ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1980, p. 943-946
0066-4804/80/06-0943/04$02.00/0

Vol. 17, No. 6

Susceptibility of *Bacterium actinomycetem comitans* to 45 Antibiotics

ULRICH HÖFFLER,* WALTER NIEDERAU, AND GERHARD PULVERER

Institute of Hygiene, University of Cologne, D-5000 Cologne 41, Germany

The minimal inhibitory concentrations of 45 antimicrobial agents were determined for 14 strains of *Bacterium actinomycetem comitans* (*Actinobacillus actinomycetem-comitans*). All the strains showed good susceptibility to tetracyclines and chloramphenicol, but not to lincomycins. Some strains were clearly resistant to penicillins, cephalosporins, and nitroimidazoles. The lowest minimum inhibitory concentrations were observed with tetracycline, rolitetracycline, methacycline, minocycline, chloramphenicol, and cotrimoxazole.

Bacterium actinomycetem comitans (*Actinobacillus actinomycetem-comitans*), listed in *Bergey's Manual* as species incertae sedis (14), is a fastidious, slow-growing, microaerophilic gram-negative rod, whose nomenclature and taxonomic position are still under discussion (5,8,11,12,14). It may be regarded not only as a part of the normal bacterial flora of the human oral cavity (5–8, 11–15), but also as facultative pathogenic organism. As indicated by the name, this bacterium often is found together with *Actinomyces israelii* in human actinomycotic processes (5,7,8, 13–15), supporting the *Actinomyces* by means of extracellular enzymes and toxins (1,6). Furthermore, in a number of well-documented cases, *B. actinomycetem comitans* was found in anaerobic, mixed-infection processes, in septicaemia, and in endocarditis (9–12), which demonstrates its pathogenic potential. Only few reports have appeared to date about its antibiotic susceptibility (2,3,10,16), and no data exist concerning its susceptibility to 30 newer antimicrobial agents. The aim of the present study was, therefore, to establish the minimal inhibitory concentrations (MICs) of 45 commonly used antibiotics to recent isolated strains of *B. actinomycetem comitans*.

MATERIALS AND METHODS

Bacterial strains. Fourteen well-defined strains of *B. actinomycetem comitans* were freshly isolated in our institute from the pus of human actinomycotic lesions (122 strains) and from other anaerobic mixed-infection pyogenic processes of the cervicofacial region (2 strains). Stock cultivation was done on CC agar medium according to Heinrich and Korth (4), using the Fortnermethod as described earlier (5). All the strains were examined according to *Bergey's Manual* by standard methods (14). The typical morphology of colonies (star-like [7]; crossed cigars [5,6]) could be seen in all the strains tested. Furthermore, the Minitek differentiation system for anaerobes (Becton, Dickinson GmbH, Heidelberg, West Germany) was used with the following reactions: indole production (negative for all strains), acid production from maltose (positive for all strains), lactose, sucrose, dulcitol, arabinose, inositol, glycerol, raffinose, and salicin (negative for all strains). On the basis of fermentation of mannitol, xylose, and galactose, one strain belonged to biotype I (11), three strains belonged to biotype II, two strains belonged to biotype III, and eight strains belonged to biotype IV.

Preparation of inocula. One loop of a 96-h-old subculture on CC agar (4) with the GasPak system (BBL Microbiology Systems, Heidelberg, West Germany) was added to 5 ml of a broth containing the following: proteose peptone (BBL Microbiology Systems, Heidelberg), 5 g; yeast extract (Difco Laboratories, Detroit, Mich.), 5 g; NaCl, 5 g; K_2HPO_4, 5 g; dextrose, 1 g; distilled water, 1,000 g; pH 7.2. After incubation for 24 h at 37°C in GasPak jars, the broth culture was homogenized (Potter-Elvehjem, B. Braun Melsungen AG) and diluted 1:10. A 25-μl amount of this broth culture was taken for inoculation with an Oxford Micro-Doser repetitive pipette (Oxford Laboratories, Athy, Ireland). Based on duplicate counts of the colony-forming units for three strains, the mean final inoculum was calculated to be 1.7×10^5 colony-forming units.

Antimicrobial agents. A total of 45 preparations of 44 antimicrobial agents were used. Potassium propicillin, disodium carbenicillin, sodium mezlocillin, sodium azlocillin, sisomicin sulfate, and metronidazole (Bayer AG, Leverkusen, West Germany); potassium azidocillin and disodium ticarcillin (Beecham GmbH, Mainz, West Germany); sodium cefapirin (Bristol-Myers, GmbH, Bensberg, West Germany); sodium cephalothin, cephalexin monohydrate, sodium cefazolin, cefamandole formiate sodium, and tobramycin sulfate (Eli Lilly GmbH, Lahn-Giessen, West Germany); sodium benzyl-penicillin, sodium ampicillin, amoxycillin trihydrate, ciclacillin, sodium cefacetril, amikacin sulfate, tetracycline hydrochloride, chloramphenicol, spiramycin sulfate, and sodium rifampin (Grünenthal GmbH, Stolberg, West Germany); cefradine monohydrate (von Heyden GmbH, München, West Germany); sodium cefuroxime, cefotaxime, and rolitetracycline gluconate (Hoechst AG, Frankfurt, West Germany);

trimethoprim and sodium sulfamethoxazole (Hoffmann-La Roche AG, Grenzach, West Germany); thiamphenicol glycinat-hydrochloride (Infarzam GmbH, München, West Germany); minocycline hydrochloride (Lederle-Cyanamid GmbH, München, West Germany); methacycline hydrochloride (Mack, Illertissen, West Germany); gentamicin sulfate (Merck, Darmstadt, West Germany); sodium cefoxitin (Sharp & Dohme GmbH, München, West Germany); doxycycline hydrochloride, oleandomycin phosphate, and tinidazole (Pfizer GmbH, Karlsruhe, West Germany); epicillin (Sandoz AG, Nürnberg, West Germany); erythromycin glucoheptonate (Schering AG, Berlin, West Germany); fusidic acid (Thomae GmbH, Biberach, West Germany); spectinomycin sulfate, lincomycin hydrochloride, and clindamycin hydrochloride (Upjohn GmbH, Heppenheim, West Germany).

Antibiotic susceptibility test. MICs were determined by a plate dilution method with Diagnostic Sensitivity Test agar (Oxoid Limited, Basingstoke, England). The results were read after incubation for 48 h at 37°C by the GasPak system. Positive controls (anaerobic incubation on drug-free Diagnostic Sensitivity Test agar), negative controls (aerobic incubation on the same agar), and antibiotic activity controls with the aerobically incubated *Staphylococcus aureus* reference strain SG 511-Jena were included in each assay. All tests were done in duplicate.

RESULTS

In Table 1 the MICs of 11 penicillins to *B. actinomycetem comitans* are shown. Most of the strains were inhibited at an MIC of 12.5 μg/ml, ampicillin being the most active penicillin tested and propicillin being the least effective. But one *B. actinomycetem comitans* biotype III strain was clearly resistant to all penicillins investigated.

In Table 2, the MICs of 10 cephalosporins to *B. actinomycetem comitans* are given. The strains showed a great variety of susceptibility, ranging over all drug concentrations tested; however, at 25 μg/ml, most of the strains were susceptible. Cefotaxime was by far the most active cephalosporin investigated, but again the same biotype III strain was clearly resistant to all cephalosporins.

The activity ranges of the five aminoglycosides examined are summarized in Table 3. All strains were inhibited by 6.25 μg of gentamicin, tobramicin, and sisomicin per ml and by 12.5 μg of amikacin and spectinomycin per ml.

The MICs of five tetracyclines (Table 4) were very low, inhibiting all tested strains at 3.1 μg/

TABLE 1. *Susceptibility of B. actinomycetem comitans to penicillins (absolute numbers, 14 strains examined)*

Antibiotic	No. of strains inhibited at MICs (μg/ml) of:									
	≥100	50	25	12.5	6.25	3.1	1.6	0.8	0.4	0.2
Benzylpenicillin	1				1	6	4	2		
Propicillin	1		4	3	2	2	2			
Azidocillin	1				8	3	1	1		
Ampicillin	1						3	9	1	
Epicillin	1					1	9	3		
Amoxycillin	1					2	4	7		
Ciclacillin	1			3	6	3	1			
Carbenicillin	1					1	1	3	8	
Ticarcillin	1					1	2	4	6	
Mezlocillin	1					3	6	4		
Azlocillin	1					1	4	5	2	1

TABLE 2. *Susceptibility of B. actinomycetem comitans to cephalosporins (absolute numbers, 14 strains examined)*

Antibiotic	No. of strains inhibited at MICs (μg/ml) of:										
	≥100	50	25	12.5	6.25	3.1	1.6	0.8	0.4	0.2	0.1
Cephalothin	1						3	7	3		
Cephalexin	1			3	10						
Cefazolin	1						8	5			
Cefradine	1		3	10							
Cephacetril	1				1		10	2			
Cefapirin	1						3	7	3		
Cefamandole	1					11	1	1			
Cefuroxime	1						1	4	8		
Cefoxitin	1				2	4	6		1		
Cefotaxime	1								7	4	2

ml. Methacycline was one of the most active antimicrobial agents investigated in our study, all strains being susceptible to 0.8 μg of this drug per ml.

Of the miscellaneous antibiotics presented in Table 5, chloramphenicol, thiamphenicol, rifampin, and 5% trimethoprim plus 95% sulfamethoxazole exhibited the highest activities. Chloramphenicol as well as thiamphenicol suppressed all tested strains at 0.8 μg/ml. The activities of the macrolide antibiotics and of lincomycin and clindamycin were low. The activity of lincomycin was clearly inferior to that of clindamycin, which inhibited only 10 of 14 strains at an MIC of 6.25 μg/ml. All strains were resistant to fusidic acid, but susceptible to 0.8 μg of rifampin per ml. Of the two nitroimidazole compounds tested, tinidazole in vitro was superior to metronidazole. But one strain, *B. actinomycetem comitans* biotype IV (not identical with the penicillin-resistant strain), was completely resistant to both drugs.

Trimethoprim was of highest effectiveness under our test conditions, and the strains were more or less susceptible to sulfamethoxazole. Cotrimoxazole was one of the most active antimicrobial agents investigated in our study, inhibiting all tested strains at 1.6 μg/ml.

TABLE 3. *Susceptibility of B. actinomycetem comitans to aminoglycoside antibiotics (absolute numbers, 14 strains examined)*

Antibiotic	No. of strains inhibited at MICs (μg/ml) of:			
	12.5	6.25	3.1	1.6
Gentamicin		4	9	1
Amikacin	2	5	7	
Spectinomycin	4	7	3	
Tobramycin			8	6
Sisomicin		6	8	

TABLE 4. *Susceptibility of B. actinomycetem comitans to tetracyclines (absolute numbers, 14 strains examined)*

Antibiotic	No. of strains inhibited at MICs (μg/ml) of:				
	3.1	1.6	0.8	0.4	0.2
Tetracycline		5	8	1	
Rolitetracycline		6	8		
Methacycline			12	2	
Doxycycline	6	4	3	1	
Minocycline		4	6	3	1

DISCUSSION

Although *B. actinomycetem comitans* is present in about 26% of cases of human actinomycosis (13), a disease more frequent in Germany than paratyphoid fever (8), little information exists about the antibiotic susceptibility of this organism. In 1960 Heinrich (3) examined 10 strains and found all isolates susceptible to 25 μg of benzylpenicillin per ml and to 2.5 μg of tetracycline per ml. Chloramphenicol, erythro-

TABLE 5. *Susceptibility of B. actinomycetem comitans to miscellaneous antibiotics (absolute numbers, 14 strains examined)*

Antibiotic	No. of strains inhibited at MICs (μg/ml) of:													
	>100	100	50	25	12.5	6.25	3.1	1.6	0.8	0.4	0.2	0.1	0.04	≤0.02
Chloramphenicol									12	2				
Thiamphenicol									10	4				
Erythromycin						7	5	2						
Oleandomycin		3	6	4		1								
Spiramycin		10	3	1										
Lincomycin		2	6	4	1									
Clindamycin					4	4	5	1						
Fusidic acid		2	8	3	1									
Rifampin									6	7	1			
Metronidazole	1				3	2	8							
Tinidazole	1							1	4	8				
Trimethoprim									3		2	2	5	2
Sulfamethoxazole			1		1	3	4	4	1					
5% Trimethoprim–95% sulfamethoxazole								3	5	5	1			

mycin, and oleandomycin showed low activities. In 1964 Fritsche (2) tested 10 strains inhibited by 4.25 μg of ampicillin per ml. In 1966 Page and King (10) performed susceptibility studies with six antibiotics against 37 strains and found all susceptible to 3.1 μg of tetracycline per ml and to 1.5 μg of chloramphenicol per ml. More than 50% of the strains required concentrations of benzylpenicillin greater than 12.5 μg/ml, and about 40% of the strains were susceptible to 12.5 μg of ampicillin per ml. In 1970 Spieckermann (16) established MICs for 10 strains. All were susceptible to 8 μg of cephalothin per ml, and nine strains were resistant to 40 μg of lincomycin per ml; the organisms were less susceptible to fusidic acid and gentamicin, all being inhibited by 25 μg of gentamicin and 100 μg of fusidic acid per ml.

Our data confirm and extend these observations with regard to 30 newer antimicrobial agents. Some differences between the results from various authors may be explained by different inoculum size and by different anaerobic cultivation procedures. Concerning the earlier results of Heinrich (3) and Fritsche (2), in our study the MICs for *B. actinomycetem comitans* were lower, perhaps depending on lower colony-forming unit count in the inoculum. As far as tetracycline and chloramphenicol are concerned, our data confirm the results of Page and King (10), but in our study most of the strains were more susceptible to benzylpenicillin and ampicillin. In agreement with these authors, one of our strains was clearly resistant to β-lactam antibiotics. Our results are in fair agreement with those of Spieckermann (16).

To summarize, contrary to most anaerobic bacteria, *B. actinomycetem comitans* cannot be suppressed with certainty by lincomycins in therapeutically achievable concentrations even though clindamycin showed a clearly higher activity than lincomycin. Some strains were clearly resistant to penicillins, cephalosporins (including the newer ones), and nitroimidazoles. But again, in our study, all the strains tested were susceptible to tetracyclines, chloramphenicol, and thiamphenicol as well as to cotrimoxazole.

LITERATURE CITED

1. **Brede, H. D.** 1959. Zur Aetiologie und Mikrobiologie der Aktinomykose I. In-vitro-Versuche zu Frage der fermentativen Unterstützung des *Actinomyces israeli* durch Begleitbakterien. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. **174**:110–122.
2. **Fritsche, D.** 1964. Untersuchungen über die Empfindlichkeit des *Actinomyces israeli* und zwei seiner häufigsten anaeroben Begleitbakterien gegen neuere Antibiotika. Z. Hyg. **150**:50–57.
3. **Heinrich, S.** 1960. Zur Aetiologie und Mikrobiologie der Aktinomycose IV. Über die Empfindlichkeit des *Actinobacillus actinomycetem-comitans* gegen die gebräuchlichen Antibiotika und Sulfonamide in vitro. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. **177**:255–263.
4. **Heinrich, S., and H. Korth.** 1967. Zur Nährbodenfrage in der Routinediagnostik der Aktinomykose. In H.-J. Heite (ed.), Krankheiten durch Aktinomyzeten und verwandte Erreger. Springer-Verlag, Berlin.
5. **Heinrich, S., and G. Pulverer.** 1959. Zur Aetiologie und Mikrobiologie der Aktinomykose II. Definition und praktische Diagnostik des *Actinobacillus actinomycetem-comitans*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. **174**:123–135.
6. **Heinrich, S., and G. Pulverer.** 1959. Zur Aetiologie und Mikrobiologie der Aktinomykose III. Die pathogene Bedeutung des *Actinobacillus actinomycetem-comitans* unter den "Begleitbakterien" des *Actinomyces israeli*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I. Orig. **176**:91–101.
7. **Holm, P.** 1950. Studies on the aetiology of human actinomycosis I. The "other microbes" of actinomycosis and their importance. Acta Pathol. Microbiol. Scand. **27**:736–751.
8. **Lentze, F.** 1969. Die Aktinomykose und die Nocardiosen. In A. Grumbach, W. Kikuth (ed.). Die Infektionskrankheiten des Menschen und ihre Erreger. 2. Aufl. G. Thieme Verlag, Stuttgart.
9. **Moorthy, K., R. Prakash, and W. S. Aronow.** 1977. Echocardiographic appearance of aortic valve vegetations in bacterial endocarditis due to *Actinobacillus actinomycetem* comitans. J. Clin. Ultrasound **5**:49–51.
10. **Page, M. I., and E. O. King.** 1966. Infection due to *Actinobacillus actinomycetem comitans* and *Haemophilus aphrophilus*. N. Engl. J. Med. **275**:181–188.
11. **Pulverer, G., and H. L. Ko.** 1970. *Actinobacillus actinomycetem-comitans*: fermentative capabilities of 140 strains. Appl. Microbiol. **20**:693–695.
12. **Pulverer, G., and H. L. Ko.** 1972. Serological studies on *Actinobacillus actinomycetem-comitans*. Appl. Microbiol. **23**:207–210.
13. **Pulverer, G., and K. P. Schaal.** 1978. Pathogenicity and medical importance of aerobic and anaerobic Actinomycetes, p. 417–427. *In* M. Modarski, W. Kurytowicz, J. Jeljaszewicz (ed.), *Nocardia* and *Streptomyces*. Fischer Verlag, Stuttgart, New York.
14. **Phillips, J. E.** 1974. Description of the species of genus *Actinobacillus*, p. 373–377. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. Williams & Wilkins Co., Baltimore.
15. **Slack, J. M., and M. A. Gerencser.** 1975. *Actinomyces*, filamentous bacteria. Biology and pathogenicity. Burgess Publishing Co., Minneapolis.
16. **Spieckermann, C.** 1970. Die In-vitro-Empfindlichkeit von *Actinomyces israelii, Actinobacillus actinomycetem-comitans* und *Bacteroides melaninogenicus* gegenüber Cephalothin, Cephaloridin, Gentamycin, Fusidinsäure und Lincomycin. Int. J. Clin. Pharmacol. **3**:318–320.