In Vitro Antimicrobial Susceptibility of Actinobacillus actinomycetemcomitans

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The agar dilution technique was used for determination of the antibiotic susceptibilities of 57 oral isolates and 2 nonoral isolates of Actinobacillus actinomycetemcomitans. Tetracycline, minocycline, and chloramphenicol inhibited more than 96% of the strains tested at a concentration of ≤2 µg/ml; 89% of the strains were inhibited by 2 µg of carbenicillin per ml. The other antimicrobial agents tested were less active. Approximately 10% of the Actinomyces strains were resistant to ampicillin, erythromycin, and penicillin G at concentrations of 32 to 64 µg/ml. These data suggest that tetracycline and minocycline may be valuable drugs in the treatment of Actinomycetemcomitans infections.

Actinobacillus actinomycetemcomitans is a facultative, gram-negative bacterium of clinical importance. It has been isolated from actinomycosis (3, 5, 9, 10), periodontitis (24; J. Slots, H. S. Reynolds, P. M. Lobbs, and R. J. Genco, J. Dent. Res., 59: 328, Special Issue A, Am. Assoc. Dent. Res., abstr. no. 244, 1980), endocarditis (6, 16, 19, 27), vertebral osteomyelitis (14), brain abscess (12), thyroid gland abscess (4), and urinary tract infection (26). A. actinomycetemcomitans is often a constituent of mixed bacterial infections, but it can also be the sole infecting agent (25, 28). A. actinomycetemcomitans can be a part of the indigenous oral microflora (9), and the portal of entry of this organism is often assumed to be the oral cavity and the upper respiratory tract.

A. actinomycetemcomitans strains vary in in vitro susceptibilities to penicillin, ampicillin, and other commonly used antibiotics (8, 11, 16). Evidence that this diversity affects therapy is suggested by the fact that a given antibiotic has been effective in certain cases of A. actinomycetemcomitans infection, but has failed in others (2, 16, 27).

Reports on the antibiotic susceptibility of A. actinomycetemcomitans are few, and only a limited number of strains have been examined. In this paper, we report the susceptibility of 59 isolates of Actinomyces actinomycetemcomitans to 15 antimicrobial agents.

MATERIALS AND METHODS

Bacteria. Of the 59 strains tested, 55 were isolated in our laboratory from samples of saliva, from tongue dorsum, and from supragingival and subgingival dental plaque of 17 subjects. Only one strain was isolated from each sample site. The strains were recovered on Trypticase soy agar (BBL Microbiology Systems) supplemented with 75 µg of bacitracin (Sigma Chemical Co.) per ml, 10% heat-inactivated horse serum, 0.1% yeast extract, and 0.2% glucose after 4 days of incubation at 37°C in 5% CO2-air or in 5% N2-10% H2-5% CO2. One strain (Y4) from a juvenile periodontitis lesion was isolated by S. S. Socransky, The Forsyth Dental Center, Boston, Mass. Strains ATCC 29522 (mandibular abscess), ATCC 29523 (blood), and ATCC 29524 (chest aspirate) were received from the American Type Culture Collection, Rockville, Md. Cultures were either maintained on Trypticase soy agar or frozen at −80°C.

All strains utilized carbohydrates fermentatively, were capnophilic, decomposed H2O2, reduced nitrate, and were benzidine positive. None of the strains required X (hebin) and V (nicotinamide adenine dinucleotide) growth factors or hydrolyzed esculin, gelatin, starch, or urea, and none produced acetyl-methylcarbinol, decarboxylases, H2S, oxidase, or indole. All strains produced acid from glucose, fructose, and mannose but not from adonitol, amygdalin, arabinose, cellobiose, dulcitol, esculin, galactose, glycero1, inositol, inulin, lactose, melizitose, melezitose, rhamnose, ribose, salicin, sorbitol, or sucrose. Various fermentation results were obtained with dextrin, maltose, mannitol, and xylose.

Antimicrobial agents. Powders of the various agents were obtained as follows: ampicillin (Sigma Chemical Co.), bacitracin (Sigma Chemical Co.), carbenicillin (J. B. Roerig, Div. of Pfizer Laboratories), chloramphenicol (Parke, Davis & Co.), clindamycin (The Upjohn Co.), erythromycin (Sigma Chemical Co.), kanamycin sulfate (Sigma Chemical Co.), lincomycin (The Upjohn Co.), metronidazole (G. D. Searle & Co.), minocycline hydrochloride (Lederle Laboratories), neomycin (Sigma Chemical Co.), penicillin G (Sigma Chemical Co.), polymyxin B (Sigma Chemical Co.), tetracycline hydrochloride (Sigma Chemical Co.), and vancomycin (Sigma Chemical Co.).

Antimicrobial susceptibility testing. Minimal inhibitory concentrations were determined by the agar dilution technique (23) with Wilkins-Chalgren medium (29). A dilution series of from 128 to 0.1 µg/ml...
was used for the antimicrobial agents tested. The Wilkins-Chalgren agar plates were made within 48 h of use.

Bacterial inocula were prepared from 48-h anaerobic cultures of the organisms in enriched brain heart infusion broth (BBL Microbiology Systems). The cultures were diluted to the turbidity of a McFarland no. 1 barium sulfate standard and dispensed with a Steers replicator (21) onto the antimicrobial agent-containing plates. The spots were allowed to dry, and the plates were incubated in an anaerobic chamber (Coy Manufacturing Co.) with 85% N₂, 10% H₂, and 5% CO₂ for 48 h. The minimal inhibitory concentration was defined as the lowest concentration allowing no detectable growth.

Penicillinase production was determined by the iodometric assay developed by Rosenblatt and Neumann (17). The test was carried out with 48-h blood agar plate cultures of the test actinobacillus strains.

RESULTS

The in vitro antibacterial activities of the 15 antimicrobial agents tested against 59 isolates of A. actinomycetemcomitans are shown in Table 1. A total of four antibiotics showed good activity at the peak serum concentrations that can be expected with frequently used clinical protocols (18). All strains examined were inhibited by chloramphenicol at 2 μg/ml (achievable serum concentrations, 3 to 12 μg/ml), by minocycline at 4 μg/ml (achievable serum concentrations, 0.7 to 4.5 μg/ml), by polymyxin B at 2 μg/ml (achievable serum concentrations, 2 to 8 μg/ml), and by tetracycline at 1 μg/ml (achievable serum concentrations by oral and intravenous routes of administration, respectively, 1 to 5 and 2 to 30 μg/ml). Carbenicillin also generally showed good activity, although about 10% of the test strains were not inhibited at 32 μg/ml.

The remaining 10 antimicrobial agents exhibited poor activity against A. actinomycetemcomitans. Only about 50% of the strains were inhibited by 4 μg of ampicillin, erythromycin, kanamycin, metronidazole, penicillin G, or vancomycin per ml, and several strains still exhibited resistance to these six antimicrobial agents at concentrations of 16 to 64 μg/ml. The concentrations of clindamycin, lincomycin, and neomycin required to inhibit the growth of 50% of the total number of strains tested were 16 to 32 μg/ml. The test strains, including ATCC 29522, ATCC 29523, ATCC 29524, and Y4, which were recovered by nonselective culturing, exhibited a high level of resistance to bacitracin. In fact, no strains were inhibited at a concentration of bacitracin of 128 μg/ml.

The test for penicillinase showed no white color developing within 5 min after mixing of the starch solution with the penicillin-iodine-bacterium mixture, indicating that the strains tested did not produce penicillinase.

DISCUSSION

The recognition that A. actinomycetemcomitans infections are potentially more serious, more difficult to cure, and probably more common than previously realized stimulates interest in the susceptibility of the organism to antimicrobial agents. Of the commonly used antibiotics examined in this study, tetracycline, minocycline, and chloramphenicol, on a weight basis, were the most effective against A. actinomycetemcomitans; all of the organisms tested were susceptible to these antibiotics at a concentration of 4 μg/ml, whereas only about 50% were susceptible to ampicillin, erythromycin, and penicillin G at this concentration. The differ-

| Table 1. Susceptibility of A. actinomycetemcomitans to selected antimicrobial agents |
|-----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Agent                            | Cumulative % of strains inhibited at following concn (μg/ml)* |
|                                  | 0.1             | 1               | 2               | 4               | 8               | 16              | 32              | 64              | 128             |
|                                  | 0               | 5               | 24              | 54              | 66              | 80              | 83              | 83              | 100             |
| Ampicillin                       | 0               | 5               | 24              | 54              | 66              | 80              | 83              | 83              | 100             |
| Bacitracin                       | 84              | 88              | 89              | 89              | 89              | 89              | 91              | 93              | 98              |
| Carbenicillin                    | 0               | 90              | 100             | 90              | 90              | 90              | 90              | 90              | 100             |
| Chloramphenicol                  | 0               | 20              | 29              | 29              | 37              | 42              | 54              | 61              | 100             |
| Erythromycin                     | 2               | 29              | 34              | 47              | 53              | 63              | 63              | 90              | 98              |
| Kanamycin                        | 37              | 39              | 44              | 58              | 76              | 95              | 100             | 100             | 100             |
| Lincomycin                       | 37              | 39              | 44              | 58              | 76              | 95              | 100             | 100             | 100             |
| Metronidazole                    | 60              | 60              | 61              | 63              | 70              | 91              | 95              | 100             | 100             |
| Minocycline                      | 13              | 91              | 96              | 100             | 100             | 100             | 100             | 100             | 100             |
| Neomycin                         | 0               | 0               | 3               | 37              | 76              | 100             | 100             | 100             | 100             |
| Penicillin G                     | 0               | 31              | 47              | 54              | 71              | 71              | 78              | 93              | 100             |
| Polymyxin B                      | 47              | 68              | 100             | 100             | 100             | 100             | 100             | 100             | 100             |
| Tetracycline                     | 63              | 100             | 100             | 100             | 100             | 100             | 100             | 100             | 100             |
| Vancomycin                       | 16              | 37              | 47              | 47              | 49              | 49              | 56              | 96              | 100             |

* A total of 59 strains were tested.
ences were even more pronounced at lower concentrations. For example, a concentration of 0.1 μg of tetracycline per ml inhibited 63% of the test strains, whereas ampicillin, erythromycin, and penicillin G were ineffective.

Previous reports of limited in vitro susceptibility testing of *A. actinomycetemcomitans* agree that this organism is susceptible to low concentrations of tetracycline (8, 11, 16, 22) and chloramphenicol (11, 16) and is frequently resistant to ampicillin (16), erythromycin (8), penicillin (8, 11, 16), clindamycin (27), lincomycin (20, 27), and vancomycin (7).

Despite favorable in vitro susceptibility data with tetracycline, there are only a few published results on the clinical efficacy of this antibiotic for *A. actinomycetemcomitans* infections. Bartlett et al. (1) reported that, in one actinomycosis patient treated with lincomycin, an infection due to *A. actinomycetemcomitans* resistant to lincomycin emerged and a cure was finally achieved with tetracycline. Page and King (16) described three patients with endocarditis who were cured after treatment with tetracycline.

Poor activity of penicillin against *A. actinomycetemcomitans* has been shown in actinomycosis-like infections which were not cured by penicillin due to the persistence of a penicillin-resistant strain of *A. actinomycetemcomitans* (9, 10, 25). Cases of *A. actinomycetemcomitans* endocarditis which were not resolved by penicillin (2, 12, 15, 16) or which followed a tooth extraction with penicillin prophylaxis (13) have also been reported. *A. actinomycetemcomitans* endocarditis has responded favorably to combinations of antibiotics, including ampicillin, chloramphenicol, erythromycin, and streptomycin, but fatal cases in which such therapeutic regimes were used have also been reported (2, 16, 19, 27), raising doubt concerning the universal efficacy of these combinations.

Since *A. actinomycetemcomitans* appears to play an important role in the progression of certain periodontal diseases (24), the present in vitro susceptibility data showing that tetracycline and minocycline were highly active against *A. actinomycetemcomitans* will be useful in periodontal treatment considerations. It should also be noted that the concentration of tetracycline is 2- to 10-fold higher in the periodontal pocket area than in the blood (J. M. Gordon, C. B. Walker, J. C. Murphy, J. M. Goodson, and S. S. Socransky, J. Dent. Res. 59: 513, Special Issue A. Am. Assoc. Dent. Res., abstr. no. 977, 1980). Therefore, if no contraindications exist, antibiotics of the tetracycline group appear to be the drugs of choice in treatment of periodontal infections of *A. actinomycetemcomitans*.

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**LITERATURE CITED**


