ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1974, p. 302-309

Vol. 5, No. 3
Printed in U.S.A.

Susceptibility of Pathogenic Actinomycetes to Antimicrobial Compounds

PHILLIP I. LERNER[1]

Department of Medicine, Case Western Reserve University School of Medicine, and Veterans Administration Hospital, Cleveland, Ohio 44106

Received for publication 3 December 1973

Current interest in antimicrobial susceptibility testing of anaerobic pathogens and recent recognition that actinomycetes other than *Actinomyces israelii* may cause actinomycosis in man prompted this in vitro survey of 74 strains of actinomycetes, representing seven species. Minimum inhibitory concentrations (MICs) for 24 antimicrobials were determined by inhibition of gross colonial enlargement in semisolid antibiotic agar after incubation at 37 C for 48 h under anaerobic conditions. Erythromycin and rifampin were the most active drugs in vitro (MICs of 0.008 to 0.25 μg/ml), although a small number of non-israelii strains were conspicuously more resistant to the latter (MICs >0.5 μg/ml). Penicillin G, cephaloridine, minocycline, and clindamycin were also very active in vitro (MICs of 0.03 to 1.0 μg/ml); for a few non-israelii strains the MICs of clindamycin were 2.0 to 8.0 μg/ml. MICs of cephalothin, ampicillin, lincomycin, tetracycline, doxycycline, and chloramphenicol were well within a therapeutic range for all strains of *A. israelii* and most other species, although the MIC of lincomycin against a few non-israelii strains and of tetracycline and doxycycline against the majority of these strains was 2.0 to 8.0 μg/ml. Oxacillin, dicloxacillin, and cephalexin were less active in vitro, particularly against strains other than *A. israelii*. Most non-israelii species were not suppressed by 125 μg of metronidazole per ml, which concentration inhibited all strains of *A. israelii;* otherwise, there were no antimicrobial susceptibility differences among the species tested. Aminoglycoside activity was negligible.

Although penicillin is the drug of choice in actinomycosis, other antimicrobials, especially tetracylines, have been employed with success either in penicillin-allergic patients or in circumstances where penicillin failed to effect a cure (30). Earlier studies from this laboratory described the in vitro susceptibility of *Actinomyces*, primarily *Actinomyces israelii*, to cephalosporins and lincomycins (23, 24). Recognition of the pathogenic role of other actinomycetes in human infection (Table 1), the introduction of a number of newer antimicrobials, and the recent widespread interest in susceptibility testing of anaerobic pathogens prompted the broader survey herein reported.

MATERIALS AND METHODS

Seventy-four strains of actinomycetes were collected from clinical isolates recovered at hospitals in the Cleveland area and from the Mycology Unit at the Center for Disease Control in Atlanta, Ga., The National Collection of Type Cultures in London, England, and the Department of Microbiology, West Virginia University, Morgantown. The following species were studied: *A. israelii*, 32 strains; *A. eriksonii*, 6 strains; *Arachnia propionica*, 7 strains; *A. naeslundii*, 5 strains; *A. viscosus*, 15 strains; *A. odontolyticus*, 5 strains; and *A. bovis*, 4 strains. The latter two species, not as yet associated with disease in man, were included for comparative purposes only.

Inhibition of gross colonial enlargement in antibiotic-containing semisolid agar (Actinomyces Broth, BBL), as previously described (23), a modification of the method of Blake (2), was employed to determine the in vitro susceptibility of actinomycetes to the following antimicrobial compounds, supplied as indicated: penicillin G, streptomycin, doxycycline (Pfizer Co., Inc.); ampicillin, oxacillin, dicloxacillin (Bristol Laboratories); cephalothin, cephaloridine, cephalexin, vancomycin, capreomycin, erythromycin, cycloserine (Eli Lilly & Co.); chloramphenicol (Parke, Davis & Co.); fusidic acid (Leo Pharmaceutical Products, Ballerup, Denmark); novobiocin, lincomycin, clindamycin (Upjohn Co.); metronidazole (Searle); rifampin (Ciba Chemical and Dye Co.); sulfisoxazole (Hoffman-La Roche, Inc.); tetracycline, minocycline

[1] Present address: Mt. Sinai Hospital, Cleveland, Ohio 44106.

TABLE 1. *Actinomycetes, other than A. israelii, associated with human disease*[a]

Actinomycete	Human disease
A. naeslundii	Gall bladder empyema, wounds, bacteremia, cervicofacial disease, suppurative thyroiditis
Arachnia propionica	Cervicofacial disease, renal abscess, infected human bite, lacrimal canaliculitis, lung abscess, empyema
A. eriksonii	Lung and wound abscess, empyema
A. viscosus	(Aerobic; catalase positive [?] *A. naeslundii*) wound, sinus tract

[a] See references 4, 7, 14-16, 22, 32.

(Lederle Laboratories); gentamicin (Schering Laboratories).

Discrete colonies grown on the surface of sheep blood agar plates (incubated at 37 C in a Brewer Gas Pak jar for 4 to 5 days) were submerged in 5 ml of semisolid actinomyces agar (0.35%) by placing an agar square (0.5 by 0.5 cm) a few millimeters below the semisolid agar surface and sealing the tube under pyrogallol carbonate seal (1). After overnight incubation at 37 C, the presence or absence of visible colonial enlargement was readily determined by gross inspection (23). To verify, when necessary, equivocal overnight results, 48-h readings were also done. To avoid bias, only 48-h minimum inhibitory concentrations (MICs) are reported, although 75 to 85% of the assay end points (varying with different antibiotics) remained unchanged after the longer incubation. Attempts to determine bactericidal end points were not successful (23); this will be discussed subsequently.

RESULTS

Figures 1 through 12 depict the range of MICs of 19 antimicrobial compounds. The number of strains inhibited by a given drug concentration is indicated within the bar. Cycloserine, sulfisoxazole, streptomycin, gentamicin, and capreomycin were also screened and will be discussed separately. The total number of strains represented in any single bar may not agree with the total number originally available for study, inasmuch as occasional contaminants, and particularly commensals, interfered with assays in some instances.

In Fig. 1, the range of MICs of penicillin G was 0.03 to 0.5 μg/ml for all 32 *A. israelii* strains and 0.06 to 0.5 μg/ml for all other actinomycetes except one strain of *A. naeslundii* and three of four *A. bovis* strains, for which the MIC was 1.0 μg/ml. Ampicillin was slightly less active, particularly against some of the non-israelii species, but was active at concentrations well within a therapeutic range.

Figure 2 demonstrates the susceptibility patterns of three cephalosporins—cephaloridine, cephalothin, and cephalexin. As is true for most gram-positive bacteria, cephaloridine was the most active in vitro, cephalexin the least, and cephalothin occupied an intermediate position. The in vitro activity of cephaloridine was almost identical to that of penicillin G. Even though the least active in vitro, cephalexin given by mouth in doses of 4.0 g per day would provide serum levels in excess of MICs for all

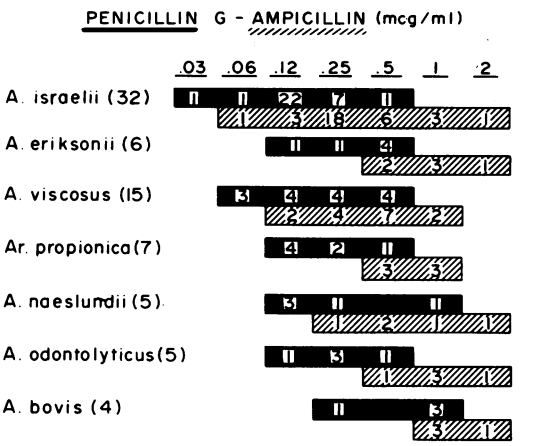

FIG. 1. *Susceptibility of actinomycetes to penicillin G and ampicillin.*

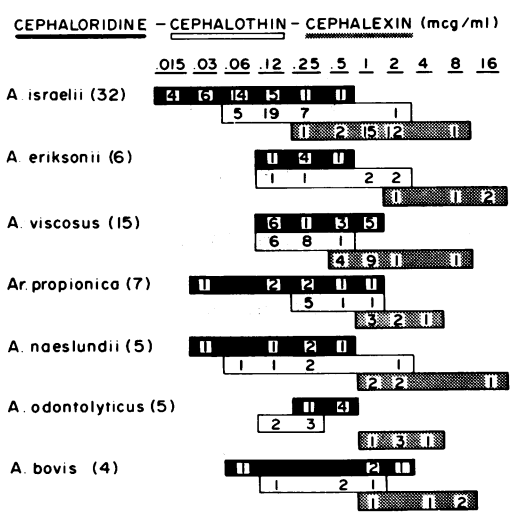

FIG. 2. *Susceptibility of actinomycetes to cephaloridine, cephalothin, and cephalexin.*

strains of *A. israelii* and most, but not all, strains of the other species. Three strains (two *A. eriksonii*; one *A. naeslundii*) were inhibited only by 16 μg of cephalexin per ml.

Figure 3 depicts the MICs of tetracycline and two newer analogues—minocycline and doxycycline. Minocycline was the most active of the three drugs against all of the species tested. Doxycycline was somewhat less active and tetracycline, as might be expected, was the least active, although all strains of *A. israelii*, *A. viscosus*, *A. propionica*, and *A. bovis* and approximately one-half of the remaining strains were suppressed by 2 μg of tetracycline per ml, an easily attained serum concentration. Only actinomycetes other than *A. israelii* had MICs exceeding 2 μg of tetracycline per ml. The uniform susceptibility of all 15 strains of *A. viscosus* to 2 μg of tetracycline per ml was noteworthy.

Figure 4 illustrates the susceptibility patterns with clindamycin and lincomycin. All *A. israelii* strains were inhibited by 0.06 to 0.5 μg of the former per ml and 0.06 to 1.0 μg of the latter per ml. A small proportion of the other species required 2.0 to 8.0 μg of either drug per ml for inhibition. The unusually wide range of MICs for strains of *A. propionica* and *A. naeslundii* was not observed with other antimicrobials in this survey.

The results obtained with oxacillin and dicloxacillin are shown in Fig. 5. Oxacillin in concentrations of 0.5 to 8.0 μg/ml suppressed all strains of *A. israelii;* only two strains of *A.*

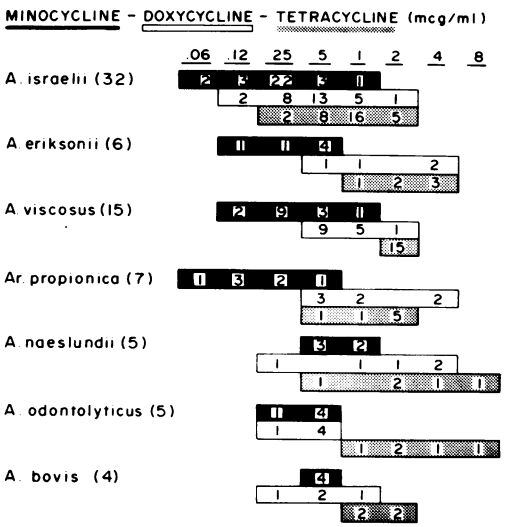

FIG. 3. *Susceptibility of actinomycetes to minocycline, doxycyline, and tetracycline.*

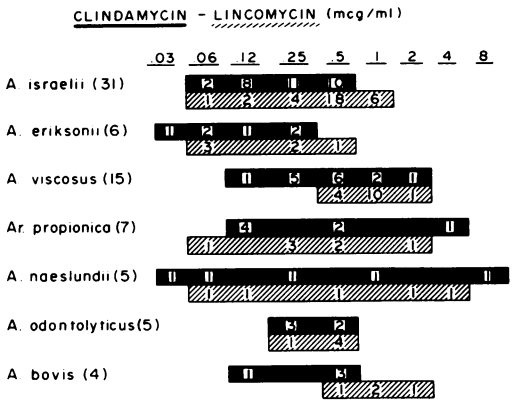

FIG. 4. *Susceptibility of actinomycetes to clindamycin and lincomycin.*

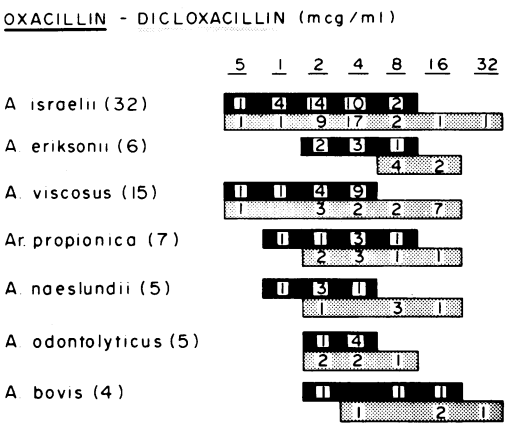

FIG. 5. *Susceptibility of actinomycetes to oxacillin and dicloxacillin.*

israelii were not suppressed by similar concentrations of dicloxacillin. *A. eriksonii* and *A. viscosus* strains were slightly more resistant to dicloxacillin, otherwise the remaining species exhibited an in vitro susceptibility pattern resembling that of *A. israelii*. Both of these semisynthetic penicillins were considerably less active in vitro than penicillin G, ampicillin, and both parenteral cephalosporins.

Erythromycin (Fig. 6) was the most active antimicrobial agent in this assay system. All strains, except one strain of *A. israelii*, were suppressed by 0.12 μg or less of this drug per ml. Other than two strains of *A. bovis*, all actinomycetes were inhibited by 1.0 to 8.0 μg of chloramphenicol per ml (Fig. 7). Vancomycin suppressed all actinomycetes tested in a narrow range of concentrations, i.e., the MICs ranged from 2.0 to 20.0 μg/ml (Fig. 8).

The pattern of MICs of rifampin differed

slightly from those of the antibiotics previously cited (Fig. 9). Although 87% (65/74) of actinomycetes were highly susceptible to rifampin (MIC of 0.008 to 0.12 μg/ml), there was a small cluster of distinctly more resistant strains (notably four of six *A. eriksonii*) that grew despite a rifampin concentration of 0.5 μg/ml, the highest employed in this assay. Fusidic acid (Fig. 10) selectively inhibited a significant number of strains, but the MICs covered a rather wide range in contrast to most of the antibiotics described in Fig. 1 through 8. Novobiocin (Fig. 11) inhibited all 28 available strains of *A. israelii* in concentrations ranging from 1.0 to 8.0 μg/ml, but the MICs for approximately 25% of strains of the other species were 16 μg or greater per ml.

Metronidazole (Fig. 12) has recently been investigated for in vitro activity against a number of anaerobic pathogens and holds particular promise because it is bactericidal for *Bacteroides fragilis* (37). Eighteen of the 25 *A. israelii*

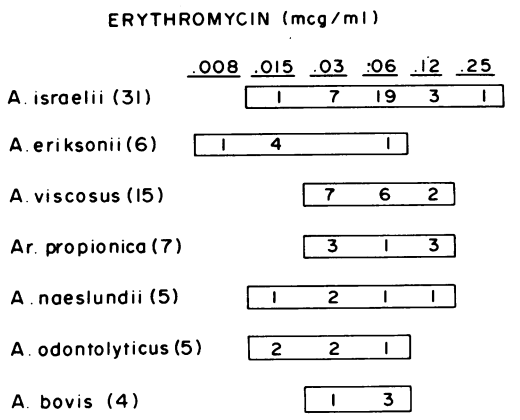

FIG. 6. *Susceptibility of actinomycetes to erythromycin.*

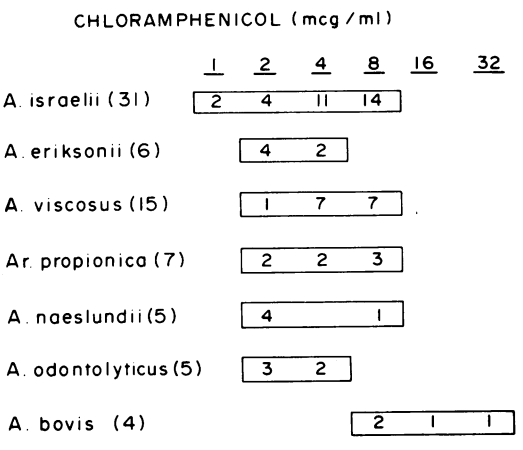

FIG. 7. *Susceptibility of actinomycetes to chloramphenicol.*

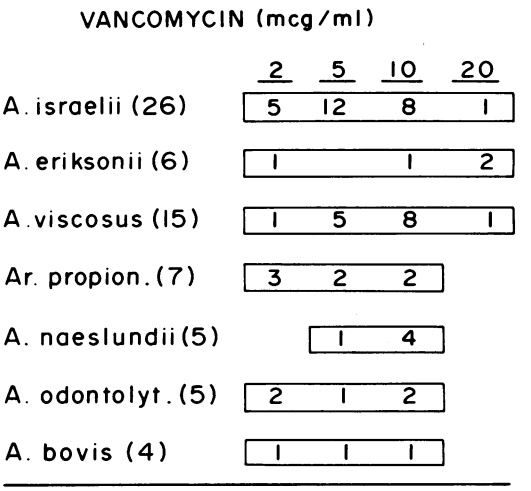

FIG. 8. *Susceptibility of actinomycetes to vancomycin.*

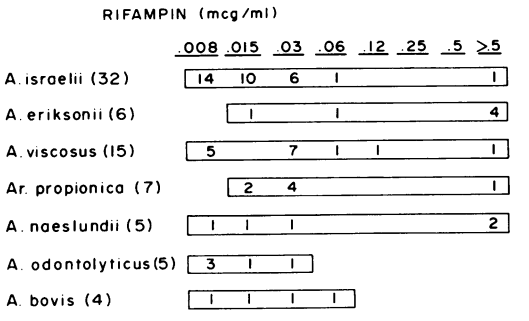

FIG. 9. *Susceptibility of actinomycetes to rifampin.*

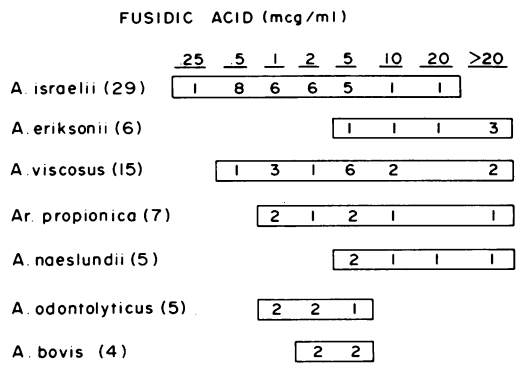

FIG. 10. *Susceptibility of actinomycetes to fusidic acid.*

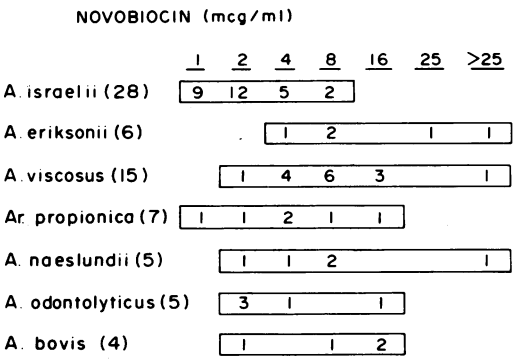

FIG. 11. *Susceptibility of actinomycetes to novobiocin.*

strains tested (72%) were suppressed by 25 to 100 μg of this drug per ml, but the majority of strains of the other species, excluding *A. bovis*, were not suppressed by 125 μg of metronidazole per ml, a concentration that did suppress all *A. israelii* strains.

Sulfisoxazole possessed selective in vitro activity against a significant number of *A. israelii* strains in concentrations ranging from 4.0 to 16.0 mg/100 ml, but these MICs were poorly reproducible in this assay system when tested a second or third time. Streptomycin in a concentration of 4 μg/ml inhibited a single strain of *A. israelii*, a second strain at 32 μg/ml and approximately 20% of the remaining strains of *A. israelii* at a concentration of 128 μg/ml; the other species were not tested. Approximately 40% of all actinomycetes, equally represented among the various species, were inhibited by 50 μg of gentamicin per ml. Cycloserine and capreomycin were essentially inactive against actinomycetes in this assay system.

DISCUSSION

Clinicians are often compelled to initiate treatment for actinomycosis on the basis of a characteristic "sulfur granule" found in tissue sections or pus, without an accompanying confirmatory culture. That actinomycetes other than *A. israelii* are sometimes associated with human disease has only recently been documented (4, 7, 14–16, 22, 32). The technical problems of routine antimicrobial susceptibility testing of these slower growing organisms prompted the present in vitro survey. If other species have antimicrobial susceptibility patterns similar to those of *A. israelii*, then infections associated with these less common species might be expected to respond to the same antibiotics even if therapy must be initiated in the absence of a positive culture. Organisms other than actinomycetes may provoke similar granule formation in tissues (34).

A. bovis, as currently classified, has never been associated with infection in man (1). Reports in the older literature carrying this designation are probably incorrect, although data concerning the isolates in question were often incomplete. The species name, *israelii* or *bovis*, often depended on the investigator's preference rather than morphology or biochemical characteristics. Consequently, reliance on species designation prior to 1960 is unwise unless complete descriptions are available (33). Difficulty in obtaining a pure culture and cultural variations due to commensalism accounted for many of these early problems in speciation.

The organism originally described as *Actinomyces propionicus* by Buchanan and Pine in 1962 has recently been removed from the genus *Actinomyces* and placed in a new genus, *Arachnia* (4, 5). Although *Arachnia propionica*, as it is now known, resembles *Actinomyces* species morphologically and biochemically, it differs serologically, has the ability to produce large amounts of propionic acid from glucose, and has diaminopimelic acid in its cell wall. The name actinomycosis remains appropriate for the similar chronic suppurative disease syndromes caused by organisms of both *Actinomyces* and *Arachnia*, because organisms in these two genera are morphologically similar. Both genera are classified in the family Actinomycetaceae, which is a further reason for retaining the term "actinomycosis" for infection due to either species (5). Accordingly, these strains were included in this in vitro survey. *A. eriksonii* may soon be reclassified in the genus *Bifidobacterium* (4).

In 1960, Peabody and Seabury reviewed the basic differences in therapy between ac-

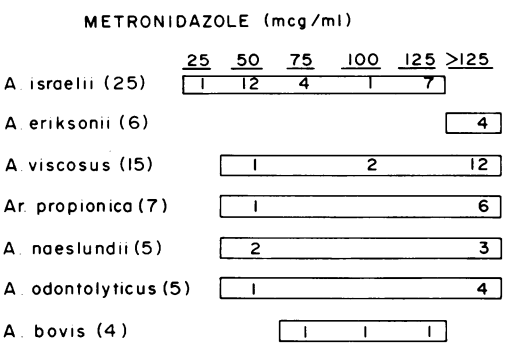

FIG. 12. *Susceptibility of actinomycetes to metronidazole.*

tinomycosis and nocardiosis (30). They summarized both clinical and in vitro experiences reported to that time and concluded that penicillin, in adequate and sometimes massive doses given over an extended period of weeks to months, was the undisputed drug of choice. They also stressed and re-emphasized the important role of surgery, not only for drainage of abscesses and empyemas, but also because of the extensive fibrosis so characteristic of actinomycotic infections. Because occasional failures followed the use of penicillin alone, they reviewed the in vitro and clinical data relative to sulfadiazine, streptomycin, erythromycin, chloramphenicol, and the tetracyclines. Cures had been achieved with each, alone or in combinations, and in vitro data suggested that *Actinomyces* were readily inhibited by chloramphenicol (0.005 to 0.1 μg/ml (26) and erythromycin (0.005 to 0.1 μg/ml) (20, 36), and were readily inhibited by several tetracyclines (13, 20, 21, 27, 30). Even streptomycin, considerably less effective in vitro, had produced excellent clinical results in some instances (3, 8, 38). The presence of other organisms, such as *Actinobacillus actinomycetemcomitans* or other gram-negative anaerobic bacilli or coccobacilli ("associates" that invariably accompany the *Actinomyces*, according to Holm, and may be penicillin resistant), might explain such unexpected results (19). Cures after the use of isoniazid and stilbamidine have also been reported (17, 28).

It is difficult, if not impossible, to compare our in vitro results with those published during the past thirty years with antibiotics other than penicillin G. The technique of colonial immersion in semisolid antibiotic agar, the use of actinomyces maintenance medium (1), now available commercially as Actinomyces Broth, the brief 24- to 48-h incubation period, and the use of complete suppression of colonial enlargement as the visual end point differs in part or in full from the methods of all, except Blake (2). Thioglycolate or various enriched broths, undefined inocula, prolonged periods of incubation (usually 5 to 20 days), and gradations of growth suppression, rather than total growth inhibition, characterized almost all other studies (3, 9, 11, 12, 18–20, 35, 36). Despite such extreme variations in assay technique, almost all investigators showed, however, that *A. israelii* was uniformly susceptible to penicillin G and was inhibited by MICs identical to those obtained in this study.

Blake tested five strains of *A. israelii* against a variety of antibiotics, in both broth suspensions and in semisolid agar (2). He employed brain heart infusion media (Oxoid), did not standardize the inoculum for his suspension dilutions, and used week-old colonies for the semisolid agar inhibition assays. End points were recorded at 5, 10, and 21 days for broth suspensions and after 72 h in semisolid agar. MICs of penicillin G, cloxacillin, ampicillin, erythromycin, and tetracyclines were similar to those reported here. Blake stated that bactericidal end points could be determined for colonies of *Actinomyces* by transferring these agar squares into antibiotic-free semisolid agar (after 72 h of incubation in antibiotic agar) and recording the absence of any growth after additional incubation for 21 days. As shown previously in this laboratory, transfer of agar squares containing antibiotic-suppressed colonies into antibiotic-free broth, followed by prolonged anaerobic incubation (up to 28 days), yielded ultimate colonial enlargement on squares originally exposed to concentrations of drug ranging from 64 to 128 μg/ml in over 95% of the assays (23). Usually only one or a few colonies, of the more than several dozen originally present on each square, ultimately enlarged as single discrete colonies. Thus, a bactericidal effect of these antibiotics was not apparent even at drug concentrations many-fold greater than those capable of producing bacteriostasis. It is possible that a technique permitting accurate quantitation of the exact number of colonies originally inoculated with each agar square might allow an estimate of the percentage of colonies killed.

Holm (19) and Garrod (11) both suggested that the concentration of antibiotic (penicillin) required to inhibit the growth of whole colonies of *Actinomyces* was five times that needed to suppress the growth of a ground-up suspension. Earlier studies in this laboratory with lincomycin, cephalothin, cephaloridine, penicillin G, and cloxacillin uncovered no such difference or, at most, a single dilution variance between MICs required for colonies in semisolid agar and those obtained by broth dilution studies with suspensions of *Actinomyces* (23). Blake also investigated this aspect of the assay system and found that MICs for suspensions determined after 5 days of incubation agreed with 72-h readings for whole colonies; subsequent readings at 10 and 21 days yielded MICs two to four times higher than 5-day readings, probably from attendant antibiotic deterioration (2).

The whole-colony immersion technique was utilized for this in vitro survey because as many as 30 to 40 strains could easily be processed, and two drugs tested during a single run with a

comparable inoculum, i.e., colonies of uniform size and age. In addition, periodic emergence of commensals was an annoying problem with a small but significant number of strains; this event was readily apparent in semisolid agar, whereas it occurred somewhat more frequently and was more difficult to appreciate (short of Gram staining all suspicious tubes) in the broth dilution method. Agar plate dilution studies were also attempted, but the slow growth of most rough strains, particularly *A. israelii*, precluded determining a clear cut end point at 48 h, let alone earlier. The single caveat in recommending this in vitro assay technique is to avoid prolonged aerobic exposure of the colonies at room temperature prior to their inoculation into semisolid media; this favors the emergence of commensals, particularly staphylococci (33).

The in vitro activity of cephaloridine, in particular, as well as cephalothin, clindamycin, lincomycin, and minocycline against *A. israelii* and most other actinomycetes, suggests that these drugs should be useful in the management of patients with actinomycosis. A limited number of reports already suggest that parenteral cephalosporins and the lincomycins can be added to the list of drugs endorsed by Peabody and Seabury (chloramphenicol, tetracyclines, and erythromycin) as alternative therapies when penicillin fails or the patient is unable to tolerate this drug (6, 10, 25, 29, 30, 31). Erythromycin, in particular, appears to be an excellent alternative to penicillin, particularly when long-term oral therapy is indicated (18, 36). Oral cephalexin and the semisynthetic penicillins, oxacillin and dicloxacillin, are considerably less active in vitro and probably should be avoided.

There are no major species variations in antimicrobial susceptibility among the first line drugs summarized above, so infection with strains other than *A. israelii* should also respond to an adequate course of treatment with penicillin G or any of the penicillin alternatives.

Sulfonamide is the drug of choice for nocardia infections, and despite our inability to demonstrate reproducible in vitro results with sulfisoxazole in this assay system, it appears that many strains of *A. israelii* are inhibited by concentrations readily attained in serum (4.0 to 8.0 mg/100 ml) and, therefore, proven cases of actinomycosis, not mistaken instance of nocardiosis, can on occasion respond to sulfonamides (9, 20).

Although this in vitro assay method is somewhat unusual, it provides a reproducible, rapid method for determining the in vitro susceptibility of these rather slowly growing organisms to penicillin and other antibiotics. This is particularly useful, because one facet in the treatment of actinomycosis has never been adequately explored, i.e., the development of in vivo-acquired antimicrobial resistance. Garrod (12) claimed that unsuccessful treatment with penicillin may be accompanied by increased in vitro resistance; the inhibitory concentration for two strains of *A. israelii* rose from 0.03 units/ml to 0.2 and >0.5 units/ml. Boand and Novak found strains of *A. bovis* (probably *A. israelii*) did not readily become adapted to or resistant to penicillin with serial passage in subinhibitory concentrations of the drug, although four of six strains developed two to fourfold resistance (3). Streptomycin resistance was rapidly induced by this method.

Because actinomycetes can now be maintained frozen at −20 C in actinomyces maintenance medium (Actinomyces Broth) agar for periods of 1 to 2 years, clinical isolates should be saved for future studies; should penicillin therapy prove ineffective, subsequent isolates can be simultaneously compared with the original for any change in degree of susceptibility.

ACKNOWLEDGMENTS

The technical assistance of Charles Shelton is gratefully acknowledged.

Lucille K. Georg of the Mycology Unit, Center for Disease Control, was most generous in supplying representative actinomycetes surveyed in this study, as well as considerable advice and encouragement. Mary A. Gerencser, Department of Microbiology, West Virginia University Medical Center, kindly supplied 12 strains of *A. viscosus* for this study.

This study was supported by Veterans Administration research fund no. 4-68.

LITERATURE CITED

1. Ajello, L., L. K. Georg, W. Kaplan, and L. Kaufman. 1966. Laboratory manual for medical mycology. Public Health Service publication no. 994. U.S. Government Printing Office, Washington, D.C.
2. Blake, G. C. 1964. Sensitivities of colonies and suspensions of *Actinomyces israelii* to penicillins, tetracyclines and erythromycin. Brit. Med. J. **1**:145-148.
3. Boand, A., and M. Novak. 1949. Sensitivity changes of *Actinomyces bovis* to penicillin and streptomycin. J. Bacteriol. **57**:501-508.
4. Brock, D. W., L. K. Georg, J. M. Brown, and M. D. Hicklin. 1973. Actinomycosis caused by *Arachnia propionica*. Amer. J. Clin. Pathol. **59**:66-77.
5. Buchanan, B. B., and L. Pine. 1962. Characterization of a propionic acid producing actinomycete, *Actinomyces propionicus*, sp. nov. J. Gen. Microbiol. **28**:305-323.
6. Caldwell, J. L. 1971. Actinomycosis treated with cephalothin. South. Med. J. **64**:987-991.
7. Coleman, R. M., L. K. Georg, and A. R. Rozzell. 1969. *Actinomyces naeslundii* as an agent of human actinomycosis. Appl. Microbiol. **18**:420-426.
8. Costigan, P. G. 1947. A case of actinomycosis treated with streptomycin. Can. Med. Ass. J. **56**:431.
9. Dobson, L., and W. C. Cutting. 1945. Penicillin and

sulfonamides in the therapy of actinomycosis. J. Amer. Med. Ass. **128**:856-863.
10. Fass, R. J., J. F. Scholand, G. R. Hodges, and S. Saslaw. 1973. Clindamycin in the treatment of serious anaerobic infections. Ann. Intern. Med. **78**:853-859.
11. Garrod, L. P. 1952. Actinomycosis of the lung. Aetiology, diagnosis and chemotherapy. Tubercle **33**:258-266.
12. Garrod, L. P. 1952. The sensitivity of *Actinomyces israelii* to antibiotics. Brit. Med. J. **1**:1263-1264.
13. Geister, R. S., and E. Meyer. 1951. The effect of aureomycin and penicillin on experimental actinomycosis infections in mice. J. Lab. Clin. Med. **38**:101-111.
14. Georg, L. K., G. W. Roberstad, and S. A. Brinkman. 1964. Identification of species of *Actinomyces*. J. Bacteriol. **88**:477-490.
15. Georg, L. K., G. W. Roberstad, S. A. Brinkman, and M. D. Hicklin. 1965. A new pathogenic anaerobic Actinomyces species. J. Infect. Dis. **115**:88-99.
16. Gerencser, M. A., and J. M. Slack. 1969. Identification of human strains of *Actinomyces viscosus*. Appl. Microbiol. **18**:80-87.
17. Greene, L. W., and W. C. Black. 1955. Treatment of cerviofacial actinomycosis with isoniazid. Rocky Mt. Med. J. **52**:43-46.
18. Herrell, W. E., A. Balows, and J. S. Dailey. 1955. Erythromycin in treatment of actinomycosis. Antibiot. Med. **2**:507-512.
19. Holm, P. 1948. Some investigations into the penicillin sensitivity of human pathogenic actinomycetes. Acta Pathol. Microbiol. Scand. **23**:376-404.
20. Howell, A., Jr. 1953. In vitro susceptibility of Actinomyces to terramycin. Antibiot. Chemother. (Washington, D.C.) **3**:378-381.
21. Lane, S. L., A. H. Kutscher, and R. Chaves. 1953. Oxytetracycline in the treatment of orocervical facial actinomycosis. J. Amer. Med. Ass. **151**:986-988.
22. Leers, W. D., J. Dussault, J. E. Mullens, R. Volpe, and K. Arthurs. 1969. Suppurative thyroiditis. An unusual case caused by *Actinomyces naeslundii*. Can. Med. Ass. J. **101**:714-718.
23. Lerner, P. I. 1968. Susceptibility of *Actinomyces* to cephalosporins and lincomycin. Antimicrob. Ag. Chemother. 1967, p. 730-735.
24. Lerner, P. I. 1969. Susceptibility of *Actinomyces* species to lincomycin and its 7-halogenated analogues. Antimicrob. Ag. Chemother. 1968, p. 461-464.
25. Lerner, P. I. 1973. Serologic screening for actinomycosis. Proceedings of the international conference on anaerobic bacteria: bacteriological and clinical considerations. C. C. Thomas, Springfield, Ill.
26. Littman, M. L., G. E. Phillips, and M. H. Fusillo. 1950. In vitro susceptibility of human pathogenic Actinomycetes to chloramphenicol (Chloromycetin). Amer. J. Clin. Pathol. **20**:1076-1078.
27. McVay, L. V., Jr., and D. H. Sprunt. 1953. A long-term evaluation of aureomycin in the treatment of actinomycosis. Ann. Intern. Med. **38**:955-966.
28. McVay, L. V., Jr., and D. H. Sprunt. 1953. Treatment of actinomycosis with isoniazid. J. Amer. Med. Ass. **153**:95-98.
29. Mohr, J. A., E. R. Rhoades, and H. G. Muchmore. 1970. Actinomycosis treated with lincomycin. J. Amer. Med. Ass. **212**:2260-2262.
30. Peabody, J. W., Jr., and J. H. Seabury. 1960. Actinomycosis and nocardiosis. A review of basic differences in therapy. Amer. J. Med. **28**:99-115.
31. Rose, H. D., and M. W. Rytel. 1972. Actinomycosis treated with clindamycin. J. Amer. Med. Ass. **221**:1052.
32. Slack, J. M., and M. A. Gerencser. 1970. Two new serological groups of *Actinomyces*. J. Bacteriol. **103**: 265-267.
33. Smith, L. D. S., and L. V. Holdeman. 1968. The pathogenic anaerobic bacteria. C. C. Thomas, Springfield, Ill.
34. Speir, W. A., J. W. Mitchener, and J. F. Galloway. 1971. Primary pulmonary botryomycosis. Chest **60**:92-93.
35. Strauss, R. E., A. M. Kligman, and D. M. Pillsbury. 1951. The chemotherapy of actinomycosis and nocardiosis. Amer. Rev. Tuberc. **63**:441-448.
36. Suter, L. S. and B. F. Vaughn. 1955. The effect of antibacterial agents on the growth of *Actinomyces bovis*. Antibiot. Chemother. **5**:557-560.
37. Tally, F. P., V. L. Sutter, and S. M. Finegold. 1972. Metronidazole versus anaerobes. Calif. Med. **117**:22-26.
38. Torrens, J. A., and M. W. W. Wood. 1949. Streptomycin in the treatment of actinomycosis. Lancet **1**:1091-1094.