

In Vitro Susceptibilities of Oxacillin-Resistant *Staphylococcus aureus* to Dapsone and Sulfamethoxazole Alone and in Combination with Trimethoprim

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Received 5 December 1989/Accepted 23 April 1990

The in vitro activity of trimethoprim plus dapsone against oxacillin-resistant *Staphylococcus aureus* was comparable to that of trimethoprim plus sulfamethoxazole. Because of its different pharmacologic properties, dapsone may be a useful agent in combination with trimethoprim for the treatment of patients colonized with oxacillin-resistant *S. aureus*.

The combination of trimethoprim (TMP)-sulfamethoxazole (SMX) (T/S) has been employed for therapy of patients colonized with oxacillin-resistant *Staphylococcus aureus*; however, the efficacy of such therapy is uncertain, and the development of resistance may complicate treatment (2, 5, 7). A factor that may contribute to therapeutic failure and to the emergence of resistance is the relatively low concentrations of SMX attained in body fluids and secretions, with reported levels rarely achieving 25% of attainable values in serum (12). Previous studies have demonstrated a reduced occurrence of TMP resistance in previously susceptible organisms when TMP is combined with a sulfonamide, such as SMX (6). Dapsone (DAP) has also been shown to have activity against gram-positive organisms (1) and, unlike SMX, may achieve concentrations in skin and other tissues 1.5 to 3.0 times peak values in serum (3, 4, 11). For this reason, we evaluated the in vitro activity of DAP with and without TMP against gram-positive cocci, specifically oxacillin-resistant *S. aureus*, and compared this with the activity of SMX.

(This study was presented in part at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, Calif. [M. Lambertus, R. Y. Y. Kwok, and M. Mulligan, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1334, 1988].)

Sixty isolates of oxacillin-resistant *S. aureus* (MIC > 16 µg/ml) recovered from different patients at the West Los Angeles V.A. Medical Center from April 1984 to October 1988 were studied. Broth microdilution testing with modifications recommended for studies of oxacillin-resistant *S. aureus* (9) was used to determine susceptibilities to TMP, DAP, and SMX alone and to the combinations of T/S and TMP-DAP (T/D). Disk diffusion susceptibilities (8) for T/S were determined prior to the study so that isolates with a range of susceptibilities to this combination could be selected. TMP (Burroughs Wellcome Co., Research Triangle Park, N.C.) was dissolved in HCl (0.05 N, 0.1% final volume). SMX (Burroughs Wellcome) and DAP (Jacobus Pharmaceutical, Princeton, N.J.) were dissolved in acetone

(5% final volume). Final concentrations for TMP were 0.016 to 256 µg/ml, for SMX they were 4.0 to 2,048 µg/ml, and for DAP they were 4.0 to 1,024 µg/ml. T/S was tested at the fixed ratio of 1:19; T/D was tested at both the 1:19 and 1:1 ratios. This latter ratio was chosen to reflect achievable levels in serum with standard oral dosing for each drug (11, 12). Final concentrations for T/D (1:1) were 0.016 to 256 µg/ml, and for T/S and T/D at 1:19 ratios final concentrations were 0.008 to 32 and 0.008 to 64 µg/ml, respectively, expressed as the TMP concentration. *S. aureus* ATCC 29213, *Streptococcus (Enterococcus) faecalis* ATCC 29212, and *Escherichia coli* ATCC 25922 were included as controls.

The MICs of TMP occurred in a bimodal distribution, with clustering at 0.062 to 1.0 and 64 to 256 µg/ml (Fig. 1). A similar bimodal clustering occurred for TMP when it was combined with either SMX or DAP. No TMP-resistant strain (MIC > 1.0 µg/ml) became susceptible with the addition of either DAP or SMX. All strains that were T/S susceptible by disk diffusion were susceptible to TMP by microdilution, with an MIC of ≤1.0 µg/ml; whereas the MICs for all strains that were resistant to T/S by disk diffusion were ≥64 µg/ml. For those isolates with intermediate T/S disk diffusion susceptibility, TMP microdilution MICs were widely scattered, ranging from 0.062 to >256 µg/ml. There were wide susceptibility ranges for SMX and DAP. DAP MICs were generally one dilution higher than those of SMX. No strain was susceptible to DAP alone when a breakpoint of 4 µg/ml was used, while 13 of 60 strains (22%) were susceptible to SMX with a breakpoint of 64 µg/ml. T/S and T/D had virtually identical activities at the ratio of 1:19 (Fig. 1). T/D at the ratio of 1:1 was less active, with MICs that were one to three dilutions higher than those of T/D and T/S at the 1:19 ratio. However, all isolates susceptible to T/S at the 1:19 ratio were susceptible to T/D at the 1:1 ratio. The relative susceptibilities to the fixed concentrations are presented in a scattergram (Fig. 2).

Previous studies indicated that DAP might have greater activity against gram-positive cocci than SMX (1). We did not find that to be the case for oxacillin-resistant *S. aureus*. The combination of T/D had in vitro activity that was comparable but not superior to that of T/S. No strains susceptible to T/S at the ratio of 1:19 were resistant to T/D at either the 1:1 or 1:19 ratio. The addition of either SMX or

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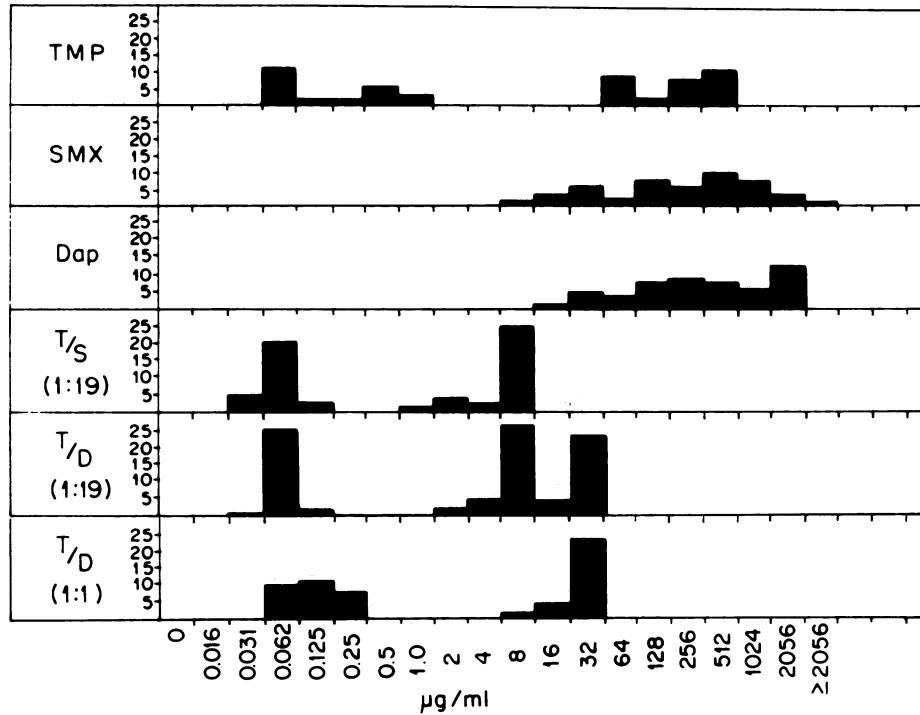


FIG. 1. Susceptibility patterns for *S. aureus* strains. Numbers of isolates for each MIC are represented on the ordinate. Concentrations of antibiotic combinations are represented as the TMP concentrations.

DAP reduced the TMP MIC by at least a twofold dilution for 62% of isolates, although no isolate resistant to TMP alone became susceptible with the addition of either SMX or DAP. It is clear that TMP is the crucial agent in determining

susceptibility, although the addition of a sulfonamide or sulfone reduces the TMP MIC and may help prevent the development of TMP resistance. Our study did not examine this issue. We found that routine disk diffusion susceptibility tests results that clearly indicated susceptibility or resistance correlated well with TMP MICs for the isolates, with MIC ranges of 0.031 to 1.0 and 64 to 512 µg/ml, respectively. Intermediate disk diffusion susceptibility (11 to 15 mm) was not predictive of microdilution results, with isolates having widely scattered MICs of TMP (0.062 to 512 µg/ml).

Differences in pharmacologic properties between DAP and SMX make DAP potentially useful in the treatment of patients colonized with oxacillin-resistant *S. aureus*. Although both drugs exhibit 65 to 70% protein binding, the volume of distribution and tissue concentrations of DAP are greater than those of SMX. The tissue-to-serum-concentration ratios for saliva are 0.2 for DAP and 0.03 for SMX, while for skin and muscle the ratio for DAP is 1.0 to 1.2. No tissue-to-serum-concentration ratio exceeds 0.5 for SMX (10, 12). The long half-life of DAP compared with that of SMX (28 and 10 h, respectively) may also offer an advantage in treatment, producing longer periods of effective antibiotic levels at sites of colonization, such as nares and surgical wounds.

Although there is debate about the need for treating patients colonized with oxacillin-resistant *S. aureus*, oral agents are commonly employed for this purpose. Because of significant variability in susceptibilities to such agents, empiric treatment of colonization without susceptibility testing seems unwise. It is important to point out that either TMP combination (with SMX or DAP) was active against only approximately 50% of the isolates in our study. These isolates were preselected to represent a wide range of susceptibility patterns and did not represent the susceptibility pattern in our hospital. Our study indicates that isolates

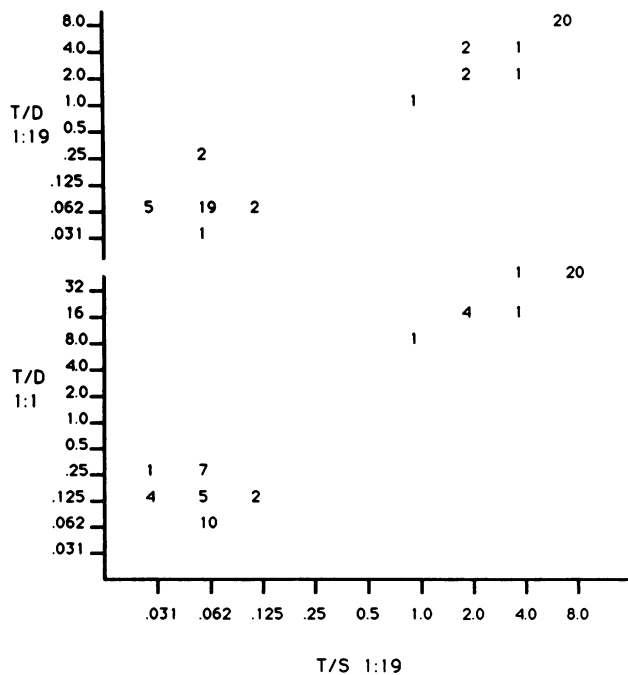


FIG. 2. Scattergram of *S. aureus* strains comparing T/S at 1:19 with T/D at 1:1 and 1:19 ratios. Numbers in the scattergram indicate numbers of isolates at each point. Regression analyses for the T/D ratios are as follows: 1:1 T/D: $y = 1.01 + 3.94x$, $R^2 = 0.965$; and 1:19 T/D: $y = 0.24 + 0.98x$, $R^2 = 0.965$.

susceptible to T/S are also susceptible to T/D at 1:1 and 1:19 concentration ratios. Because DAP has a long half-life and achieves concentrations with tissue-to-serum ratios greater than those of SMX, T/D may be worthy of further study for the treatment of colonized patients.

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