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Activity of Four Cephalosporin Antibiotics In Vitro Against Bovine Udder Pathogens and Pathogenic Bacteria Isolated from Newborn Calves

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The in vitro activity of chephaloridine, cephalexin, cefatrizine (BL-S640), and cephapirin (BL-P-1322) was evaluated by the serial dilution method against pathogenic gram-positive and gram-negative bacteria isolated from bovine udders and neonatal calf diseases. Cephapirin showed the comparatively greatest activity against the most common streptococcal species associated with bovine mastitis, whereas cephaloridine exhibited the best activity against *Staphylococcus aureus*. Cefatrizine was more active than the other cephalosporins against the gram-negative bacteria studied. In general, the minimal bactericidal concentration of each cephalosporin was two- to fourfold lower than the comparative value reported in the literature against the same type of pathogen of human origin.

Past experience has shown that microbial resistance to antibiotics emerges commonly in closed animal populations, such as dairy herds kept under intensive practices, where these drugs are used routinely (9). As a result, it is imperative to constantly monitor susceptibilities of commonly encountered pathogens to new antimicrobial agents that offer promise for the treatment of the economically most important infectious diseases of modern dairying, i.e., bovine mastitis and neonatal calf diseases.

The cephalosporin antibiotics are widely acceptable for the treatment of a broad spectrum of gram-positive and gram-negative pathogens in humans. A number of new semisynthetic cephalosporins have been reported to be of potential value, and these include cefatrizine (BL-S640) (7, 10) and cephapirin (BL-1322) (3, 4, 11). The present communication describes comparative in vitro activity of these new cephalosporins and two other members of this group; cephaloridine, an established parenteral cephalosporin, and cephalexin, a widely used oral cephalosporin against pathogenic bacteria isolated from bovine udders and from calves with neonatal diseases.

MATERIALS AND METHODS

Cephalosporins. Cephaloridine and cephalexin were products of Glaxo Laboratories, England. Cefatrizine and cephapirin were kindly provided by G. J. Christie, Bristol Laboratories, Syracuse, N.Y.

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Bacterial strains. *Streptococcus agalactiae, S. dysgalactiae, S. uberis, Enterococcus, Staphylococcus aureus, Corynebacterium pyogenes, Escherichia coli,* and *Pseudomonas aeruginosa* strains were isolated from cases of clinical and subclinical bovine mastitis in several dairy herds throughout Israel and were preponderantly of recent origin. The *S. aureus* strains were classified as either sensitive or resistant to benzylpenicillin by the method of Bauer et al. (1). *E. coli, Salmonella* sp., *Pasteurella multocida,* and *P. aeruginosa* strains were all recently isolated from clinical cases of diarrhea, pneumonia, and septicemia in newborn dairy calves. The *Salmonella* sp. strains were typed serologically and were either type B or D.

Antibiotic susceptibility tests. Antibiotic susceptibility of the bacteria isolated from the bovine udder was determined by the serial dilution method, using sterile whole milk as fluid medium. Overnight cultures of *S. uberis, Enterococcus, S. aureus, E. coli,* and *P. aeruginosa* grown in Trypticase soy broth (Difco) were diluted 10^{-4} in the same medium, and 0.1-ml amounts were inoculated into tubes containing serial twofold dilutions of antibiotics in sterile whole milk to give a final volume of 2 ml. Overnight cultures of *S. agalactiae, S. dysgalactiae,* and *C. pyogenes* in Trypticase soy broth were undiluted and similarly inoculated. The inoculum contained approximately 10^5 of each bacterial species. Inoculated tubes were incubated for 18 h at 37 C and were subcultured with a calibrated loop (0.01 ml) onto blood agar plates containing 5% sheep erythrocytes. Plates were incubated for 18 h at 37 C, and the minimal bactericidal concentration (MBC) was defined as the lowest concentration of antibiotic giving a count of less than 10 colonies per plate.

Antibiotic susceptibility of the bacteria isolated from newborn calves was determined by the tube

TABLE 1. *MBC of four cephalosporins against gram-positive and gram-negative udder pathogens*

Species[a]	Antibiotic	Cumulative % of strains killed at MBC (μg/ml):												Median (μg/ml)
		<0.05	0.05	0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25	50	>50	
S. agalactiae (18)	Cephaloridine		22	89	100										0.1
	Cephalexin			11	22	56	83	100							0.4
	Cefatrizine		11	33	61	78	94	100							0.2
	Cephapirin	11	61	78	89	100									0.05
S. dysgalactiae (12)	Cephaloridine		8	75		100									0.1
	Cephalexin			25	67	83	100								0.2
	Cefatrizine		8	33	75	92	100								0.2
	Cephapirin	8	33	75	100										0.1
S. uberis (16)	Cephaloridine			13	44	75	88	94	100						0.4
	Cephalexin			6	19	56	81	100							0.4
	Cefatrizine		13	38	69	88	100								0.2
	Cephapirin		25	63	81	100									0.1
Enterococcus (6)	Cephaloridine									17	33	67		100	25
	Cephalexin											17	33	100	>50
	Cefatrizine									17		67	83	100	25
	Cephapirin									17	33	67		100	25
S. aureus, penicillin sensitive (20)	Cephaloridine	20	50	90	100										0.05
	Cephalexin						20	50	70	90	100				1.6
	Cefatrizine			15	45	85	100								0.4
	Cephapirin			15	55	80	90	100							0.4
S. aureus, penicillin resistant (16)	Cephaloridine	19	31	69	88	100									0.1
	Cephalexin						13	38	69	88	100				3.1
	Cefatrizine				13	38	75	94	100						0.8
	Cephapirin			13	38	75	94	100							0.4
C. pyogenes (6)	Cephaloridine	33	67	83	100										0.05
	Cephalexin		17	33		50	67	83	100						0.4
	Cefatrizine		17	50		83	100								0.1
	Cephapirin		17	67	100										0.1
E. coli (24)	Cephaloridine							17	67	79	92	96	100		3.1
	Cephalexin								13	38	71	83	92	100	12.5
	Cefatrizine				8	17	58	83	92	100					0.8
	Cephapirin					8		25	50	79	88	92	100		3.1

[a] Numbers in parentheses indicate the number of strains.

dilution method using Trypticase soy broth. The minimal inhibitory concentration (MIC) was determined as the lowest concentration of antibiotic preventing visible turbidity after overnight incubation at 37 C.

RESULTS

The bactericidal activity of the four cephalosporins against 94 gram-positive strains and against 24 *E. coli* strains of bovine udder origin is given in Table 1. Against 18 strains of *S. agalactiae*, 12 strains of *S. dysgalactiae*, and 16 strains of *S. uberis*, cephapirin was two to four times more active than were the other cephalosporins. Cephaloridine was most active against penicillin-resistant and penicillin-sensitive staphylococci and *C. pyogenes*, whereas cefatrizine was the most active antibiotic against the *E. coli* strains. Neither cephalosporin was very active in killing the *Enterococcus* strains; the

TABLE 2. *MBC of four cephalosporins against gram-negative bacteria associated with neonatal calf diseases*

Species[a]	Antibiotic	Cumulative % of strains killed by MBC (μg/ml):										Median (μg/ml)
		0.4	0.8	1.6	3.1	6.2	12.5	25	50	100	>100	
E. coli (26)	Cephaloridine			19	57	80	84		92	100		3.1
	Cephalexin			15	27	46	92	96			100	12.5
	Cefatrizine	12	50	81	96	100						0.8
	Cephapirin		15	50	73	96		100				1.6
Salmonella type B (20)	Cephaloridine		5	20	50	90	100					3.1
	Cephalexin			10	20	50	70	85		95	100	6.2
	Cefatrizine	3	70	85	95		100					0.8
	Cephapirin		10	50	75		85	95	100			1.6
Salmonella type D (20)	Cephaloridine	10	15	55	70	85	95	100				1.6
	Cephalexin		10	20	40	80		90	95	100		6.2
	Cefatrizine	40	75	85	90	95	100					0.8
	Cephapirin	20	50	70	80	90	95	100				0.8
P. multocida, (6)	Cephaloridine			17		67	100					6.2
	Cephalexin				17	33	50	83	100			12.5
	Cefatrizine		17	50	83	100						1.6
	Cephapirin		17		50	83	100					3.1

[a] Numbers in parentheses indicate the number of strains.

TABLE 3. *MIC and MBC of four cephalosporins against selected gram-negative bacteria associated with neonatal calf diseases*

Species	Strain no.	Cephaloridine		Cephalexin		Cefatrizine		Cephapirin	
		MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
E. coli	1	1.6	1.6	3.1	3.1	0.2	0.4	0.8	0.8
	2	3.1	3.1	6.2	6.2	0.8	1.6	0.8	1.6
	3	12.5	12.5	12.5	12.5	1.6	3.1	3.1	3.1
	4	25	25	50	50	1.6	3.1	6.2	6.2
Salmonella type B	1	0.8	1.6	3.1	3.1	0.2	0.4	0.8	1.6
	2	3.1	3.1	6.2	6.2	0.2	0.8	1.6	3.1
	3	3.1	6.2	12.5	25	0.4	3.1	3.1	6.2
Salmonella type D	1	0.4	0.4	0.8	0.8	0.2	0.4	0.4	0.4
	2	0.8	0.8	1.6	1.6	0.2	0.8	0.8	0.8
	3	1.6	1.6	3.1	3.1	0.4	1.6	1.6	1.6
	4	1.6	3.1	6.2	6.2	0.8	3.1	1.6	3.1
	5	6.2	12.5	12.5	25	0.8	6.2	3.1	6.2
P. multocida	1	1.6	1.6	3.1	3.1	0.4	0.8	0.8	0.8
	2	6.2	6.2	6.2	6.2	0.4	1.6	3.1	3.1
	3	12.5	12.5	12.5	12.5	0.8	3.1	3.1	6.2

median MBC values were ≥25 μg/ml. The MBC of each antibiotic against the six *P. aeruginosa* strains studied was >50 μg/ml. Cephalexin exhibited the poorest killing effect for each of the bacterial species examined.

Gram-negative bacteria associated with neonatal calf diseases were most sensitive to cefatrizine and least sensitive to cephalexin (Table 2). The majority of these strains, however, were relatively sensitive to the cephalosporins studied, with median MBC values ≤12.5 μg/ml. The activity of cefatrizine and cephapirin against the *Salmonella* type D strains was the same, but the median MBC of cephapirin was twice as high as that of cefatrizine for the other bacterial species. The MBC of the four cephalosporins against four *P. aeruginosa* strains of calf origin was >100 μg/ml.

Among the antibiotics studied, cefatrizine appeared to inhibit the growth of gram-negative bacteria associated with neonatal calf diseases at concentrations that were two- and threefold lower than the MBC of the drug (Table 3). Against these strains the MIC and MBC of cephaloridine and cephalexin were generally the same over wide concentration values, and the MIC of cephapirin was generally onefold lower than the MBC.

DISCUSSION

The in vitro data presented here indicate that the antibacterial activity of cefatrizine and cephapirin against gram-positive and gram-negative bacteria is essentially similar to that of cephaloridine but somewhat greater than that of cephalexin. Cephapirin was the most active derivative studied against the most common streptococcal species associated with udder diseases in cattle and slightly less active than was cephaloridine against *S. aureus*. Cefatrizine was most active in vitro against the gram-negative species examined. These findings are in full accord with published data on the comparative activity of these cephalosporins against the similar bacterial species isolated from humans (2-8, 10, 11). In general, however, each of the cephalosporins evaluated was effective against the strains of animal origin at concentrations that were two- to fourfold lower than those reported for the same type of organisms isolated from humans, and this may reflect their very limited application in veterinary medicine.

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