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Yersinia enterocolitica: In Vitro Antimicrobial Susceptibility

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Using 21 antimicrobial agents, agar dilution susceptibility tests were carried out against 190 strains of *Yersinia enterocolitica*. Isolates from human, environmental, and animal sources had essentially equal susceptibility patterns.

Yersinia enterocolitica can produce human and animal diseases with a variety of syndromes, enterocolitis being the most frequently encountered manifestation in humans (1). Although the epidemiology of *Y. enterocolitica* infections has not been completely elucidated, present evidence indicates that wild and domestic animals may serve as reservoirs for the production of human infections (1). Furthermore, isolations of the organism from water (3, 7) and from foods of animal origin (6, 9) have recently been made in increasing numbers. Despite the increase in both environmental and clinical isolations of *Y. enterocolitica*, comprehensive antimicrobial susceptibility data are sparse (2, 5, 8). The purpose of this study was to obtain quantitative antimicrobial information using the agar dilution method for determining minimal inhibitory concentrations (MICs). Our aim was also to evaluate differences in susceptibility patterns among strains isolated from human, environmental, and animal sources.

One hundred ninety strains of *Y. enterocolitica* encompassing all previously identified biotypes were studied. In addition to our own isolates (4, 7), strains were obtained from the following sources: M. Bissett, California State Department of Health, Berkeley, Calif.; B. Chester, Microbiology Department, Clinical Laboratory, VA Hospital, Miami, Fla.; Analytab Products Inc., Plainview, N.Y.; and the American Type Culture Collection, Rockville, Md. Ninety-one strains were human clinical isolates, 66 were from surface waters, and 8 were isolates from wild and domestic animals. The origins of the other 25 strains are not known. All were identified as *Y. enterocolitica* according to Sonnenwirth (12). The MIC determinations were made using the agar replicator technique of Steers et al. (13). Mueller-Hinton medium (Difco) was used as the agar medium. Plates for the majority of the MIC determinations were prepared by the Clinical Microbiology Laboratory of the UCLA Hospital and Clinics according to their routine procedure. A stock culture solution of each drug was prepared, and dilutions in distilled water were added in ratios ranging from 0.2 to 2.6 ml per 100 ml of agar to obtain the final concentrations shown in Table 1. The plates were kept refrigerated in plastic bags and used within 7 days. Antimicrobial agents have been shown to maintain their activity under these conditions (11). Each overnight culture in Mueller-Hinton broth (Difco) was adjusted to a density intermediate between McFarland standards 1 and 0.5. It was then diluted 1:1,000 to obtain an inoculum of approximately 10^5 cells per ml, which was immediately used for the MIC determination. Control strains were included. The plates were dried and then incubated at 35°C for 16 to 18 h. The MIC was considered to be the lowest antimicrobial concentration that inhibited growth.

The results of the susceptibility tests for all the strains are shown in Table 1. Each strain was tested once. Table 2 presents cumulative percentages of the human, water, and animal isolates susceptible to various MICs. All of our isolates are highly susceptible to most aminoglycosides (gentamicin, netilmicin, tobramycin, neomycin, amikacin), and these observations are similar to those previously reported (1, 2, 5, 8, 10). The MICs of the aminoglycosides in our study were slightly less than those reported by Hammerberg et al. (5), but our data on chloramphenicol and tetracycline were comparable to theirs. Despite recent reports (1) that in vitro resistance to tetracyclines and streptomycin is increasing, we found no strains resistant to tetracycline and only one strain (a clinical isolate) resistant to streptomycin (MIC, ≥16 μg/ml).

Using MIC methods, Hausnerova et al. (8) found significant differences in antimicrobial susceptibilities between *Y. enterocolitica* iso-

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lates of Nilehn biotype 4 (clinical strains) and biotype 1 (clinical, water, and fish isolates). Our strains represented all biotypes and were isolated from human, environmental, and animal sources. These biochemically diverse strains exhibited relatively uniform susceptibility patterns (Table 2), although the water isolates showed slightly greater susceptibility than did the strains of human and animal origin. Furthermore, we also failed to confirm other reports that there might be correlations between specific biochemical features and antimicrobial susceptibility patterns. Chester and Stotzky (2), for example, using the disk diffusion method at 37°C, found that all of their 13 rhamnose-positive strains were susceptible to ampicillin and cephalothin. In contrast, only 33% of our 73 rhamnose-positive strains were susceptible to ampicillin (MIC, ≤8 μg/ml), and 22% of our 117 rhamnose-negative strains were susceptible. Our corresponding figures for cephalothin were 1 and 7%. Similarly, Toma and Lafleur (14) reported

TABLE 1. *Antimicrobial susceptibility of 190 strains of Y. enterocolitica*

Antimicrobial agent	Cumulative % of strains susceptible at drug concn (μg/ml):										
	0.25	0.5	1	2	4	8	16	32	64	128	256
Gentamicin	47		97		100						
Netilmicin		73	96	98	100						
Tobramycin	52		96		100						
Neomycin		28	67	97	99	100					
Amikacin				96	99	100					
Tetracycline hydrochloride				86		100					
Kanamycin					83	100					
Cefamandole		38		78		100					
Chloramphenicol				19		100					
Nalidixic acid								100			
Streptomycin		1	10	55	93	99		99			
Nitrofurantoin									100		
Polymyxin B sulfate				10	22	59	98	99	99		
Ampicillin				4		25	58				
Carbenicillin							24	27	33	62	81
Erythromycin				1	1	9					
Cephalothin		1		2		5	12				
Clindamycin					3						
Penicillin					2						
Oxacillin					1						
Novobiocin									1	4	

TABLE 2. *Y. enterocolitica: cumulative percentage of strains susceptible to designated MICs*

Antimicrobial agent	MICs (μg/ml)					
	Clinical strains		Water strains		Animal strains	
	75%	90%	75%	90%	75%	90%
Gentamicin[a]	1	1	1	1	1	1
Netilmicin	1	1	0.5	0.5	0.5	0.5
Tobramycin	1	1	0.25	1	1	1
Neomycin	2	2	1	1	2	2
Amikacin	2	2	2	2	2	4
Tetracycline hydrochloride[b]	2	8	2	2	2	8
Kanamycin	8	8	4	4	4	4
Cefamandole	8	8	2	2	2	2
Chloramphenicol	8	8	8	8	8	8
Nalidixic acid	32	32	32	32	32	32
Streptomycin	4	8	2	4	2	4
Nitrofurantoin	64	64	64	64	64	64
Polymyxin B sulfate	16	16	16	16	16	32

[a] 90% of the 91 clinical strains were inhibited by 1 μg of gentamicin per ml.
[b] 75% of the 92 clinical strains were inhibited by 2 μg/ml, and 90% were inhibited by 8 μg/ml.

that none of their indole-negative serotype O:3 strains (78% of their 278 isolates belonged to this serotype) was susceptible to ampicillin. However, 25% of our 67 indole-negative strains were susceptible to ampicillin, as well as 26% of our 123 indole-positive strains.

At the present time we conclude that biochemical and antimicrobial tests do not reliably identify isolates of *Y. enterocolitica* according to their origin, nor do these tests definitively evaluate the homogeneity of this species. Experiments using numerical taxonomy and deoxyribonucleic acid hybridizations are underway to clarify these problems.

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