

Susceptibilities of *Bacteroides* and *Fusobacterium* spp. from Foot Rot in Goats to 10 β -Lactam Antibiotics

S. PIRIZ DURAN, J. VALLE MANZANO, R. CUENCA VALERA, AND S. VADILLO MACHOTA*

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

Received 4 October 1989/Accepted 17 January 1990

The agar dilution method was used to determine the bacteriostatic activities of 10 β -lactam antibiotics against 132 strains belonging to the genus *Bacteroides* and 25 strains belonging to the genus *Fusobacterium*, all isolated from clinical cases of caprine foot rot. The three ureidopenicillins studied proved to be the most effective antimicrobial agents.

Foot rot is an infectious disease caused by the synergic action of different microbial species and specific to sheep and goats, although it is also reported in cattle. Microorganisms of the genus *Bacteroides* (mainly *B. nodosus*) and the genus *Fusobacterium* (mainly *F. necrophorum*) have been reported as the etiological agents of ovine (7, 14, 16, 25), bovine (6), and caprine (10) foot rot. A wide range of chemotherapeutic agents for topical and parenteral use have been recommended to check this disease, but few quantitative studies have been done to determine the in vitro susceptibilities of these microorganisms to different antimicrobial agents (15, 18, 30). The purpose of this study was to determine the susceptibilities of *Bacteroides* and *Fusobacterium* strains isolated from clinical cases of caprine foot rot to 10 β -lactam antibiotics (penicillin G, ampicillin, azlocillin, mezlocillin, piperacillin, cefuroxime, cefoperazone, cefotaxime, ceftiofur, and imipenem).

The study was done with 132 strains belonging to the genus *Bacteroides* and 25 strains from the genus *Fusobacterium* isolated from 120 goats with clinical signs of foot rot. The goats came from 13 different flocks in the province of Cáceres, Spain. The strains were identified by the criteria in the *Wadsworth Anaerobic Bacteriology Manual* (32) and the *Virginia Polytechnic Institute Anaerobe Laboratory Manual* (19).

MICs were determined by the proposed standard reference agar dilution procedure for antimicrobial susceptibility testing of anaerobic bacteria (22). Wilkins-Chalgren agar (Oxoid Ltd., Basingstoke, United Kingdom) was used. The plates were incubated at 37°C in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) for 48 h. 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 MICs of 10 β -lactam antibiotics for 132 strains of the genus *Bacteroides* isolated from foot rot in goats are shown in Table 1. The susceptibilities to β -lactam antibiotics of the 25 *Fusobacterium* strains studied are shown in Table 2.

The susceptibilities to penicillin G of the *Bacteroides* strains isolated in this study are similar to those reported by Benno and Mitsouka (5) for *B. helcogenes* isolated from abscesses in swine and by Gradin and Schmitz (18) for *B. nodosus* obtained from foot rot in sheep. Results similar to those obtained in the present study have been reported (1, 9,

29, 34) for bacteria belonging to the *Bacteroides melaninogenicus*-*B. oralis* group. The 12 *F. necrophorum* strains studied here were more resistant to penicillin G than were those isolated by Baba et al. (2) from bovine hepatic abscesses and by Ikawa et al. (20) from uterine pyemia in cattle. Ampicillin showed good activity against the microorganisms isolated in this study. This is not commonly the case with bacteria isolated from humans (1, 4, 34, 35).

The degrees of effectiveness of azlocillin, mezlocillin, and piperacillin found in this study are similar to those reported by Sutter and Finegold (34) for nine *B. melaninogenicus* strains and several *Fusobacterium* species. Others (8, 24) have reported similar results. This suggests that, a priori, the use of some of these ureidopenicillins (azlocillin, mezlocillin, and piperacillin) might be of value in the treatment of foot rot.

The rate of resistance of *B. nodosus* to cefuroxime in this study is not comparable to the values reported by Gradin and Schmitz (18) for 18 *B. nodosus* strains tested against cefamandole (MIC range, 0.06 to 1 μ g/ml). The other two species which were resistant to 16 μ g of cefuroxime per ml were *Bacteroides buccae* (6.1%) and *B. ruminicola* subsp. *brevis* (4.5%). Of the *Fusobacterium* strains studied, 20% were resistant at a concentration of 16 μ g/ml, and 16% were resistant at a greater concentration. George et al. (17) and Sutter and Finegold (33) reported similar results. The values obtained for cefoperazone and cefotaxime were practically the same for the 132 *Bacteroides* strains studied, with 6.8% resistant to 16 μ g/ml in both cases. Similar resistance rates have been described by Sedallian (28) with 39 *B. oralis* and *B. bivius* strains and 33 *B. asaccharolyticus* strains. However, Cuchural et al. (11), Rosenblatt (27), and Tally et al. (36) reported considerably higher resistance rates in *B. fragilis* group strains, although it should be borne in mind that these strains are β -lactamase producers. Rates of resistance to cefoperazone and cefotaxime obtained in this study for 25 *Fusobacterium* strains are lower than those reported by George et al. (17) for *F. varium* and *F. naviforme* isolated from normal populations and from human infections.

Ceftiofur activity against the 132 *Bacteroides* strains (12.9% of strains resistant to 16 μ g/ml) was similar to that described by others (3, 11, 12, 24, 27, 28, 31, 33, 36). In all cases, resistance rates were around 15%. The rates of resistance to ceftiofur recorded in this study for *F. necrophorum*, *F. mortiferum*, and *F. naviforme* were higher than those reported by Dubreuil et al. (13) for 14 *Fusobacterium*

* Corresponding author.

TABLE 1. Susceptibilities of 132 *Bacteroides* strains isolated from cases of caprine foot rot

Species (no. of strains tested)	Antimicrobial agent	MIC ^a		
		Range	50%	90%
<i>B. buccae</i> (65)	Penicillin G	≤0.06–≥256	0.5	32
	Ampicillin	≤0.06–64	0.5	8
	Azlocillin	≤0.06–32	0.5	8
	Piperacillin	≤0.06–8	0.25	4
	Mezlocillin	≤0.06–32	0.12	8
	Cefuroxime	≤0.06–32	4	16
	Cefoperazone	≤0.06–64	1	32
	Cefotaxime	≤0.06–64	1	32
	Cefoxitin	≤0.06–≥256	0.25	16
	Imipenem	≤0.06–16	0.12	4
	<i>B. nodosus</i> (37)	Penicillin G	≤0.06–32	0.12
Ampicillin		≤0.06–4	0.12	2
Azlocillin		≤0.06–32	0.25	4
Piperacillin		≤0.06–2	0.25	2
Mezlocillin		≤0.06–4	0.25	4
Cefuroxime		≤0.06–32	0.25	32
Cefoperazone		≤0.06–32	1	16
Cefotaxime		≤0.06–32	0.12	8
Cefoxitin		≤0.06–64	1	64
Imipenem		≤0.06–16	0.5	16
<i>B. ruminicola</i> subsp. <i>brevis</i> (22)		Penicillin G	≤0.06–128	0.5
	Ampicillin	≤0.06–4	0.5	4
	Azlocillin	≤0.06–64	0.5	16
	Piperacillin	≤0.06–128	0.25	4
	Mezlocillin	≤0.06–32	≤0.06	4
	Cefuroxime	≤0.06–128	4	8
	Cefoperazone	≤0.06–8	1	2
	Cefotaxime	≤0.06–8	2	4
	Cefoxitin	≤0.06–≥256	0.12	4
	Imipenem	≤0.06–0.5	0.12	0.5
	Other <i>Bacteroides</i> (8) ^b	Penicillin G	≤0.06–1	≤0.06
Ampicillin		≤0.06–4	≤0.06	
Azlocillin		≤0.06–1	≤0.06	
Piperacillin		≤0.06–0.5	≤0.06	
Mezlocillin		≤0.06	≤0.06	
Cefuroxime		≤0.06–4	≤0.06	
Cefoperazone		≤0.06–16	2	
Cefotaxime		≤0.06–1	≤0.06	
Cefoxitin		≤0.06–1	0.12	
Imipenem	≤0.06–1	0.12		

^a 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.

^b Including three *B. levii*, three *B. splanchnicus*, one *B. putredinis*, and one *B. macacae* isolate.

strains. Phillips et al. (24), Sedallian (28), and Sutter and Finegold (33) found no strains resistant to cefoxitin.

Finally, the activity recorded for imipenem against *Bacteroides* strains has also been reported by others (21, 23, 24, 26). However, this antimicrobial agent was less effective against the 25 *Fusobacterium* strains, resistance being recorded in *F. necrophorum*, *F. mortiferum*, and *F. naviforme*. Values obtained for other *Fusobacterium* species were similar to those reported by Phillips et al. (24), all such species being susceptible to imipenem.

We thank E. Fernández Corrales for technical assistance.

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

TABLE 2. Susceptibilities of 25 *Fusobacterium* strains isolated from cases of caprine foot rot

Species (no. of strains tested)	Antimicrobial agent	MIC ^a		
		Range	50%	90%
<i>F. necrophorum</i> (12)	Penicillin G	≤0.06–128	0.12	128
	Ampicillin	≤0.06–16	≤0.06	4
	Azlocillin	≤0.06–8	≤0.06	8
	Piperacillin	≤0.06–8	0.25	4
	Mezlocillin	≤0.06–4	0.25	4
	Cefuroxime	≤0.06–≥256	1	≥256
	Cefoperazone	≤0.06–128	0.5	64
	Cefotaxime	≤0.06–≥256	1	≥256
	Cefoxitin	≤0.06–128	0.5	64
	Imipenem	≤0.06–64	2	32
	Other <i>Fusobacterium</i> (13) ^b	Penicillin G	≤0.06–32	2
Ampicillin		≤0.06–≥256	0.5	4
Azlocillin		≤0.06–≥256	0.25	64
Piperacillin		≤0.06–32	0.12	8
Mezlocillin		≤0.06–32	0.12	16
Cefuroxime		≤0.06–64	0.25	32
Cefoperazone		≤0.06–16	≤0.06	16
Cefotaxime		≤0.06–32	0.5	8
Cefoxitin		≤0.06–64	0.5	64
Imipenem		≤0.06–128	0.5	16

^a 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.

^b Including four *F. mortiferum*, three *F. naviforme*, two *F. prausnitzii*, two *F. sulci*, one *F. necrogenes*, and one *F. perfoetens* isolate.

LITERATURE CITED

- Appelbaum, P. C., and S. A. Chatterton. 1978. Susceptibility of anaerobic bacteria to ten antimicrobial agents. *Antimicrob. Agents Chemother.* 14:371–376.
- Baba, E., T. Fukata, A. Arakawa, H. Ikawa, and M. Takeda. 1989. Antibiotic susceptibility of *Fusobacterium necrophorum* from bovine hepatic abscesses. *Br. Vet. J.* 145:195–197.
- Barry, A. L., and R. R. Packer. 1984. Determination of susceptibility of anaerobic bacteria to cefotetan and cefoxitin by the thioglycolate disk elution method. *J. Clin. Microbiol.* 20:912–916.
- Bawdon, R. E., L. R. Crane, and S. Palchaudhuri. 1982. Antibiotic resistance in anaerobic bacteria: molecular biology and clinical aspects. *Rev. Infect. Dis.* 4:1075–1095.
- Benno, Y., and T. Mitsouka. 1984. Susceptibility of *Bacteroides* from swine abscesses to 13 antibiotics. *Am. J. Vet. Res.* 45:2631–2633.
- Berg, J. N., and R. W. Loan. 1975. *Fusobacterium necrophorum* and *Bacteroides melaninogenicus* as etiologic agents of foot rot in cattle. *Am. J. Vet. Res.* 36:1115–1122.
- Beveridge, W. I. B. 1941. Foot rot in sheep: a transmissible disease due to infection with *Fusiformis nodosus* (n. sp.). *Bull. Coun. Scient. Ind. Res.* 140:1–56.
- Brown, J. E., V. E. Del Bene, and C. D. Collins. 1981. In vitro activity of *N*-formimidoyl thienamycin, moxalactam, and other new beta-lactam agents against *Bacteroides fragilis*: contribution of beta-lactamase to resistance. *Antimicrob. Agents Chemother.* 19:248–252.
- Brown, W. J., and P. E. Waatti. 1980. Susceptibility testing of clinically isolated anaerobic bacteria by an agar dilution technique. *Antimicrob. Agents Chemother.* 17:629–635.
- Claxton, P. D., and K. C. O'Grady. 1986. Foot rot in goats and characterisation of caprine isolates of *Bacteroides nodosus*, p. 119–123. In D. J. Stewart, J. E. Peterson, N. M. Mckern, and D. L. Emery (ed.), *Foot rot in ruminants*, 1st ed. Australian Wool Corporation technical publication. Australian Wool Corporation, Melbourne.
- Cuchural, G., N. Jacobus, S. L. Gorbach, and F. P. Tally. 1981. A survey of *Bacteroides* susceptibility in the United States. *J.*

- Antimicrob. Chemother. 8(Suppl. D):27-31.
12. Dubreuil, L., J. Devos, C. Neut, and C. Romond. 1983. Etude de la sensibilité antibiotique des anaérobies stricts. *Med. Mal. Infect.* 13:478-483.
 13. Dubreuil, L., J. Devos, C. Neut, and C. Romond. 1984. Susceptibility of anaerobic bacteria from several French hospitals to three major antibiotics. *Antimicrob. Agents Chemother.* 25:764-766.
 14. Egerton, J. R. 1979. Treatment of ovine foot rot by vaccination with the specific aetiological agent *Bacteroides nodosus*. *Comp. Immunol. Microbiol. Infect. Dis.* 2:61-67.
 15. Egerton, J. R., I. M. Parsonson, and N. P. H. Graham. 1968. Parenteral chemotherapy of ovine foot rot. *Aust. Vet. J.* 44:275-283.
 16. Egerton, J. R., and D. S. Roberts. 1971. Vaccination against ovine foot rot. *J. Comp. Pathol.* 81:179-185.
 17. George, W. L., B. D. Kirby, V. L. Sutter, D. M. Citron, and S. M. Finegold. 1981. Gram-negative anaerobic bacilli: their role in infection and patterns of susceptibility to antimicrobial agents. II. Little-known *Fusobacterium* species and miscellaneous genera. *Rev. Infect. Dis.* 3:599-626.
 18. Gradin, J. L., and J. A. Schmitz. 1983. Susceptibility of *Bacteroides nodosus* to various antimicrobial agents. *J. Am. Vet. Med. Assoc.* 183:434-437.
 19. 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.
 20. Ikawa, H., H. Kitada, K. Kubo, M. Takeda, and E. Baba. 1987. An uncommon source of bovine *Fusobacterium* pyemia. *Vet. Med.* August:844-847.
 21. 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.
 22. National Committee for Clinical Laboratory Standards. 1985. Reference dilution procedure for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard MII-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
 23. Owens, W. E., and S. M. Finegold. 1983. Comparative in vitro susceptibilities of anaerobic bacteria to cefmenoxime, cefotetan, and *N*-formimidoyl thienamycin. *Antimicrob. Agents Chemother.* 23:626-629.
 24. 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.
 25. Roberts, D. S., and J. R. Egerton. 1969. The aetiology and pathogenesis of ovine foot rot. II. The pathogenic association of *Fusiformis nodosus* and *Fusiformis necrophorus*. *J. Comp. Pathol.* 79:217-227.
 26. Rolfe, R. D., and S. M. Finegold. 1981. Comparative in vitro activity of new beta-lactam antibiotics against anaerobic bacteria. *Antimicrob. Agents Chemother.* 20:600-609.
 27. Rosenblatt, J. E. 1984. Antimicrobial susceptibility testing of anaerobic bacteria. *Rev. Infect. Dis.* 6(Suppl. 1):242-248.
 28. Sedallian, A. 1986. Susceptibility of anaerobes to eight antibiotics. *Pathol. Biol.* 34:645-647.
 29. Stanek, J. L., and J. A. Washington II. 1974. Antimicrobial susceptibilities of anaerobic bacteria: recent clinical isolates. *Antimicrob. Agents Chemother.* 6:311-315.
 30. Stewart, D. F. 1954. The treatment of contagious foot rot in sheep by the topical application of chloromycetin. *Aust. Vet. J.* 30:209-212.
 31. Sutter, V. L., A. L. Barry, T. D. Wilkins, and R. J. Zabransky. 1979. Collaborative evaluation of a proposed reference dilution method of susceptibility testing of anaerobic bacteria. *Antimicrob. Agents Chemother.* 16:495-502.
 32. 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.
 33. Sutter, V. L., and S. M. Finegold. 1975. Susceptibility of anaerobic bacteria to carbenicillin, cefoxitin, and related drugs. *J. Infect. Dis.* 131:417-422.
 34. Sutter, V. L., and S. M. Finegold. 1976. Susceptibility of anaerobic bacteria to 23 antimicrobial agents. *Antimicrob. Agents Chemother.* 10:736-752.
 35. Sutter, V. L., M. J. Jones, and A. T. M. Ghoneim. 1983. Antimicrobial susceptibilities of bacteria associated with periodontal disease. *Antimicrob. Agents Chemother.* 23:483-486.
 36. Tally, F. P., G. J. Cuchural, N. V. Jacobus, S. L. Gorbach, K. E. Aldridge, T. J. Cleary, S. M. Finegold, G. B. Hill, P. B. Iannini, R. V. McCloskey, J. P. O'Keefe, and C. L. Pierson. 1983. Susceptibility of the *Bacteroides fragilis* group in the United States in 1981. *Antimicrob. Agents Chemother.* 23:536-540.