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Activity of Tetracycline, Doxycycline, and Minocycline Against Methicillin-Susceptible and -Resistant Staphylococci

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Tetracycline, doxycycline, and minocycline were evaluated for their antibacterial activity against methicillin-susceptible and -resistant isolates of *Staphylococcus*. At clinically achievable levels both doxycycline and minocycline were more active than tetracycline against methicillin-susceptible organisms. Tetracycline and doxycycline had no activity against methicillin-resistant staphylococci, whereas minocycline at 2 μg/ml inhibited six of 13 strains and, at 3 μg/ml, 10 of 13 strains.

Staphylococci that are resistant to semisynthetic penicillins and cephalosporins have been isolated with increasing frequency in Europe and the Middle East and have been shown to cause infections that respond poorly to antibiotic treatment (3, 5). O'Toole and Barrett suggested that infection due to methicillin-resistant staphylococci may gradually be increasing in this country (2, 13). Our study was designed to compare the activity of tetracycline and two new tetracycline derivatives against methicillin-susceptible and -resistant staphylococci.

MATERIALS AND METHODS

Bacteria. Thirty-seven isolates of staphylococci (28 of *Staphylococcus aureus* and nine of *Staphylococcus epidermidis*) were obtained over a 6-month period from clinical specimens at the Veterans Administration Hospital, Houston, Tex., and the Texas Children's Hospital. Except for an attempt to obtain these isolates from different hospital areas and at different times, the bacteria were selected at random. They were identified and classified by conventional laboratory methods; all were susceptible to methicillin. Thirteen strains of *S. aureus* which were resistant to methicillin were obtained from Scandinavia, Ethiopia, and Israel. These strains were kindly provided by M. Barza, J. Sherris, and G. Altmann.

Antibiotics. Standard tetracycline and minocycline powders of known potency were obtained from Lederle Laboratories, Pearl River, N.Y.; doxycycline powder was provided by Pfizer Laboratories, New York, N.Y. In order to verify resistance to methicillin, commercially available sodium methicillin monohydrate was used. All antibiotic solutions were made up the day of testing and used only once.

Media. Freshly prepared Trypticase soy broth (BBL) at pH 7.3 was used.

Susceptibility testing. For tetracycline, minocycline, doxycycline, and methicillin, tubes containing 1.5 ml of Trypticase soy broth with 10, 7, 4, 3, 2, 1, 0.5, 0.1, and 0.05 μg of antibiotic per ml were made up. For methicillin-resistant staphylococci the concentrations were 200, 100, 50, 25, 15, 10, 7, 5, and 2 μg/ml. To each tube bacteria were added to give an inoculum size of 10^5 organisms/ml. The lowest concentration of antibiotic at which there was no visible growth after 18 h of incubation at 37 C was considered to be the minimal inhibitory concentration (MIC). Twenty-five microliters from each clear tube was transferred to brain heart infusion agar, and the lowest concentration of antibiotic at which no growth could be observed after overnight incubation was considered to be the minimal bactericidal concentration (MBC).

Antibiotic disk susceptibility. Petri dishes (15 by 100 mm) containing 20 ml of Mueller-Hinton agar were made up the day before use. All bacteria were allowed to come to full growth by overnight incubation; repeated studies using serial 10-fold dilutions showed this concentration to be between 2×10^9 and 3×10^9 colony-forming units/ml. The bacteria were then diluted to 10^6 colony-forming units/ml in distilled water. The Mueller-Hinton agar plates were flooded with 3 ml of this bacterial suspension and allowed to stand for about 3 min. The plates were then tilted and excess fluid was aspirated by using Pasteur pipettes, and the plates were allowed to dry for 4 to 10 min. Antibiotic disks containing 30 μg of tetracycline, doxycycline, or minocycline and 5 μg of methicillin were then placed on the agar and allowed to stand for 0.5 h at room temperature. Overnight incubation was carried out at 37 C. A duplicate set of plates were incubated at 35 C. Plates prepared in this fashion showed a uniform pattern of bacterial growth in which individual colonies were just touching each other, but were distinct. The margins of the zone of growth inhibition were easily measurable to within 1 mm by several different observers. Results for the same strain were reproducible from day to day.

RESULTS

Of 28 isolates of *S. aureus*, 22 and 20 had an MIC and MBC, respectively, ≤3 μg of tetracycline per ml. An MIC and MBC ≤2 μg/ml was observed, respectively, with doxycycline for 26

and 17 of the isolates and with minocycline for 28 and 27 of the isolates. Of nine *S. epidermidis* strains, five were resistant to 3 μg of tetracycline per ml; all had MICs ≤1 μg of doxycycline and minocycline per ml, and six and eight had MBCs ≤2 μg/ml, respectively, of these two antibiotics. Cumulative data summarizing the effect of these three tetracyclines on staphylococci are presented in Fig. 1.

In the clinical microbiology laboratory, all 37 strains of staphylococci which had been isolated in Houston were found to be susceptible to methicillin and/or cloxacillin by the Kirby-Bauer disk-sensitivity method. This finding was confirmed by tube-dilution studies which showed the mean MIC and MBC of methicillin with 12 of these strains to be 0.27 and 0.4 μg/ml, respectively. (The cloxacillin disk does not reliably detect resistant organisms [7]). Insusceptibility of the methicillin-resistant strains of *S. aureus* to methicillin was verified by studies in broth at 37 C. Results are shown in Fig. 2. The most susceptible organism had an MIC of 2 μg/ml and an MBC of 5 μg/ml. These organisms all were inhibited by 100 μg/ml, but seven were not killed by 200 μg/ml. The zones measured after incubation at 35 C correlated better with the MIC than those measured after incubation at 37 C. All 13 strains were found to be resistant to 10 μg of tetracycline per ml and to 3 μg of doxycycline per ml. By contrast, the MIC and MBC, respectively, of minocycline were ≤2 μg/ml in six and three of 13 strains (Fig. 3).

Figure 4 compares the zones of inhibition produced by tetracycline and minocycline disks with 25 strains of tetracycline-resistant staphylococci (including both methicillin-susceptible and -resistant strains), plotted against the MIC

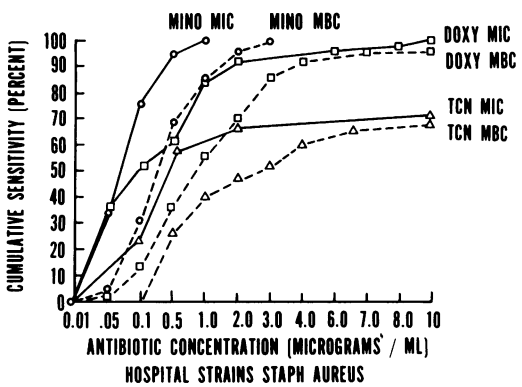

FIG. 1. *Cumulative susceptibility of 37 hospital-acquired staphylococcal isolates to tetracycline, doxycycline, and minocycline.*

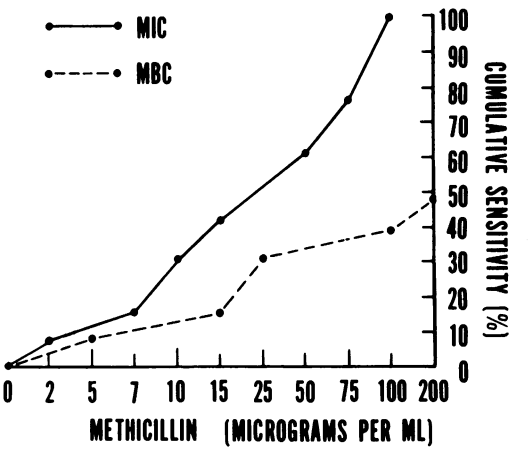

FIG 2. *Cumulative susceptibility of 13 methicillin-resistant staphylococci to methicillin.*

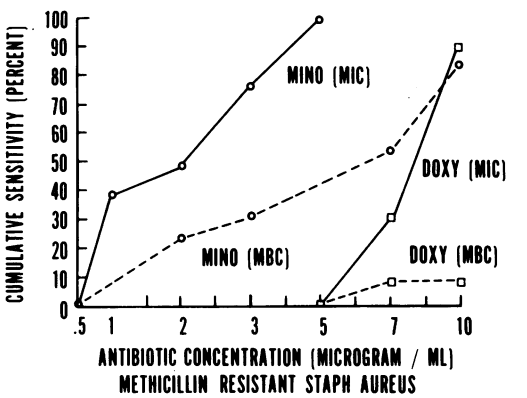

FIG. 3. *Cumulative susceptibility of 13 methicillin-resistant staphylococci to doxycycline and minocycline.*

of minocycline. The zones around tetracycline disks were all <11 mm. Eighteen of these strains were susceptible to ≤2 μg of minocycline per ml; all except one had exhibited zones of inhibition of ≥20 mm around minocycline disks. Strains that were susceptible to doxycycline but resistant to tetracycline had zones of ≥13 mm around the doxycycline disk.

DISCUSSION

Doxycycline and minocycline are two tetracycline analogues that are reportedly more effective against gram-positive bacteria than were earlier tetracycline derivatives (8, 9). The exceptions to this remain the groups B and D streptococci, only 50 and 10% of which, respectively, are susceptible to achievable concentrations in serum (14). Both of these antibiotics have been reported to be more effective against

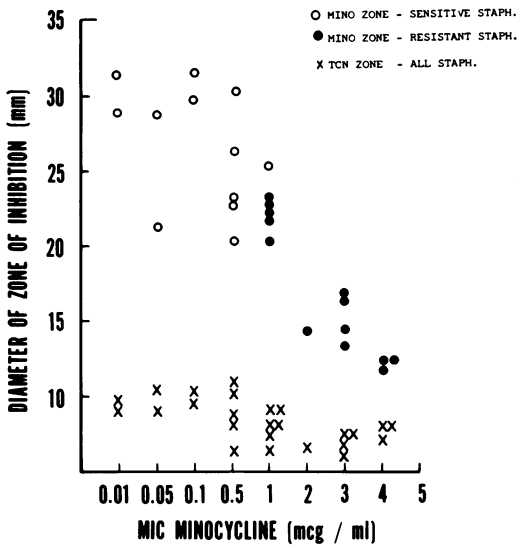

FIG. 4. *Comparison of tetracycline and minocycline zones of inhibition for both methicillin-susceptible and -resistant staphylococci, all of which are resistant to tetracycline, plotted against the MIC of minocycline.*

S. aureus (9, 14). Our data on 37 strains of staphylococci which were isolated by our clinical laboratories in Houston show that 67% were inhibited by tetracycline compared with 95 and 100%, respectively, by doxycycline and minocycline. Thirteen methicillin-resistant strains obtained from abroad were all resistant to tetracycline and doxycycline; by contrast, six had an MIC ≤ 2 μg of minocycline per ml. These results suggest that doxycycline as well as minocycline may be effective in vivo against tetracycline-resistant staphylococci (9). They further suggest that minocycline may find a role in treating infections caused by staphylococci which are resistant to semisynthetic penicillins and cephalosporins.

Antibiotic susceptibility determined by using the tetracycline disk correlated well with data obtained when tetracycline was studied by tube dilution against all 50 strains, but, predictably, failed to distinguish the isolates that were resistant to tetracycline yet susceptible to doxycycline or minocycline. Our findings agree with previous studies (1, 9) in showing that the tetracycline disk does not detect susceptibility of staphylococci to all members of the tetracycline family, as has been maintained by the Food and Drug Administration and others (11). Further studies are needed to understand the clinical significance of this finding.

Although 3 μg/ml is often used as a cut-off point to express susceptibility to deoxycycline and minocycline, published data suggest that this level would only be reached in patients who can tolerate a dosage higher than that which is recommended (4, 10, 12, 15); a level of 2 μg/ml appears to be more appropriate and was used to report data in these studies.

Observations on MICs in the present study were made after the conventional overnight (18-h) incubation. When tests were incubated for 42 to 48 h, the MIC tended to approach the MBC. Whereas tetracyclines are generally not considered to be bactericidal, it has been shown that there is slow killing of both wall-deficient and normal staphylococci after prolonged incubation with tetracyclines (6, 14). In other studies (unpublished data) we have shown that subculturing 0.1-ml samples after 48 h of exposure to tetracyclines shows no colony-forming units.

These data suggest that minocycline may be a useful drug in treating infection caused by staphylococci which are resistant to the semisynthetic penicillins and cephalosporins.

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