methicillin-susceptible staphylococci, the two middle panels represent those for oxacillin-resistant strains, and the two lower panels represent those for oxacillin-resistant and ofloxacin-moderately susceptible and -resistant strains. With the oxacillin-susceptible staphylococci, the combination was indifferent, providing limited changes (≤0.9 $\log_{10}$ CFU per ml) in the bactericidal effect as compared with the most active drug after 24 h of incubation. The combination acted synergistically against oxacillin-resistant *S. epidermidis* G47 (an increased killing of 2.8 $\log_{10}$-fold CFU per ml compared with ofloxacin alone). The effect of the combination oxacillin-ofloxacin against the two MRSA strains could not be interpreted when ofloxacin was used at 1 μg/ml, as it had by itself an excellent bactericidal activity at this concentration. Thus, further experiments were performed at an ofloxacin concentration of 0.25 μg/ml (Fig. 2). Here the combination acted synergistically and additively (an increased killing of 2.6 and 1.5 $\log_{10}$ CFU per ml). Similarly, the time-kill curves for the methicillin-susceptible strains *S. aureus* J7 and *S. epidermidis* F26 were established in experiments with lower concentrations of oxacillin (0.5 μg/ml) and ofloxacin (0.5 μg/ml). The above-mentioned indifference could be confirmed (an increased inhibition of 0.4 and 0.3 $\log_{10}$ CFU per ml). For the strain *S. epidermidis* 87562 we chose the higher concentration of 4 μg of ofloxacin per ml (2). Here, synergism could be demonstrated for the combination (an increased inhibition of 2.1 $\log_{10}$ CFU per ml) (Fig. 3).

TABLE 1. MICs and FICs (oxacillin-ofloxacin) for 6 methicillin-susceptible and 11 methicillin-resistant *Staphylococcus* strains

Strain	MIC (μg/ml)			FIC with incubation at:		
	Oxacillin		Ofloxacin (no[a])	35°C		30°C (no[a])
	NaCl[a]	No[a]		NaCl[a]	No[a]	
S. aureus J7	0.12	0.25	0.25	1	2	1
S. aureus B32	1	0.5	0.25	1	2	0.75
S. aureus G5	0.25	0.25	0.25	1	1	2
S. epidermidis F26	0.12	0.12	0.25	1	2	1
S. epidermidis 7580	0.06	0.12	8	2	1	2
S. haemolyticus K51	0.12	0.12	0.25	2	2	2
S. aureus MRGR2	128	64	0.12	0.63	0.53	0.51
S. aureus MR3	128	16	0.25	0.56	0.63	0.28
S. aureus 680	128	16	0.25	0.63	0.53	0.31
S. aureus MR15	256	32	0.25	0.51	0.56	0.38
S. aureus MR45	128	16	0.5	0.51	0.5	0.5
S. aureus MR63	128	8	0.25	0.5	0.38	0.31
S. epidermidis K36	32	4	0.25	0.38	0.63	0.31
S. epidermidis G47	64	16	1	0.51	0.56	0.56
S. epidermidis 7875	4	4	8	0.53	0.63	0.56
S. epidermidis 87562	32	8	8	0.63	0.63	0.63
S. haemolyticus K54	512	256	0.25	0.51	0.38	0.51

[a] No, Without 2% NaCl supplement; NaCl, with 2% NaCl supplement.

DISCUSSION

According to the FIC index, the combination of a quinolone with oxacillin was regularly synergistic or additive against methicillin-resistant staphylococci. This resistance was intrinsic for the tested strains (10). Little or no interaction occurred when the same combinations were tested against methicillin-susceptible strains. In good accordance with previous investigations (17, 18, 24, 26), the combination of a quinolone with vancomycin, gentamicin, or rifampin was indifferent in terms of FIC indices.

The synergism, described here for the first time, was somewhat surprising since oxacillin acts on the bacterial cell wall, while the quinolones block the bacterial gyrase, an enzyme which interferes with the chromosomal DNA.

Several hypotheses may be advanced to explain the synergistic effect of a quinolone combined with a β-lactam. We

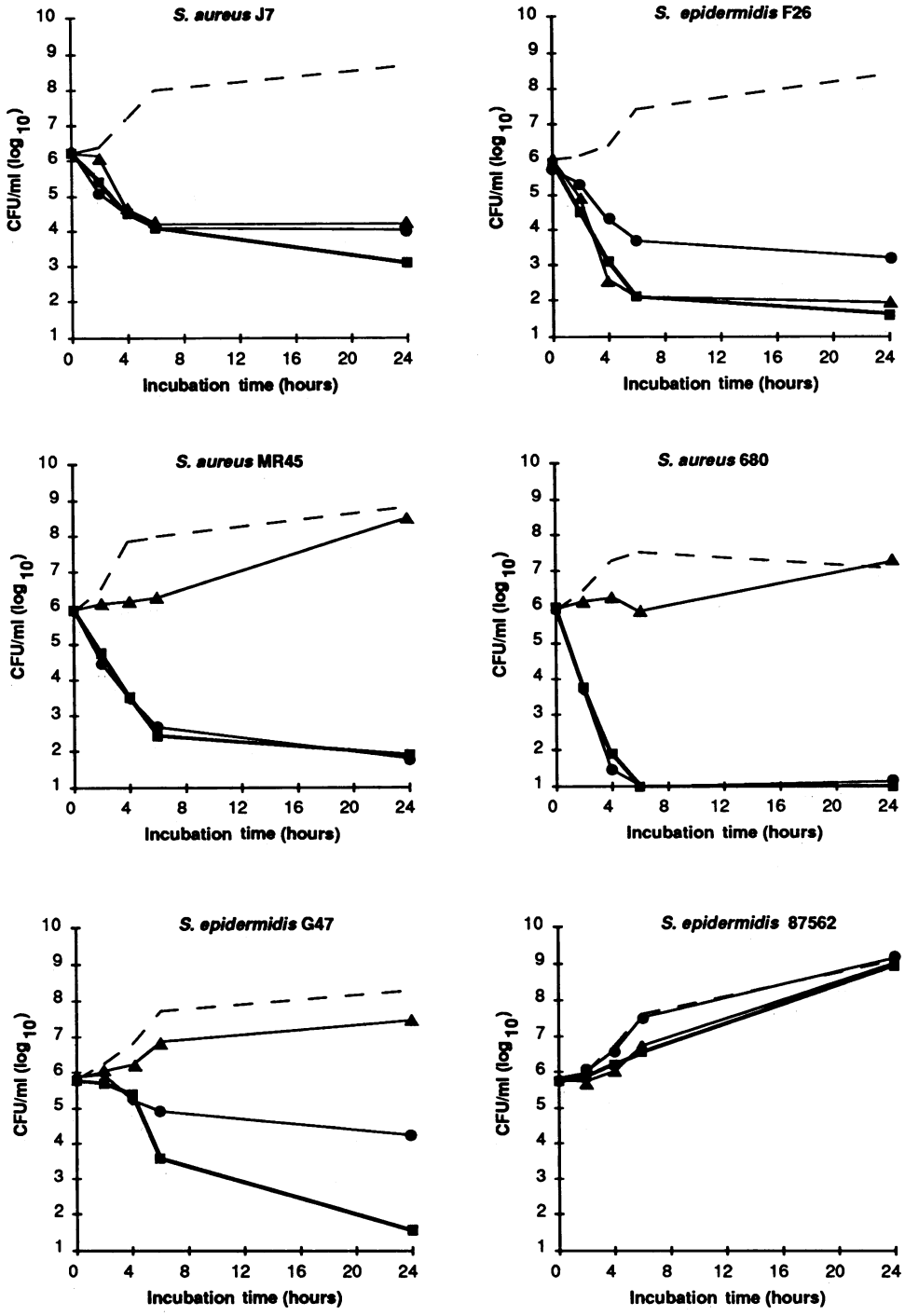

FIG. 1. Bactericidal kinetics of oxacillin (2 μg/ml [▲]) and ofloxacin (1 μg/ml [●]) alone and in combination (■) against six *Staphylococcus* strains. The growth control with no drug is also indicated (– – –).

may postulate that the population of methicillin-resistant staphylococci was heterogeneous with regard to antimicrobial susceptibility, with cells resistant to one drug remaining susceptible to the second. This interpretation would fit with the observation that the synergisms were more readily demonstrated at 30°C than at 35°C, i.e., under conditions which favor the expression of methicillin-resistant organisms (15, 18). Another possibility would be that the quinolone altered the expression of the penicillin-binding protein (PBP) patterns of methicillin-resistant staphylococci, thereby restoring the oxacillin activity. In this respect, methicillin resistance in staphylococci is known to be related to the synthesis of abnormal PBPs (such as PBP 2a or 2′) with a low affinity for β-lactam antibiotics (5, 6, 16, 22, 23). We also know that the antistaphylococcal activity of quinolones results in a morphological alteration of the bacterial cell, consisting of cell enlargement and the cessation of separation. These changes are similar to those produced by ceph-

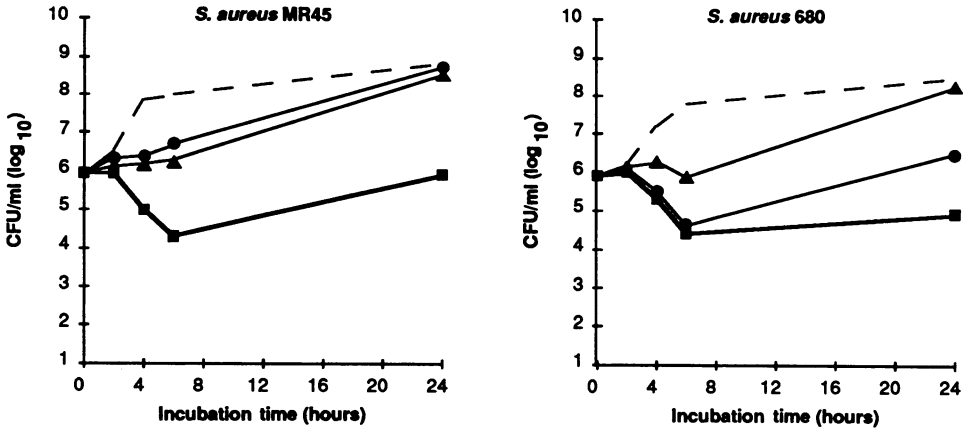

FIG. 2. Bactericidal kinetics of oxacillin (2 μg/ml [▲]) and ofloxacin (0.25 μg/ml [●]) alone and in combination (■) against two MRSA strains susceptible to ofloxacin. – – –, Drug-free control.

alexin (3). Therefore, some direct or indirect interference between the quinolone activity and the metabolism of PBPs cannot be excluded. These hypotheses would fit with the temperature dependence of the described synergism, since more of the abnormal PBP is expressed at 30°C, and with the observation that no synergism occurred in methicillin-susceptible strains.

A third potential mechanism for the synergism could be related to the presence of plasmids in nearly all clinical isolates of methicillin-resistant staphylococci. According to recent studies (13, 22), regulatory genes capable of altering the expression of methicillin resistance seem to be located on these plasmids, which often code for β-lactamase. Quinolones might potentiate the activity of oxacillin owing to their known ability to cure bacteria from extrachromosomal DNA (27). A further possibility, the suppression of β-lactamase production by ofloxacin, could improve the activity of oxacillin.

The successful treatment of infections due to staphylococci with quinolones has been reported. However, there is evidence showing the emergence of quinolone-resistant staphylococci during or after therapy (4). This would limit the clinical application of quinolones alone in the treatment of staphylococcal infections. It remains to be determined to what degree of quinolone-oxacillin combination could reduce the emergence of quinolone-resistant isolates.

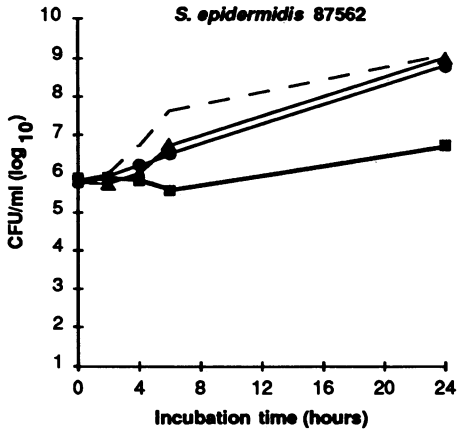

FIG. 3. Killing kinetics of oxacillin (2 μg/ml [▲]) and ofloxacin (4 μg/ml [●]) alone and in combination (■) against a methicillin-resistant *S. epidermidis* strain resistant to ofloxacin. – – –, Drug-free control.

Our study could provide a new approach to the empiric therapy of staphylococcal infections. These infections could be treated with a β-lactamase-resistant penicillin in combination with a quinolone since no antagonism could be demonstrated, whereas a synergism or additive effect for methicillin-resistant strains was observed. This strategy would be especially important for infections due to coagulase-negative staphylococci, characterized by a higher incidence of resistance to oxacillin, in patients with prosthetic materials or compromised immune defenses. However, the recently documented occurrence of quinolone resistance among MRSA strains (19) may compromise the future usefulness of a quinolone-oxacillin combination.

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