ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1992, p. 198–201
0066-4804/92/010198-04$02.00/0

Vol. 36, No. 1

Susceptibilities of Anaerobic Bacteria Isolated from Animals with Ovine Foot Rot to 28 Antimicrobial Agents

SEGUNDO PIRIZ, RAFAELA CUENCA, JORGE VALLE, AND SANTIAGO VADILLO*

Unidad de Microbiología e Inmunología, Facultad de Veterinaria, Universidad de Extremadura, Carretera de Trujillo, s/n, 10.071 Cáceres, Spain

Received 19 June 1991/Accepted 17 October 1991

The agar dilution method was used to determine the inhibitory activities of 28 antimicrobial agents against 35 strains of the genus *Peptostreptococcus*, 4 strains of the species *Peptococcus niger*, 20 strains of the species *Megasphaera elsdenii*, 7 strains from the species *Acidaminococcus fermentans*, 8 strains of the genus *Clostridium*, 11 strains of the genus *Eubacterium*, and 1 strain of the species *Propionibacterium acidipropionici*, all of which were isolated from 125 clinical cases of ovine foot rot between January 1987 and December 1988. The three ureidopenicillins studied proved to be the most active antimicrobial agents, with a high percentage of strains being susceptible at a concentration of 64 μg/ml. Penicillin G, ampicillin, and the three cephalosporins studied also had good activity. Fosfomycin showed a high degree of activity among the 116 anaerobic bacteria tested.

Foot rot is a disease, or more properly, an infectious syndrome, that is caused by the synergic action of certain microbial species. It is characterized by an exudative inflammation with a strongly characteristic odor; this is followed by necrosis of the epidermal tissues of the hoof, leading in some cases to the complete separation of the horn (7). Although the etiology of foot rot has been widely debated, *Bacteroides nodosus* and *Fusobacterium necrophorum* are generally considered to be the main causal agents. However, since the limbs of affected animals are constantly exposed to bacteria in the environment, and therefore to the possibility of contamination, the range of bacterial species isolated from lesions is wide and varied (5).

Gram-positive anaerobic cocci have been isolated from a variety of animal infections caused by anaerobic bacteria (2, 17). Brunner et al. (3, 4) isolated strains of *Peptostreptococcus* and *Peptococcus* from cases of bovine interdigital dermatitis, and these genera have also been reported to form part of the etiologic complex responsible for summer mastitis (20). The pathogenic role of gram-negative anaerobic cocci has yet to be fully identified; only *Veillonella parvula* has been isolated with any frequency from human infections (16, 18). Katitch (7) and Brunner et al. (3, 4) isolated *Clostridium* species from cases of ovine foot rot and bovine interdigital dermatitis.

Species of the genera *Eubacterium*, *Propionibacterium*, *Leptotrichia*, *Wolinella*, and *Tissierella* have been isolated only from ruminants with foot rot by Píriz Durán et al. (12, 13), who isolated them from affected sheep and goats.

The purpose of this study was to analyze the susceptibilities of 39 gram-positive cocci, 27 gram-negative cocci, 20 gram-positive bacilli, and 30 gram-negative bacilli, all of which are strict anaerobes that were isolated from ovine foot rot lesions, to 28 antimicrobial agents.

The study was carried out with 116 strains belonging to the genera *Peptostreptococcus*, *Peptococcus*, *Acidaminococcus*, *Megasphaera*, *Clostridium*, *Eubacterium*, *Propionibacterium*, and *Tissierella* isolated from 125 sheep with clinical signs of foot rot. Sheep came from 16 different flocks in the province of Cáceres, Spain. Strains were identified in accordance with the criteria laid down in the *Wadsworth Anaerobic Bacteriology Manual* (21) and the Virginia Polytechnic Institute's *Anaerobe Laboratory Manual* (6).

MICs were determined by the proposed standard reference agar dilution procedure for antimicrobial susceptibility testing of anaerobic bacteria (9) by using Wilkins-Chalgren agar (Oxoid Ltd., Basingstoke, United Kingdom). Plates were incubated at 37°C in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) for 48 h. At the beginning and end of each series of antibiotic dilutions, two plates of Wilkins-Chalgren agar were inoculated, one as an anaerobic growth control and the other as an aerobic contamination control. Two control strains from the American Type Culture Collection (Rockville, Md.) were included in all MIC determinations: *Bacteroides fragilis* ATCC 25285 and *Clostridium perfringens* ATCC 13124. The MIC was interpreted as the lowest concentration of each antimicrobial agent that permitted no growth, one discrete colony, or a barely visible haze.

The abilities of the 28 antimicrobial agents studied to inhibit the in vitro growth of bacteria belonging to the genera *Peptostreptococcus*, *Peptococcus*, *Megasphaera*, and *Acidaminococcus* are shown in Table 1. Breakdown and analysis of results in terms of antimicrobial agent groups revealed that only 3% of *Peptostreptococcus* spp. and 10% of *Megasphaera elsdenii* strains were resistant to penicillin G at a concentration of 16 IU/ml; no *Acidaminococcus fermentans* strains showed resistance to the antibiotic at this concentration. Ampicillin showed less activity than penicillin G, with resistance shown by 34.6% of *Peptostreptococcus anaerobius* and 40% of *Peptostreptococcus prevotii* strains. Our results are similar to those reported by Phillips et al. (10) for susceptibility studies involving 225 gram-positive anaerobic cocci isolated from human infections. Lower resistance rates to ampicillin, however, have been reported by Sutter and Finegold (22), Appelbaum and Chatterton (1), and Píriz Durán et al. (11).

A high degree of activity was recorded for the three ureidopenicillins studied, and in particular for piperacillin. We agree with Wilkinson (23) and Píriz Durán et al. (15) that

* Corresponding author.

TABLE 1. Susceptibilities of 39 anaerobic gram-positive cocci and 27 anaerobic gram-negative cocci isolated from cases of ovine foot rot

Antimicrobial agent[a]	*Peptostreptococcus* spp. (35)[b]				*Peptococcus niger* (4)			*Megasphaera elsdenii* (20)				*Acidaminococcus fermentans* (7)		
	MIC (μg/ml)[c]			% S[d]	MIC (μg/ml)		% S	MIC (μg/ml)			% S	MIC (μg/ml)		% S
	Range	50%	90%		Range	50%		Range	50%	90%		Range	50%	
Penicillin G (16)	≤0.06–128	0.2	4	97	0.1–0.5	0.1	100	≤0.06–32	1	16	90	≤0.06–16	1	100
Ampicillin (4)	≤0.06–128	2	32	66	≤0.06–2	0.1	100	0.1–32	0.5	2	95	0.1–4	0.5	100
Azlocillin (64)	≤0.06–64	0.5	16	100	0.5–4	2	100	≤0.06	0.5	2	100	≤0.06–4	0.2	100
Piperacillin (64)	≤0.06–32	0.5	8	100	≤0.06–0.5	0.2	100	≤0.06–16	1	8	100	≤0.06–4	0.5	100
Mezlocillin (64)	≤0.06–128	0.5	8	97	0.2–1	0.2	100	≤0.06–64	0.5	8	100	≤0.06–4	0.2	100
Cefuroxime (16)	≤0.06–≥256	0.5	16	94	≤0.06–1	0.2	100	≤0.06–32	1	8	90	≤0.06–4	1	100
Cefoperazone (32)	≤0.06–16	0.5	4	100	≤0.06–8	0.2	100	≤0.06–16	1	8	100	≤0.06–16	2	100
Cefotaxime (32)	≤0.06–≥256	2	8	97	≤0.06–2	1	100	≤0.06–16	0.5	8	100	≤0.06–32	2	100
Cefoxitin (32)	≤0.06–64	1	16	97	0.2–4	0.5	100	≤0.06–128	1	32	95	0.2–32	4	100
Imipenem (8)	≤0.06–≥256	2	32	80	0.5–8	1	100	0.2–16	1	16	80	≤0.06–8	0.5	100
Dihydrostreptomycin (32)	0.5–128	16	64	77	8–≥256	32	50	≤0.06–≥256	8	≥256	70	0.2–≥256	16	71
Neomycin (32)	≤0.06–≥256	64	≥256	40	4–≥256	16	75	≤0.06–128	8	64	75	2–128	16	71
Spectinomycin (32)	≤0.06–≥256	16	64	71	8–128	32	75	≤0.06–≥256	32	128	50	2–≥256	16	71
Josamycin (4)	≤0.06–≥256	2	32	63	1–16	2	75	≤0.06–≥256	0.2	32	75	≤0.06–≥256	2	71
Spiramycin (4)	≤0.06–≥256	2	32	69	1–16	2	75	≤0.06–32	2	16	75	≤0.06–≥256	2	57
Chloramphenicol (16)	≤0.06–≥256	1	64	86	1–16	2	100	≤0.06–128	4	64	85	≤0.06–64	1	86
Thiamphenicol (16)	≤0.06–≥256	8	64	77	4–32	16	75	≤0.06–≥256	8	≥256	60	≤0.06–≥256	8	71
Nalidixic acid (16)	1–≥256	128	≥256	20	8–≥256	32	25	1–≥256	16	128	50	4–≥256	32	29
Pipimedic acid (16)	≤0.06–≥256	32	128	40	1–64	4	75	≤0.06–≥256	16	64	55	1–128	8	57
Norfloxacin (4)	≤0.06–128	4	32	54	0.5–8	1	75	≤0.06–≥256	2	32	65	0.5–128	16	43
Lincomycin (4)	≤0.06–≥256	4	≥256	54	0.5–32	8	25	≤0.06–≥256	1	≥256	80	0.2–≥256	2	57
Metronidazole (16)	≤0.06–≥256	2	64	86	0.5–8	1	100	≤0.06–≥256	0.5	≥256	80	1–≥256	16	57
Tinidazole (16)	≤0.06–≥256	2	32	83	1–16	1	100	0.1–≥256	1	≥256	80	8–≥256	32	29
Rifampin (2)	≤0.06–32	1	32	80	0.5–4	2	75	≤0.06–16	1	8	65	≤0.06–32	2	71
Tetracycline (4)	≤0.06–32	2	16	69	0.2–8	1	75	≤0.06–16	0.5	4	95	0.5–32	8	43
Sulfametoxypyridiazine (256)	16–≥256	128	≥256	66	8–≥256	64	50	16–≥256	64	≥256	60	1–≥256	128	86
Trimethoprim (8)	≤0.06–≥256	128	≥256	11	2–≥256	32	25	8–≥256	128	≥256	5	32–≥256	64	0
Fosfomycin (128)	≤0.06–128	4	32	100	0.2–4	1	100	≤0.06–≥256	16	64	90	1–≥256	8	86

[a] Numbers in parentheses are MIC breakpoints (in micrograms per milliliter) indicating susceptibility.
[b] Includes 26 *P. anaerobius*, 5 *P. prevotii*, and 4 *P. asaccharolyticus* isolates.
[c] MICs of all agents except penicillin G are given in micrograms per milliliter. Penicillin G MICs are in units per milliliter. 50% and 90%, MICs for 50 and 90% of strains tested, respectively.
[d] % S, percentage of strains susceptible at the MIC breakpoint.

TABLE 2. Susceptibilities of 20 anaerobic gram-positive rods strains and 30 anaerobic gram-negative rods strains isolated from cases of ovine foot rot

Antimicrobial agents[a]	Anaerobic gram-positive rods (20)[b]				*Tissierella praeacuta* (30)			
	MIC (μg/ml)[c]			% S[d]	MIC (μg/ml)			% S
	Range	50%	90%		Range	50%	90%	
Penicillin G (16)	≤0.06–32	4	32	90	≤0.06–64	1	64	83
Ampicillin (4)	≤0.06–64	1	16	80	0.1–128	1	64	77
Azlocillin (64)	≤0.06–32	1	32	100	≤0.06–16	1	16	100
Piperacillin (64)	≤0.06–8	0.2	1	100	≤0.06–64	0.2	4	100
Mezlocillin (64)	≤0.06–4	0.2	1	100	≤0.06–16	1	4	100
Cefuroxime (16)	≤0.06–64	0.5	32	85	≤0.06–32	4	32	83
Cefoperazone (32)	≤0.06–64	1	16	95	0.1–64	2	8	93
Cefotaxime (32)	≤0.06–64	1	16	95	≤0.06–128	2	64	87
Cefoxitin (32)	≤0.06–32	1	8	100	≤0.06–128	2	128	80
Imipenem (8)	≤0.06–64	1	32	80	0.1–4	0.5	2	100
Dihydrostreptomycin (32)	≤0.06–64	16	64	85	8–≥256	≥256	≥256	10
Neomycin (32)	≤0.06–128	32	128	65	16–≥256	≥256	≥256	17
Spectinomycin (32)	≤0.06–≥256	32	128	55	≤0.06–≥256	16	≥256	67
Josamycin (4)	≤0.06–8	1	4	90	≤0.06–64	0.5	8	87
Spiramycin (4)	≤0.06–32	2	32	60	≤0.06–16	1	16	67
Chloramphenicol (16)	≤0.06–32	0.5	8	95	≤0.06–128	2	16	90
Thiamphenicol (16)	≤0.06–64	2	32	85	≤0.06–≥256	4	≥256	70
Nalidixic acid (16)	≤0.06–≥256	32	≥256	45	2–≥256	128	≥256	7
Pipimedic acid (16)	≤0.06–≥256	16	128	50	2–≥256	32	≥256	27
Norfloxacin (4)	≤0.06–128	2	32	65	≤0.06–64	8	64	30
Lincomycin (4)	≤0.06–64	0.5	64	75	0.2–≥256	8	128	47
Metronidazole (16)	≤0.06–128	0.5	32	85	≤0.06–16	1	4	100
Tinidazole (16)	≤0.06–32	0.2	8	95	≤0.06–16	1	4	100
Rifampin (2)	≤0.06–16	0.5	16	85	≤0.06–64	1	64	53
Tetracycline (4)	≤0.06–32	1	32	85	≤0.06–64	1	16	73
Sulfametoxypyridiazine (256)	32–≥256	128	≥256	60	8–≥256	128	≥256	63
Trimethoprim (8)	8–≥256	128	≥256	5	2–≥256	128	≥256	3
Fosfomycin (128)	≤0.06–64	1	16	100	2–≥256	32	≥256	80

[a] Numbers in parentheses are MIC breakpoints (in micrograms per milliliter) indicating susceptibility.

[b] Numbers in parentheses are the numbers of strains tested. The value for anaerobic gram-positive rods includes four *Clostridium malenominatum*, two *Clostridium scatologenes*, one *Clostridium sporogenes*, one *Clostridium tyrobutyricum*, four *Eubacterium nodatum*, four *Eubacterium combesii*, two *Eubacterium brachy*, one *Eubacterium nitritogenes*, and one *Propionibacterium acidipropionici* isolates.

[c] MICs of all agents except penicillin G are given in micrograms per milliliter. Penicillin G MICs are in units per milliliter. 50% and 90%, MICs for 50 and 90% of strains tested, respectively.

[d] % S, percentage of strains susceptible at the MIC breakpoint.

of the penicillin group of antibiotics, ureidopenicillins possess the greatest activity against anaerobic bacteria.

Of the three cephalosporins studied, the most active was cefoperazone, with an MIC for 50% of isolates studied (MIC_{50}) of 0.5 μg/ml for gram-positive anaerobic cocci and 1 μg/ml for gram-negative species; no strain was resistant at the established breakpoint (16 μg/ml). Sedallian (19) obtained lower MICs when studying the susceptibilities of 35 *Peptostreptococcus* and *Peptococcus* strains isolated from human infections to cefotaxime. Of the β-lactam antibiotics, cefoxitin performed slightly better than imipenem, although fairly high rates of resistance to both antibiotics were found.

Resistance rates to the remainder of the antimicrobial agents studied were also high: 35% of strains were resistant to the three aminoglycosides and 30% were resistant to both macrolides. Many strains were resistant to the quinolones nalidixic acid and pipimedic acid while the MIC_{50} of the fluoroquinolone norfloxacin was 2 μg/ml for the 39 gram-positive cocci tested; this concentration was similar to that reported by Mandell and Neu (8) in studies of *Peptostreptococcus* species and by Píriz Durán et al. (11) in studies of 65 gram-positive anaerobic cocci. Norfloxacin activity against gram-negative cocci was also moderate. Fosfomycin proved effective in inhibiting the growth of most of the strains tested; only 10% of *Megasphaera elsdenii* and 14% of *Acidaminococcus fermentans* strains were resistant to 128 μg/ml.

Table 2 shows the susceptibilities of 20 gram-positive and 30 gram-negative obligate anaerobic bacilli to the same 28 antimicrobial agents. Again, the ureidopenicillins were the most active group; no resistance to any of the three antibiotics was recorded, a finding which coincides with the results obtained by Píriz Durán et al. (14) in studies of gram-positive and gram-negative anaerobic bacilli isolated from goats with foot rot.

Penicillin G was more active that ampicillin. The behaviors of the three cephalosporins were similar, with susceptibility rates of about 90%. One *Clostridium* strain (12.5%) was resistant to imipenem. These values are similar to those reported by Phillips et al. (10) and Píriz Durán et al. (14).

The three aminoglycosides showed very little activity, with resistance rates of over 25% for all four genera. Píriz Durán et al. (14) reported equally high rates of resistance to these antimicrobial agents among microorganisms isolated from goats with foot rot.

The two macrolides yielded uneven results: josamycin was much more active than spiramycin. Similarly, chloramphenicol showed greater activity than thiamphenicol, which

had an MIC_{50} of 4 μg/ml for members of the genus *Propionibacterium*; Sutter and Finegold (21) reported a comparable value (8 μg/ml) for the same genus.

The quinolones sulfametoxypyridiazine and trimethoprim failed to inhibit the growth of the bacteria studied. High resistance rates were also encountered for the remainder of the antimicrobial agents tested. The most effective of these was tinidazole, which inhibited all *Clostridium* strains, and only 5% of the other genera were resistant to this antimicrobial agent. Finally, the 20 anaerobic gram-positive rods studied were susceptible to fosfomycin at a concentration of 128 μg/ml.

We thank E. Fernández Corrales for excellent technical assistance.

This study was part of a research project supported by the Extremadura Regional Government Council for Education and Science.

REFERENCES

1. **Appelbaum, P. C., and S. A. Chatterton.** 1978. Susceptibility of anaerobic bacteria to ten antimicrobial agents. Antimicrob. Agents Chemother. **14**:371–376.
2. **Berg, J. N., W. H. Fales, and C. M. Scanlan.** 1979. Occurrence of anaerobic bacteria in diseases of the dog and cat. Am. J. Vet. Res. **40**:876–881.
3. **Brunner, F., G. Marca, A. Nelli, and G. Ruffo.** 1982. Isolamento e identificazione di microrganismo anaerobi de episodi di dermatite digitale del bovino. Atti Soc. Sci. Vet. **36**:595–599.
4. **Brunner, F., A. Zecconi, C. M. Mortellaro, G. Marca, and G. Ruffo.** 1989. Isolamento e identificazione di germi anaerobi da lesioni digitali del bovini da latte. Arch. Vet. Ital. **40**:185–187.
5. **Egerton, J. R., D. S. Roberts, and I. M. Parsonson.** 1969. The aetiology and pathogenesis of ovine footrot. I. A histological study of the bacterial invasion. J. Comp. Pathol. **79**:207–215.
6. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
7. **Katitch, R. V.** 1979. Les problemes de l'etiologie et de l'immunoprophylaxie dans le pietin du mouton. Comp. Immunol. Microbiol. Infect. Dis. **2**:55–59.
8. **Mandell, W., and H. C. Neu.** 1986. In vitro activity of CI-934, a new quinolone, compared with that of other quinolones and other antimicrobial agents. Antimicrob. Agents Chemother. **29**:852–857.
9. **National Committee for Clinical Laboratory Standards.** 1989. Methods for antimicrobial susceptibility testing of anaerobic bacteria, 2nd ed. Tentative standard M11-T2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
10. **Phillips, I., C. Warren, E. Taylor, R. Timewell, and S. Eykyn.** 1981. The antimicrobial susceptibility of anaerobic bacteria in a London teaching hospital. J. Antimicrob. Chemother. **8**(Suppl. D):17–26.
11. **Píriz Durán, S., R. Cuenca Valera, J. Valle Manzano, T. Torroba Pérez, and S. Vadillo Machota.** 1989. Sensibilidad de cocos grampositivos y gramnegativos aislados de casos de pedero caprino a 27 antimicrobianos. Acta Vet. **3**:37–41.
12. **Píriz Durán, S., R. Cuenca Valera, J. Valle Manzano, and S. Vadillo Machota.** 1990. The isolation and identification of anaerobic bacteria from ovine footrot in Spain. Res. Vet. Sci. **49**:245–247.
13. **Píriz Durán, S., J. Valle Manzano, R. Cuenca Valera, and S. Vadillo Machota.** 1990. Obligately anaerobic bacterial species isolated from footrot lesions in goats. Br. Vet. J. **146**:551–558.
14. **Píriz Durán, S., J. Valle Manzano, R. Cuenca Valera, and S. Vadillo Machota.** 1990. Sensibilidad de microorganismos anaerobios obligados aislados a partir de lesiones de pedero caprino a 27 antimicrobianos. Med. Vet. **7**:99–104.
15. **Píriz Durán, S., J. Valle Manzano, R. Cuenca Valera, and S. Vadillo Machota.** 1990. Susceptibilities of *Bacteroides* and *Fusobacterium* spp. from foot rot in goats to 10 β-lactam antibiotics. Antimicrob. Agents Chemother. **34**:657–659.
16. **Popoff, M. R.** 1982. Importance et diversité des bactéries anaérobies en pathologie animale. Rev. Med. Vet. **4**:231–236.
17. **Prescott, J. F.** 1979. Identification of some anaerobic bacteria in nonspecific anaerobic infections in animals. Can. J. Comp. Med. **43**:194–199.
18. **Rosenblatt, J. E.** 1985. Anaerobic cocci, p. 445–449. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
19. **Sedallian, A.** 1986. Susceptibility of anaerobes to eight antibiotics. Pathol. Biol. **34**:645–647.
20. **Sorensen, G. H.** 1976. Studies on the ocurrence of *Peptococcus indolicus* and *Corynebacterium pyogenes* in apparently healthy cattle. Acta Vet. Scand. **17**:15–24.
21. **Sutter, V. L., D. M. Citron, M. A. C. Edelstein, and S. M. Finegold.** 1985. Wadsworth anaerobic bacteriology manual, 4th ed. Star Publishing Co., Los Angeles.
22. **Sutter, V. L., and S. M. Finegold.** 1976. Susceptibility of anaerobic bacteria to 23 antimicrobial agents. Antimicrob. Agents Chemother. **10**:736–752.
23. **Wilkinson, P. J.** 1982. Beta-lactam antibiotics in the newborn. J. Antimicrob. Chemother. **9**(Suppl. B):21–29.