ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1995, p. 268–270 Vol. 39, No. 1
0066-4804/95/$04.00+0

Antimicrobial Susceptibilities of *Stomatococcus mucilaginosus* and of *Micrococcus* spp.

CHRISTOF VON EIFF,* MATHIAS HERRMANN, AND GEORG PETERS

Institute of Medical Microbiology, Westfälische Wilhelms-Universität Münster, 48149 Münster, Germany

Received 28 March 1994/Returned for modification 20 July 1994/Accepted 11 October 1994

The in vitro susceptibilities of 63 isolates of *Stomatococcus mucilaginosus* and of 188 isolates of *Micrococcus* spp. to 18 antimicrobial agents were determined by the agar dilution method. Many β-lactams, imipenem, rifampin, and the glycopeptides were shown to be active in vitro against *Stomatococcus* and *Micrococcus* isolates, whereas the activities of antibiotics such as some aminoglycosides, erythromycin, and fosfomycin against an important number of these microorganisms are limited.

The predominant class of microorganisms causing bacteremia, especially in neutropenic patients, has changed over the past 15 years from aerobic gram-negative bacilli to gram-positive cocci. Although *Staphylococcus aureus* remains the most commonly isolated agent, the list of gram-positive pathogens now includes a growing number of commensal bacteria: coagulase-negative staphylococci, viridans group streptococci, and, most recently, *Stomatococcus mucilaginosus* and *Micrococcus* spp.; both genera are members of the family *Micrococcaceae* (12, 13, 16). *S. mucilaginosus* is considered part of the normal flora of the human mouth and the upper respiratory tract (3). Though it appears to be an organism of low virulence, the recent increase in the number of reported cases of *S. mucilaginosus* bacteremia has established the pathogenic potential of this organism. The most frequent clinical presentations include septicemia, endocarditis, and catheter-related sepsis (2, 8, 9, 12, 13, 15). *Micrococcus* spp. are found as normal inhabitants of human skin and mucous membranes and are usually disregarded as contaminants in clinical isolates (6). Nevertheless, strains identified as *Micrococcus* spp. have been involved as causative organisms in septicemia, endocarditis, central nervous system infection, peritonitis, and pneumonia (1, 10, 12, 18).

Previous studies have examined the susceptibilities either of single isolates or of a strain collection against selected compounds only (5, 12, 13, 17). However, data on susceptibility patterns of a large number of isolates against a panel of antimicrobial agents are so far not available. Therefore, we tested the in vitro activities of a representative number of commonly used antibiotics against 63 isolates of *S. mucilaginosus* and against 188 isolates of micrococci.

Bacterial strains. *S. mucilaginosus* was isolated from the mucous membrane of the cheek and gingiva; *Micrococcus* spp. were isolated additionally from the skin. A total of 36 isolates of *S. mucilaginosus* and 104 isolates of micrococci were obtained from swabs of 50 healthy persons; a total of 27 isolates of *S. mucilaginosus* and 84 isolates of micrococci were obtained from swabs of 50 neutropenic patients. At the time of specimen isolation, patients did not reveal any clinical signs of infections and had not been treated previously with antibiotics for at least 7 days.

* Corresponding author. Mailing address: Institute of Medical Microbiology, Westfälische Wilhelms-Universität Münster, Domagkstraße 10, 48149 Münster, Germany. Phone: 49-251-835360. Fax: 49-251-835350.

Isolates of *S. mucilaginosus* were identified on the basis of the following characteristics: the presence of gram-positive cocci in clusters; strong adherence of the usually mucoid, transparent, or whitish organisms to the agar surface; weakly positive or negative catalase reactivity; and the inability of the organisms to grow on nutrient agar containing 5% NaCl. When heart infusion broth was used as a basal medium, the isolates hydrolyzed esculin and produced acid from glucose, fructose, sucrose, trehalose, and mannose (3, 16). Identification as *Micrococcus* spp. was based on typical Gram stain morphology with large gram-positive cocci in tetrads and irregular clusters and aerobic growth of catalase-positive, usually circular, entire, convex, and smooth colonies on furazolidone agar. In biochemical testing, production of acid was weak and limited to a few sugars. In the API-Staph system (API System, La Balme les Grottes, France), typical profiles were 0004006 and 0000005 (*Micrococcus* spp.). For additional differentiation of *Staphylococcus* spp., resistance to lysostaphin (Lysostaphin Test; Hoffmann-La Roche, Grenzach-Wyhlen, Federal Republic of Germany [FRG]) was employed (7). *Micrococcus* spp. were identified only to genus level by the conventional method, since attempts at further species identification by means of the simple biochemical tests performed in a routine clinical laboratory are mostly inconclusive (7, 11, 16).

Susceptibility testing. The MICs were determined by the standard agar dilution method (14). Mueller-Hinton agar (Difco, Augsburg, FRG), supplemented with 5% sheep blood was used for both *S. mucilaginosus* and *Micrococcus* spp. A 1:100 dilution of a fresh overnight culture was applied to agar plates by using a multipoint inoculator (Mast Laboratories Ltd., Bootle, England), yielding a final inoculum of 10^4 CFU per spot. The results were read after 18 h of incubation at 37°C. The isolates of *S. mucilaginosus* were incubated in 6% CO_2. Sterility and growth controls, as well as activity controls using reference strains, were always performed. The MIC of each antibiotic was defined as the lowest concentration which inhibited visible growth of the organism.

Compounds. The following 18 antimicrobial agents in the form of standard powders of known potency for laboratory use were supplied by the indicated manufacturers: penicillin (Grünenthal, Stolberg, FRG), ampicillin (Hoechst, Frankfurt, FRG), cefazolin (Eli Lilly, Giessen, FRG), cefotiam (Takeda, Aachen, FRG), cefuroxime (Hoechst), cefotaxime (Hoechst), imipenem-cilastatin (MSD, Munich, FRG), gentamicin (Merck, Darmstadt, FRG), amikacin (Bristol-Myers Squibb, Munich, FRG), netilmicin (Essex Pharma, Munich, FRG), erythromy-

TABLE 1. In vitro susceptibilities of 63 *S. mucilaginosus* strains to 18 antimicrobial agents

Antibiotic	MIC (μg/ml)[a]		
	Range	50%	90%
Penicillin	≤0.031–1	≤0.031	0.125
Ampicillin	≤0.031–0.25	≤0.031	≤0.031
Cefazolin	≤0.031–8	0.063	0.5
Cefotiam	≤0.031–16	0.125	1
Cefuroxime	≤0.031–1	≤0.031	0.125
Cefotaxime	≤0.031–0.5	≤0.031	0.063
Imipenem	≤0.031–0.25	≤0.031	0.125
Gentamicin	≤0.031–16	2	4
Amikacin	≤0.031–>16	4	8
Netilmicin	≤0.031–>16	16	>16
Erythromycin	≤0.031–>16	1	1
Clarithromycin	≤0.031–4	≤0.031	≤0.031
Clindamycin	≤0.031–>16	0.5	2
Vancomycin	≤0.031–1	0.5	1
Teicoplanin	≤0.031–0.5	0.25	0.5
Rifampin	≤0.031	≤0.031	≤0.031
Fosfomycin	≤0.031–>16	1	>16
Fusidic acid	≤0.031–0.5	0.5	0.5

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

TABLE 2. In vitro susceptibilities of 188 *Micrococcus* strains to 18 antimicrobial agents

Antibiotic	MIC (μg/ml)[a]		
	Range	50%	90%
Penicillin	≤0.031–2	0.063	0.125
Ampicillin	≤0.031–4	0.125	0.25
Cefazolin	0.063–8	0.25	2
Cefotiam	0.125–16	0.5	1
Cefuroxime	≤0.031–>16	0.5	1
Cefotaxime	≤0.031–2	0.5	1
Imipenem	≤0.031–2	0.063	0.125
Gentamicin	0.125–2	0.5	1
Amikacin	0.5–>16	2	2
Netilmicin	0.5–>16	2	4
Erythromycin	0.5–>16	4	>16
Clarithromycin	≤0.031–16	0.125	1
Clindamycin	≤0.031–4	0.125	0.25
Vancomycin	0.25–2	0.25	1
Teicoplanin	0.063–2	0.25	1
Rifampin	≤0.031–0.063	≤0.031	≤0.031
Fosfomycin	0.5–>16	>16	>16
Fusidic acid	0.063–16	2	4

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

cin (Abbott, Wiesbaden, FRG), clarithromycin (Abbott), clindamycin (Upjohn, Heppenheim, FRG), vancomycin (Eli Lilly), teicoplanin (Marion Merrell Dow, Rüsselsheim, FRG), rifampin (Grünenthal), fosfomycin (Boehringer, Mannheim, FRG), and fusidic acid (Thomae, Biberach an der Riss, FRG). These agents were dissolved in appropriate solvents to prepare stock solutions and were then diluted in water and added to molten supplemented Mueller-Hinton agar to provide a final range of twofold concentrations from 0.031 to 16 μg/ml when tested.

S. mucilaginosus. The MIC ranges and the MICs at which 50% (MIC_{50}s) and 90% (MIC_{90}s) of the 63 strains of *S. mucilaginosus* are inhibited are shown in Table 1. Among the β-lactam antibiotics tested, ampicillin was the most active compound in vitro (MIC_{90}, ≤0.031 μg/ml). The activity of penicillin was similar to that of imipenem, with MIC_{90}s of ≤0.125 μg/ml. Among the cephalosporins tested, cefotaxime was most active (MIC_{90}, 0.063 μg/ml). Several strains were inhibited only by high concentrations of cefazolin and cefotiam. Compared to β-lactam antibiotics, aminoglycosides, particularly netilmicin (MIC_{90}, >16 μg/ml) and amikacin (MIC_{90}, 8 μg/ml), were clearly less active, with elevated MICs for many isolates. Among the macrolides tested, erythromycin was less active than clarithromycin (MIC_{90}s, 1 and ≤0.031 μg/ml, respectively). The MIC_{90} of clindamycin for *S. mucilaginosus* was 2 μg/ml. The activity of teicoplanin against *S. mucilaginosus* was slightly higher than that of vancomycin, the other glycopeptide tested (MIC_{90}s, 0.5 and 1 μg/ml), and comparable to the activity of fusidic acid. Rifampin was the most active compound. All strains of *S. mucilaginosus* were inhibited by a concentration of ≤0.031 μg/ml. Of the 63 strains tested, 17 were not inhibited by concentrations of <16 μg of fosfomycin per ml.

When data were analyzed with regard to the origins of the isolates, i.e., neutropenic patients versus healthy persons, careful comparisons of the MIC ranges, MIC_{50}s, and MIC_{90}s yielded similar results among the subgroups, with the MIC_{50}s and MIC_{90}s being identical for most antimicrobial agents or differing by at most one dilution step. These differences were detectable particularly for aminoglycosides, with isolates from patients being slightly less susceptible than isolates from volunteers (data not shown).

***Micrococcus* spp.** The ranges of MICs, the MIC_{50}s, and the MIC_{90}s for the 188 strains of *Micrococcus* spp. tested are shown in Table 2. Among the β-lactam antibiotics tested, penicillin and imipenem were most active against micrococci (MIC_{90}, 0.125 μg/ml). The in vitro activities of the four cephalosporins tested were similar (MIC_{90}s, 1 μg of cefotiam, cefuroxime, and cefotaxime per ml and 2 μg of cefazolin per ml). Single strains which required elevated cefazolin, cefotiam, and cefuroxime MICs were observed. Similarly, a number of isolates were not inhibited even in the presence of high aminoglycoside concentrations. Gentamicin (MIC_{90}, 1 μg/ml) was more active than amikacin or netilmicin, with MICs for the majority of strains being two dilutions lower than those of amikacin or netilmicin. Among the macrolides tested, erythromycin was again less active than clarithromycin (MIC_{90}, >16 and 1 μg/ml, respectively). The MICs of clindamycin for micrococci were comparable to those of ampicillin (MIC_{90}, 0.25 μg/ml). No differences in susceptibility regarding the activities of the glycopeptides against micrococci (MIC_{90}, 1 μg/ml) were found. Again, rifampin was the most active compound among the agents tested. All strains of micrococci except one were inhibited by a concentration of ≤0.031 μg/ml. Fosfomycin showed limited activity against many strains: the MIC_{50} was higher than 16 μg/ml. Similarly, the activity of fusidic acid against micrococci was low, yet with a larger proportion of moderately susceptible strains (MIC_{90}, 4 μg/ml). Comparison of the patient and volunteer subgroups yielded similar results (data not shown).

Our study presents for the first time a thorough evaluation of MICs of a representative panel of antimicrobial agents against a large number of *S. mucilaginosus* and *Micrococcus* isolates obtained both from volunteers and from patients at risk for invasive infection.

The results concerning the antimicrobial susceptibilities of our strains were generally in agreement with those reported by other investigators. Nearly all *S. mucilaginosus* strains described in the present communication as well as clinical isolates of *S. mucilaginosus* reported in the literature were susceptible

to penicillin (2, 9, 12, 13), ampicillin (2), cefotaxime (8, 9), imipenem (8), rifampin (8, 13), and the glycopeptides (2, 9, 12, 13); however, isolates revealing diminished susceptibility or resistance to penicillin have been described (2, 8, 13, 15). Some of these strains were implicated in serious infections such as endocarditis (15) and septicemia (2, 13). Our data, as well as the results reported by other investigators (5, 9, 13, 17), show that low-level resistance towards aminoglycosides is a common feature of *S. mucilaginosus*. Among the macrolides tested, clarithromycin was more active than erythromycin, with MICs for the majority of strains being at least two dilutions less than those of erythromycin. Concerning erythromycin, limited in vitro activity towards *S. mucilaginosus* is reported (2, 4, 17).

Regarding *Micrococcus* spp., data on susceptibilities are rare. Strains recovered from clinical sources were susceptible to most antibiotics (10, 12, 18); however, clinical isolates resistant to penicillin and to erythromycin have been reported (1, 12). This has been confirmed by our results, in which we found strains that required elevated penicillin and erythromycin MICs as well. Additionally, we observed a large number of strains that required elevated MICs of fosfomycin and of the aminoglycosides.

In conclusion, our study demonstrates that many β-lactams, imipenem, rifampin, and the glycopeptides show increased in vitro activity against *Stomatococcus* and *Micrococcus* isolates, whereas activities of antibiotics such as some aminoglycosides, erythromycin, and fosfomycin against an important number of these microorganisms are limited. For the first time, these findings may provide a basis for studies evaluating in vitro synergism, animal infection models, and future clinical trials.

We sincerely thank M. Brück and S. Weber for technical assistance.

This study was supported by a grant from the Deutsche Forschungsgemeinschaft, Bonn, FRG.

REFERENCES

1. **Adang, R. P., H. C. Schouten, F. H. van Tiel, and G. H. Blijham.** 1992. Pneumonia due to Micrococcus spp. in a patient with acute myeloid leukaemia. Leukemia **6:**224–226.
2. **Ascher, D. P., C. Zbick, C. White, and G. W. Fischer.** 1991. Infections due to Stomatococcus mucilaginosus: 10 cases and review. Rev. Infect. Dis. **13:** 1048–1052.
3. **Bergan, T., and M. Kocur.** 1982. *Stomatococcus mucilaginosus* gen.nov., sp.nov., ep. rev., a member of the family *Micrococcaceae*. Int. J. Syst. Bacteriol. **32:**374–377.
4. **Chomarat, M., C. Martin, and F. Breysse.** 1989. Sensibilité aux antibiotiques de trente-deux souches de *Stomatococcus mucilaginosus* chez des patients de stomatologie en pratique de ville. Pathol. Biol. **37:**378–381.
5. **Chomarat, M., M. G. Vital, and J. P. Flandrois.** 1991. Susceptibility to aminoglycosides of 63 strains of Stomatococcus mucilaginosus isolated from sputum. Int. J. Med. Microbiol. **276:**63–67.
6. **Gahrn-Hansen, B.** 1985. Etiologic importance of coagulase-negative Micrococcaceae isolated from blood cultures. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **93:**1–6.
7. **Gahrn-Hansen, B., O. Heltberg, V. T. Rosdahl, and P. Sogaard.** 1987. Evaluation of a conventional routine method for identification of clinical isolates of coagulase-negative Staphylococcus and Micrococcus species. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **95:**283–292.
8. **Henwick, S., M. Koehler, and C. C. Patrick.** 1993. Complications of bacteremia due to Stomatococcus mucilaginosus in neutropenic children. Clin. Infect. Dis. **17:**667–671.
9. **Kaufhold, A., R. R. Reinert, and W. Kern.** 1992. Bacteremia caused by Stomatococcus mucilaginosus: report of seven cases and review of the literature. Infection **20:**213–220.
10. **Kim, E. L., D. L. Ching, and F. D. Pien.** 1990. Bacterial endocarditis at a small community hospital. Am. J. Med. Sci. **299:**87–93.
11. **Kloos, W. E., T. G. Tornabene, and K. H. Schleifer.** 1974. Isolation and characterization of micrococci from human skin, including two new species: *Micrococcus lylae* and *Micrococcus kristinae*. Int. J. Syst. Bacteriol. **24:**79–101.
12. **Magee, J. T., I. A. Burnett, J. M. Hindmarch, and R. C. Spencer.** 1990. Micrococcus and Stomatococcus spp. from human infections. J. Hosp. Infect. **16:**67–73.
13. **McWhinney, P. H., C. C. Kibbler, S. H. Gillespie, S. Patel, D. Morrison, A. V. Hoffbrand, and H. G. Prentice.** 1992. Stomatococcus mucilaginosus: an emerging pathogen in neutropenic patients. Clin. Infect. Dis. **14:**641–646.
14. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. NCCLS document M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
15. **Pinsky, R. L., V. Piscitelli, and J. E. Patterson.** 1989. Endocarditis caused by relatively penicillin-resistant *Stomatococcus mucilaginosus*. J. Clin. Microbiol. **27:**215–216.
16. **Rhoden, D. L., G. A. Hancock, and J. M. Miller.** 1993. Numerical approach to reference identification of *Staphylococcus*, *Stomatococcus*, and *Micrococcus* spp. J. Clin. Microbiol. **31:**490–493.
17. **Rochette, A., M. Chomarat, and M. de Montclos.** 1988. Sensibilité aux antibiotiques de soixante quatre souches de *Stomatococcus mucilaginosus* isolées en clinique humaine. Mise en évidence d'une résistance à l'érythromycine. Pathol. Biol. **36:**394–397.
18. **Selladurai, B. M., S. Sivakumaran, S. Aiyar, and A. R. Mohamad.** 1993. Intracranial suppuration caused by Micrococcus luteus. Br. J. Neurosurg. **7:**205–207.