

Susceptibilities of Bovine Summer Mastitis Bacteria to Antimicrobial Agents

HANNELE JOUSIMIES-SOMER,^{1*} SATU PYÖRÄLÄ,² AND ARJA KANERVO¹

*Anaerobe Reference Laboratory, National Public Health Institute, FIN-00300 Helsinki,¹ and
The Department of Clinical Veterinary Sciences, College of Veterinary
Medicine, FIN-04840 Hautjärvi,² Finland*

Received 8 May 1995/Returned for modification 11 July 1995/Accepted 17 October 1995

The susceptibility to 9 antimicrobial agents of 32 aerobic bacterial isolates and to 10 antimicrobial agents of 37 anaerobic bacterial isolates from 23 cases of bovine summer mastitis (16 *Actinomyces pyogenes* isolates, 8 *Streptococcus dysgalactiae* isolates, 3 *S. uberis* isolates, 3 *S. acidominimus* isolates, 2 *Streptococcus* spp., 15 *Peptostreptococcus indolicus* isolates, 10 *Fusobacterium necrophorum* isolates, and 12 isolates of anaerobic gram-negative rods) was determined by the agar dilution method. All isolates except one *Bacteroides fragilis* isolate (β -lactamase producer) were susceptible to penicillin G, amoxicillin, amoxicillin-clavulanate, cefoxitin, clindamycin, and chloramphenicol (the *B. fragilis* strain was susceptible to the last four), which had MICs at which 90% of isolates were inhibited (MIC_{90s}) of ≤ 0.06 , ≤ 0.06 , ≤ 0.06 , 0.25, ≤ 0.06 , and 4.0 $\mu\text{g/ml}$, respectively. Spiramycin was active against the gram-positive aerobes (MIC₉₀, 1.0 $\mu\text{g/ml}$) but not against the anaerobes (MIC₉₀, 16.0 $\mu\text{g/ml}$). Similar trends were noted for susceptibilities of aerobic and anaerobic bacteria to ofloxacin (MIC_{90s}, 2.0 and 8 $\mu\text{g/ml}$, respectively). Occasional strains of aerobic streptococci were resistant to oxytetracycline, but all anaerobes were susceptible. Tinidazole was active against all anaerobes (MIC₉₀, 2.0 $\mu\text{g/ml}$). β -Lactamase was produced only by the *B. fragilis* isolate.

Aerobic-anaerobic mastitis, often termed summer mastitis, is a suppurative infection of the udder quarters of heifers and nonlactating cows. The infection is characterized by production of copious quantities of foul-smelling pus and a rapidly progressing course of hardening, tenderness, and eventual tissue destruction of the infected quarters (25). Most cases of aerobic-anaerobic mastitis occur during the summer pasture season and a fly, *Hydrotaea irritans*, is generally considered the vector (25). However, we have recently shown that a clinically and bacteriologically comparable disease also occurs indoors in winter (18). Microaerophilic *Actinomyces pyogenes* and anaerobic bacteria, especially *Peptostreptococcus indolicus* and *Fusobacterium necrophorum*, are the pathogens most often implicated in the bacterial etiology of summer mastitis. The most common aerobic isolates are *Streptococcus dysgalactiae* strains (25).

The prognosis of aerobic-anaerobic mastitis is poor; the affected quarter is lost for milk production, and common practice is to cull the animal (25). Therapeutic approaches with antimicrobial agents have been unsuccessful. Contributing factors for treatment failures include pharmacokinetic problems associated with particular drugs, the unknown nature of susceptibility patterns of isolates of animal origin, and the ability of ruminants to metabolize many drugs very rapidly (25). In addition, antimicrobial therapy in cattle carries the risk of leaving drug residues. Therefore, susceptibility results based on MICs and pharmacokinetics determined in humans are not directly applicable to bacterial strains of animal origin.

The objective of this study was to collect in vitro antimicrobial susceptibility data for major groups of bacteria involved in

aerobic-anaerobic mastitis to identify potential candidates for future pharmacokinetic studies and eventually to permit directed therapeutic approaches. Aerobic and anaerobic bacterial strains isolated during our recent investigation on the clinical, bacteriologic, and therapeutic aspects of bovine mastitis (18) were used for the in vitro susceptibility tests.

MATERIALS AND METHODS

The 32 aerobic and 37 anaerobic isolates (see Tables 1 and 2) investigated originated from udder secretion samples taken from 3 heifers and 20 cows with summer mastitis. The animals and the methods used to collect and process udder secretion samples, as well as isolation and identification of the organisms, were described previously (18). Briefly, the animals were selected on the basis of medical histories and typical symptoms of summer mastitis (25): the affected quarter was swollen, hard, and tender, and the secretion was purulent or serous, with a characteristic foul odor. After careful disinfection of the teat end, milk samples from the affected quarters were aspirated via the teat canal with a blunt cannula and a disposable syringe. Each sample was injected into a gas-tight sealed vial (Port-A-germ; Bio Mérieux, Charbonnières, France) and transported within 24 h to the laboratory for quantitative aerobic and anaerobic culture. Several selective and nonselective media, as well as thioglycolate enrichment broth, were inoculated with the samples (24). The plates were incubated in conducive environments for up to 10 days and examined frequently. Bacteria were isolated, counted, and identified by established methods (7, 8, 10, 12, 24).

The MICs of penicillin G, amoxicillin, amoxicillin-clavulanate, cefoxitin, oxytetracycline, spiramycin, clindamycin, chloramphenicol, ofloxacin, and tinidazole (anaerobes only) were determined by the agar dilution method outlined by the National Committee for Clinical Laboratory Standards (13, 14). β -Lactamase production (by anaerobic gram-negative rods) was assessed by the chromogenic cephalosporin disk test (Biodisk, Solna, Sweden). The antimicrobial agents were received as standard powders from individual pharmaceutical manufacturers. The medium used for testing aerobic isolates was Iso-Sensitest agar (Oxoid, London, England) supplemented with 7% hemolysed sheep blood, and that used for testing the anaerobes was vitamin K₁- and hemin-supplemented brucella blood agar (24). The inocula were delivered with a multipoint inoculator. Aerobes were incubated for 1 to 2 days, in air, and anaerobes were incubated at 36°C for 2 days in jars filled with a mixed gas (80% N₂, 10% CO₂, 10% H₂), after which the plates were interpreted.

In addition to the mastitis isolates, the following reference strains were included as controls: *Staphylococcus aureus* ATCC 25923 and ATCC 25929, *Escherichia coli* ATCC 25922, *Bacteroides fragilis* ATCC 25285, *Fusobacterium necro-*

* Corresponding author. Mailing address: Anaerobe Reference Laboratory, National Public Health Institute, Mannerheimintie 166, 00300 Helsinki, Finland. Phone: 358-0-4744242. Fax: 358-0-4744238. Electronic mail address: Hannele.Somer@ktl.fi.

TABLE 1. In vitro susceptibility of aerobic bacteria isolated from udder secretions

Bacterial isolate (no. of isolates)	Antimicrobial agent	MIC ($\mu\text{g/ml}$)		
		Range	MIC ₅₀	MIC ₉₀
<i>Actinomyces pyogenes</i> (16)	Penicillin G	≤ 0.06	≤ 0.06	≤ 0.06
	Oxytetracycline	0.125–1.0	0.25	0.5
	Clindamycin	≤ 0.06	≤ 0.06	≤ 0.06
	Spiramycin	0.06–0.25	0.125	0.25
	Chloramphenicol	0.25–8.0	0.5	0.5
	Cefoxitin	≤ 0.06	≤ 0.06	≤ 0.06
	Amoxicillin	≤ 0.06	≤ 0.06	≤ 0.06
	Amoxicillin-clavulanate	≤ 0.06	≤ 0.06	≤ 0.06
<i>Streptococcus dysgalactiae</i> (8)	Penicillin G	≤ 0.06		
	Oxytetracycline	0.5–16		
	Clindamycin	≤ 0.06		
	Spiramycin	0.5–1		
	Chloramphenicol	1–4		
	Cefoxitin	0.25		
	Amoxicillin	≤ 0.06		
	Amoxicillin-clavulanate	≤ 0.06		
<i>Streptococcus</i> species (8) [<i>S. uberis</i> (3), <i>S. acidominimus</i> (3), <i>Streptococcus</i> species (2)]	Penicillin G	≤ 0.06		
	Oxytetracycline	0.25–8		
	Clindamycin	≤ 0.06		
	Spiramycin	0.125–1		
	Chloramphenicol	0.5–2		
	Cefoxitin	≤ 0.06 –1		
	Amoxicillin	≤ 0.06		
	Amoxicillin-clavulanate	≤ 0.06		
	Ofloxacin	1		

phorum ATCC 25286, *Prevotella oralis* ATCC 33269, *Bacteroides levii* ATCC 27147, and *Peptostreptococcus magnus* ATCC 29328.

RESULTS

Tables 1 and 2 contain the susceptibility results. All bacterial isolates, except one β -lactamase-producing *B. fragilis* isolate, were susceptible to penicillin G, amoxicillin, amoxicillin-clavulanate, cefoxitin, clindamycin, and chloramphenicol (the *B. fragilis* strain was susceptible to the last four), which had MICs at which 90% of isolates were inhibited (MIC₉₀s) of ≤ 0.06 , ≤ 0.06 , ≤ 0.06 , 0.25, ≤ 0.06 , and 4.0 $\mu\text{g/ml}$, respectively. All anaerobic isolates were susceptible to oxytetracycline, but occasional aerobic streptococci were resistant. Spiramycin was highly active against the gram-positive aerobes (MIC₉₀, 1.0 $\mu\text{g/ml}$), but its activity against the anaerobes was low (MIC₉₀, 16.0 $\mu\text{g/ml}$). The susceptibilities of aerobes and anaerobes to ofloxacin followed the same trends (MIC₉₀, 2.0 and 8 $\mu\text{g/ml}$). Tinidazole was active against all anaerobes (MIC₉₀, 2.0 $\mu\text{g/ml}$). β -Lactamase was produced by the one *B. fragilis* isolate.

DISCUSSION

The susceptibility profiles of most aerobic and anaerobic bacterial strains recovered in this study compared well with MICs reported earlier (2, 15, 16, 20). However, major differences were noted between published susceptibilities to spiramycin of *Bacteroides*, *Prevotella*, and *Fusobacterium* species isolated from goats with footrot (16) and those of the isolates from bovine summer mastitis in the present study. In that study, the MICs at which 50% of isolates were inhibited (MIC₅₀s) for *Bacteroides* and *Prevotella* spp. were 0.06 to 1.0

$\mu\text{g/ml}$ and those for fusobacteria were 0.06 to 2.0 $\mu\text{g/ml}$. In our study, the MICs were consistently much higher. On the other hand, no resistance to tinidazole, oxytetracycline, or chloramphenicol was detected among the anaerobic bacteria in the present study. Surprisingly, 20 to 50% of footrot strains were resistant to tinidazole and also to oxytetracycline and chloramphenicol (16). Knowledge of the antimicrobial susceptibility profiles of the bacteria involved in a particular disease is one facet of rational therapy, which also includes knowledge of the pharmacokinetics and safety of the drugs in question in the target animal. In acute infections, it is preferable to give antimicrobial treatment parenterally, especially for infections such as summer mastitis, in which adequate distribution of antimicrobial agents administered by the local route is unlikely because of the massive purulent reaction. For systemic treatment of acute mastitis, knowledge of drug concentrations in udder secretions is useful. Unfortunately, pharmacokinetic data for the antimicrobial agents in lactating cattle, discussed here, are not comprehensive (17). In this study, most of the aerobes and anaerobes were very sensitive in vitro to penicillin G, amoxicillin and the combination amoxicillin-clavulanic acid. Generally, β -lactam drugs are rapidly eliminated in ruminants if used as water-soluble formulations. Penicillins also distribute poorly in the milk gland because they are weak acids. However, using the recommended dose of penicillin G, concentrations above the MICs and MBCs for the susceptible pathogens can be maintained in serum and milk (3, 4). Some representatives of

TABLE 2. In vitro susceptibility of anaerobic bacteria isolated from udder secretions

Bacterial isolate (no. of isolates)	Antimicrobial agent	MIC ($\mu\text{g/ml}$)		
		Range	MIC ₅₀	MIC ₉₀
<i>Peptostreptococcus indolicus</i> (15)	Penicillin G	≤ 0.06	≤ 0.06	≤ 0.06
	Oxytetracycline	0.25–4	0.25	0.5
	Clindamycin	0.25–0.5	0.25	0.25
	Spiramycin	16	16	16
	Chloramphenicol	4	4	4
	Cefoxitin	≤ 0.06 –0.25	≤ 0.06	≤ 0.06
	Amoxicillin	≤ 0.06	≤ 0.06	≤ 0.06
	Amoxicillin-clavulanate	≤ 0.06	≤ 0.06	≤ 0.06
	Ofloxacin	8–16	8.0	16.0
	Tinidazole	0.5–4	2	2
	<i>Fusobacterium necrophorum</i> (10)	Penicillin G	≤ 0.06	≤ 0.06
Oxytetracycline		0.125–0.5	0.5	0.5
Clindamycin		≤ 0.06 –0.25	≤ 0.06	0.25
Spiramycin		16	16	16
Chloramphenicol		2–4	2	4
Cefoxitin		≤ 0.06 –16	0.125	2
Amoxicillin		≤ 0.06 –8	≤ 0.06	2
Amoxicillin-clavulanate		≤ 0.06 –2	≤ 0.06	1
Ofloxacin		2–8	8	8
Tinidazole		0.25–1	1	1
Gram-negative rods (12) [MCR <i>Prevotella hep/zoo</i> ^a (7), MCR <i>B. levii</i> (4), <i>B. fragilis</i> (1)]	Penicillin G	≤ 0.06 –16	0.06	0.125
	Oxytetracycline	0.125–0.5	0.25	0.5
	Clindamycin	≤ 0.06 –0.5	≤ 0.06	≤ 0.06
	Spiramycin	4–16	8	16
	Chloramphenicol	2–8	4	4
	Cefoxitin	≤ 0.06 –8	0.5	1
	Amoxicillin	≤ 0.06 –16	≤ 0.06	0.25
	Amoxicillin-clavulanate	≤ 0.06 –0.25	≤ 0.06	≤ 0.06
	Ofloxacin	0.25–165	1	8
	Tinidazole	≤ 0.06 –2	≤ 0.06	2

^a Most closely related (MCR) to *Prevotella heparinolytica/zooglooformans* group.

the cephalosporin group have also been studied in lactating cows (22, 23). The fraction secreted into milk is very small, and therapeutic levels cannot be obtained in milk.

Alone among the gram-negative rods investigated here, the *B. fragilis* strain was β -lactamase producing. All other *Bacteroides*, *Prevotella*, and *Fusobacterium* spp. tested were susceptible to penicillin G, a feature dissimilar to that of corresponding isolates of human origin. When penicillin-resistant bacteria are involved in the infection, use of β -lactamase inhibitors often solves the problem, provided that the mechanism of resistance is β -lactamase mediated. Limited data are available on pharmacokinetics in cattle of the amoxicillin-clavulanic acid combination. The dosage recommendation made by the manufacturer appears too low, because the compounds are eliminated rapidly in adult cattle. The concentrations of both components in milk seem to remain low and are short-lived (1).

A widely used alternative to penicillin G in treatment of anaerobic infections in cattle is macrolides. Many macrolides have favorable pharmacokinetics in lactating cattle, because, as lipophilic drugs, they distribute well throughout the body and concentrate in milk. There are no difficulties in maintaining therapeutic concentrations in milk with spiramycin administered parenterally once a day (4, 21). However, macrolide therapy of infections such as summer mastitis involving *F. necrophorum* may not be successful, because fusobacteria are frequently resistant to macrolides such as erythromycin (5). The MICs of spiramycin against all anaerobes evaluated in this study were high. Some newer representatives of this group of antimicrobial agents might be more potent (5). Oxytetracycline remains the most common drug used against mixed and anaerobic infections in cattle. Anaerobes investigated here showed no resistance to this drug, but occasional aerobic isolates were resistant. Levels of oxytetracycline in serum can be maintained by administering recommended doses at a dose interval of 1 or 2 days. However, this drug is not ideal, because it is difficult to maintain sufficient concentrations and because the casein component of milk also interferes with its action (11, 17).

Tinidazole and other nitroimidazole derivatives are active against a wide range of anaerobes, as also demonstrated here. Among nitroimidazoles, the pharmacokinetics of tinidazole has been studied in several animal species, including lactating cows (19). This is a good example of a drug with highly variable pharmacokinetics in different animal species. With administration of high doses, therapeutic levels of the drug can be maintained in the serum and milk of cows. Unfortunately, approval of tinidazole is unlikely because of its potentially carcinogenic and mutagenic residues (17). In a recent study, the combination of tinidazole and penicillin G was not superior to penicillin G alone in the treatment of experimentally induced summer mastitis (6).

Fluoroquinolones are generally not very active against anaerobes (5). This was observed with ofloxacin in the present study. Therefore, despite favorable pharmacokinetic properties (9, 17), the currently available fluoroquinolones are not likely to be suitable drugs for treating these infections.

In conclusion, all β -lactam compounds and several other antimicrobial agents had low MICs for aerobic and anaerobic isolates originating from cases of summer mastitis. However, spiramycin and ofloxacin are not good candidates for the antimicrobial therapy of summer mastitis because of their low activity against the key anaerobes involved. The drug of choice for anaerobic and mixed infections in cattle remains penicillin G. Cases in which β -lactamase-producing bacteria are present, especially summer mastitis, in which *F. necrophorum* is

prevalent and is generally resistant to macrolides, are problematic. Clindamycin and ceftiofur have not been approved for use in cattle, and chloramphenicol has recently been banned for food animal use in the European Union. Tetracyclines, although not ideal, remain a good choice, although occasional resistance occurs. Despite the high in vitro activity of tinidazole, it is not a good supplement because of its potential mutagenicity and its unapproved status. Other compounds for future clinical trials include the new macrolides and cephalosporin derivatives, but careful pharmacokinetic assessments in the animal species for which their use is intended must be done.

REFERENCES

1. **Blanchflower, S. E.** 1983. Antibiotic concentrations in milk from normal, endotoxin challenged and mastitic quarters of cows after parenteral dosing with amoxicillin. *Vet. Res. Commun.* 7:259–260.
2. **Chirino-Trejo, J. M., and J. F. Prescott.** 1983. The identification and antimicrobial susceptibility of anaerobic bacteria from pneumonic cattle lungs. *Can. J. Comp. Med.* 47:270–276.
3. **Franklin, A., O. Holmberg, M. Horn af Ranzen, and G. Åström.** 1984. Effect of procaine benzylpenicillin alone or in combination with dihydrostreptomycin on udder pathogens in vitro and in experimentally infected bovine udders. *Am. J. Vet. Res.* 45:1398–1402.
4. **Franklin, A., M. Horn af Ranzen, N. Obel, K. Östenson, and G. Åström.** 1986. Concentrations of penicillin, streptomycin, and spiramycin in bovine udder tissue liquids. *Am. J. Vet. Res.* 47:804–807.
5. **Goldstein, E. J. C., C. A. Nesbit, and D. M. Citron.** 1995. Comparative in vitro activities of azithromycin, Bay y 3118, levofloxacin, sparfloxacin, and 11 other oral antimicrobial agents against 194 aerobic and anaerobic bite wound isolates. *Antimicrob. Agents Chemother.* 39:1097–1100.
6. **Hirvonen, J., S. Pyörälä, A. Heinäsoo, and H. Jousimies-Somer.** 1994. Penicillin G and penicillin G-tinidazole treatment of experimentally induced summer mastitis—effect on elimination rates of bacteria and outcome of the disease. *Vet. Microbiol.* 42:307–315.
7. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore.** 1977. *Anaerobe laboratory manual*, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
8. **Jousimies-Somer, H. R., and S. M. Finegold.** 1991. Anaerobic gram-negative bacilli and cocci, p. 538–553. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
9. **Kaartinen, L., L. Älli, and S. Pyörälä.** 1994. Pharmacokinetics of enrofloxacin in lactating cows, p. 232. *In* Proceedings of the 6th Congress of the European Association for Veterinary Pharmacology and Toxicology. Blackwell Scientific Publications, Oxford.
10. **Lenette, E. H., A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.).** 1985. *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.
11. **Mevius, D. J., J. F. M. Nouws, H. J. Breuking, T. B. Vree, F. Driessens, and R. Verkaik.** 1986. Comparative pharmacokinetics, bioavailability and renal clearance of five parenteral oxytetracycline-20% formulations in dairy cows. *Vet. Q.* 8:285–294.
12. **Moore, L. V. H., E. P. Cato, and W. E. C. Moore.** 1991. *Anaerobe laboratory manual update: a supplement to the VPI anaerobe laboratory manual*, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
13. **National Committee for Clinical Laboratory Standards.** 1990. *Methods for antimicrobial susceptibility testing of aerobic bacteria*, 2nd ed. Approved standard M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
14. **National Committee for Clinical Laboratory Standards.** 1990. *Methods for antimicrobial susceptibility testing of anaerobic bacteria*, 2nd ed. Approved standard M11-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
15. **Piriz, S., R. Cuenca, J. Valle, and S. Vadillo.** 1992. Susceptibilities of anaerobic bacteria isolated from animals with ovine foot rot to 28 antimicrobial agents. *Antimicrob. Agents Chemother.* 36:198–201.
16. **Piriz Duran, S., J. Valle Manzano, R. Cuenca Valera, and S. Vadillo Machota.** 1990. In-vitro antimicrobial susceptibility of *Bacteroides* and *Fusobacterium* isolated from footrot in goats. *Br. Vet. J.* 146:437–442.
17. **Prescott, J. F., and J. D. Baggot (ed.).** 1993. *Antimicrobial therapy in veterinary medicine*, 2nd ed. Iowa State University Press, Ames.
18. **Pyörälä, S., H. Jousimies-Somer, and M. Mero.** 1992. Clinical, bacteriological, and therapeutic aspects of bovine mastitis caused by aerobic and anaerobic pathogens. *Br. Vet. J.* 148:54–62.
19. **Pyörälä, S., P. Silvennoinen, U. Hänninen, and M. Mero.** 1990. Pharmacokinetics of tinidazole in cows—a preliminary study. *J. Vet. Pharmacol. Ther.* 13:425–427.
20. **Robertsson, J. Å., and A. Franklin.** 1987. Antibiotikaresistens hos bak-

- terier isolerade från akuta mastiter på kor. *Sven. Vet.* **39**:115–120. (In Swedish.)
21. **Sanders, P., G. Moulin, P. Guillot, M. Dagorn, P. Perjant, B. Delepine, C. Gaudiche, and D. Mourot.** 1992. Pharmacokinetics of spiramycin after intravenous, intramuscular and subcutaneous administration in lactating cows. *J. Vet. Pharmacol. Ther.* **15**:53–61.
 22. **Soback, S., S. Bright, and M. Paape.** 1991. Disposition kinetics of ceftiofur in lactating cows. *Acta Vet. Scand. Suppl.* **87**:93–95.
 23. **Soback, S., G. Ziv, A. Bor, and M. Shapira.** 1988. Pharmacokinetics of cephalexin glycinate in lactating cows and ewes. *J. Vet. Med. Assoc.* **35**:755–760.
 24. **Sutter, V. L., D. M. Citron, M. A. C. Edelstein, and S. M. Finegold.** 1985. *Wadsworth anaerobic bacteriology manual*, 4th ed. Star Publishing Co., Belmont, Calif.
 25. **Thomas, G., H. J. Oliver, U. Vecht, and P. Nansen (ed.).** 1987. *Summer mastitis*. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.