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Antimicrobial Susceptibility of Pathogenic *Yersinia enterocolitica* Isolated in Canada from 1972 to 1990

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***Yersinia enterocolitica* has emerged as an enteropathogen associated with several types of human infections that often require antimicrobial therapy, but little is known about the antimicrobial susceptibilities of pathogenic strains isolated from humans in Canada. To determine the present patterns of antimicrobial susceptibility, to identify changes in these patterns that occurred during the past two decades, and to investigate the relationships between O serotypes and patterns of susceptibility, we tested a total of 1,105 pathogenic *Y. enterocolitica* strains isolated during 1972 to 1976, 1980, 1985, and 1990 for their susceptibilities to 22 antimicrobial agents. Susceptibility testing was performed by using a single breakpoint concentration in agar procedure. The results showed that all strains were susceptible to ciprofloxacin and piperacillin, and 98% or more of the strains from each period were susceptible to trimethoprim, sulfamethoxazole, cotrimoxazole, tetracycline, chloramphenicol, cefamandole, cefotaxime, aztreonam, and four aminoglycosides. In contrast, all strains were nonsusceptible to erythromycin, furazolidone, and clindamycin and 90% or more of the strains from each period were nonsusceptible to ampicillin, carbenicillin, ticarcillin, and cephalothin. Strains belonging to serotypes O:3, O:5,27, and O:8 had different patterns of susceptibility to ampicillin, carbenicillin, ticarcillin, and amoxicillin-clavulanic acid. No major difference in susceptibility was observed between any of the groups of human or animal strains included in the study, but nonsusceptibility to tetracycline increased from 0.4% in 1985 to 2% in 1990 in human strains isolated in those years. Our results indicate that between 1972 and 1990 there was no marked decrease in susceptibility to agents commonly used for therapy among pathogenic *Y. enterocolitica* strains isolated in Canada.**

Yersinia enterocolitica has emerged as a significant human pathogen associated with several clinical syndromes, including enterocolitis, mesenteric adenitis, focal extraintestinal infections, septicemia, and posttransfusion septic shock (3, 5, 17). Secondary immunological conditions, including erythema nodosum and reactive arthritis, are recognized complications of *Y. enterocolitica* infection (5). Systemic and extraintestinal infections and enterocolitis in immune-compromised patients require prompt antibiotic therapy, and the agents used most commonly include chloramphenicol, gentamicin, tetracycline, cotrimoxazole, and, more recently, ciprofloxacin (3, 7, 14).

Compared with other bacterial enteropathogens, relatively little is known about the antimicrobial susceptibilities of pathogenic *Y. enterocolitica* isolated from humans in Canada. *Y. enterocolitica* infection is essentially a zoonosis, and the serotypes that cause human infection are also isolated from domestic animals, including pigs, dogs, and cats; wild animals; and the environment (9). In 1990, Kwaga and Iversen (11) investigated the in vitro susceptibilities of yersiniae isolated from slaughtered pigs and pork products in Canada, but to our knowledge, there has been no systematic study of the susceptibilities of pathogenic *Y. enterocolitica* isolated from humans in this country since the 1970s (6, 21). Our laboratory maintains an extensive collection of *Y. enterocolitica* strains isolated from both humans and animals in Canada since 1972. In order to determine the current antimicrobial susceptibility patterns of pathogenic *Y. enterocolitica*, to evaluate trends in susceptibility during the past two decades, and to identify serotype-specific susceptibility patterns, we tested collections of human and animal strains isolated at regular intervals between 1972 and 1990 for their susceptibilities to a broad range of antimicrobial agents.

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MATERIALS AND METHODS

Bacterial strains. A total of 1,105 strains of *Y. enterocolitica*, including 1,060 strains isolated from humans and 45 strains isolated from animals, were examined in this study. The strains belonged to the four most common pathogenic serotypes (O:3 [biotype 4 and biotype 3], O:5,27 [biotype 2], O:8 [biotype 1B], and O:9 [biotype 2]) associated with human infections in Canada and were isolated during four different periods, namely, 1972 to 1976, 1980, 1985, and 1990. The serotype distributions of the human strains examined in the study were as follows: O:3, 89%; O:5,27, 5%; O:8, 5%; and O:9, 0.3%. All human strains, with the exception of three strains isolated from blood specimens, were isolated from stool specimens. Among the 45 strains isolated from animals, 37 (82%) were from pigs, 5 (11%) were from dogs, 2 (4%) were from cats, and 1 (2%) was from a cow. Multiple isolates associated with the same outbreak were excluded from the study. All cultures were identified as *Y. enterocolitica* by standard laboratory criteria.

Biogrouping and serotyping. *Y. enterocolitica* strains were subdivided into biogroups according to a revised biogrouping schema (24), and O serotypes were determined by slide agglutination with the antigenic schema established by Wauters et al. (24).

Antibiotic susceptibility testing. Susceptibility testing was performed by using a single breakpoint concentration in agar procedure with Mueller-Hinton agar (BBL, Becton Dickinson, Mississauga, Ontario, Canada). The preparation of agar plates

TABLE 1. Comparative number of susceptible *Y. enterocolitica* strains from humans

Antimicrobial agent	No. (%) of susceptible strains isolated in:			
	1972–1976 (n = 291)	1980 (n = 257)	1985 (n = 267)	1990 (n = 245)
Ampicillin	18 (6.2)	12 (4.7)	10 (3.8)	10 (4.1)
Carbenicillin	20 (6.9)	12 (4.7)	14 (5.2)	5 (2.0)
Piperacillin	291 (100)	257 (100)	267 (100)	245 (100)
Ticarcillin	27 (9.3)	13 (5.1)	22 (8.2)	12 (4.9)
Amoxicillin-clavulanic acid	176 (60.5)	178 (69.3)	209 (78.3)	187 (76.3)
Cephalothin	3 (1.0)	1 (0.4)	0 (0)	0 (0)
Cefamandole	291 (100)	256 (99.6)	264 (98.9)	242 (98.8)
Cefotaxime	291 (100)	256 (99.6)	267 (100)	243 (99.2)
Aztreonam	291 (100)	256 (99.6)	264 (98.9)	243 (99.2)
Kanamycin	291 (100)	256 (99.6)	267 (100)	243 (99.2)
Gentamicin	291 (100)	256 (99.6)	267 (100)	243 (99.2)
Tobramycin	291 (100)	256 (99.6)	267 (100)	243 (99.2)
Amikacin	291 (100)	256 (99.6)	267 (100)	243 (99.2)
Sulfamethoxazole	291 (100)	256 (99.6)	267 (100)	242 (98.8)
Trimethoprim	291 (100)	257 (100)	267 (100)	244 (99.6)
Trimethoprim-sulfamethoxazole	291 (100)	257 (100)	267 (100)	244 (99.6)
Clindamycin	0 (0)	0 (0)	0 (0)	0 (0)
Erythromycin	0 (0)	0 (0)	0 (0)	0 (0)
Furazolidone	0 (0)	0 (0)	0 (0)	0 (0)
Chloramphenicol	291 (100)	257 (100)	263 (98.5)	244 (99.6)
Tetracycline	290 (99.7)	256 (99.6)	266 (99.6)	240 (98.0)
Ciprofloxacin	291 (100)	257 (100)	267 (100)	245 (100)

and inoculum and the inoculation and incubation of agar plates were performed with methods recommended by the National Committee for Clinical Laboratory Standards (13). Agar plates containing sulfamethoxazole and/or trimethoprim were supplemented with lysed horse blood (5% [vol/vol]). The following antibiotics were obtained from Sigma Chemical Co. (St. Louis, Mo.) or their respective manufacturers and used at the concentrations indicated: amikacin, 16 μg/ml; amoxicillin-clavulanic acid, 8/4 μg/ml; ampicillin, 8 μg/ml; aztreonam, 8 μg/ml; carbenicillin, 16 μg/ml; cefamandole, 8 μg/ml; cefotaxime, 8 μg/ml; cephalothin, 8 μg/ml; chloramphenicol, 8 μg/ml; ciprofloxacin, 1 μg/ml; clindamycin, 0.5 μg/ml; erythromycin, 0.5 μg/ml; furazolidone, 1.25 μg/ml; gentamicin, 4 μg/ml; kanamycin, 16 μg/ml; piperacillin, 16 μg/ml; sulfamethoxazole, 256 μg/ml; tetracycline, 4 μg/ml; ticarcillin, 16 μg/ml; tobramycin, 4 μg/ml; trimethoprim, 8 μg/ml; and trimethoprim-sulfamethoxazole, 0.5 and 9.5 μg/ml. The concentrations of all antimicrobial agents used, except for trimethoprim-sulfamethoxazole, were those recommended by the National Committee for Clinical Laboratory Standards for the susceptible category (13), and strains completely inhibited at these concentrations were considered to be susceptible. The following strains were used as reference control strains throughout the study: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213.

RESULTS

The susceptibilities of 1,060 *Y. enterocolitica* strains isolated from humans during 1972 to 1976, 1980, 1985, and 1990 to 22 antimicrobial agents are shown in Table 1. All strains were uniformly nonsusceptible to clindamycin, erythromycin, and furazolidone at the breakpoint concentrations tested. More than 90% of the strains from each time period were also nonsusceptible to ampicillin, carbenicillin, ticarcillin, and cephalothin. Only four strains (three from the 1972-to-1976 period and one from 1980) were susceptible to cephalothin at a concentration of 8 μg/ml. Susceptibility to amoxicillin-clavulanic acid varied from a minimum of 60.5% of the strains analyzed from the 1972-to-1976 period to a maximum of 78.3% of the strains sampled from 1985. There were marked variations in the susceptibilities to ampicillin, carbenicillin, ticarcillin, and amoxicillin-clavulanic acid among strains belonging to different serotypes (Table 2). In contrast, all of the strains were uniformly susceptible to piperacillin and ciprofloxacin at the concentrations tested. In addition, 98% or more of the strains from each period were susceptible to cefamandole, cefotaxime, aztreonam, sulfamethoxazole, trimethoprim, cotrimoxazole, chloramphenicol, tetracycline, and the four aminoglycosides tested.

TABLE 2. Comparative number of susceptible *Y. enterocolitica* strains of serotypes O:3, O:5,27, and O:8

Antimicrobial agent	No. (%) of susceptible strains of serotype:		
	O:3 (n = 945)	O:5,27 (n = 58)	O:8 (n = 54)
Ampicillin	1 (0.1)	2 (3.4)	47 (87.0)
Carbenicillin	2 (0.2)	46 (79.3)	3 (5.6)
Ticarcillin	2 (0.2)	46 (79.3)	26 (48.2)
Amoxicillin-clavulanic acid	696 (73.7)	1 (1.7)	53 (98.2)
Cephalothin	1 (0.1)	1 (1.7)	2 (3.7)

No marked decrease in susceptibility to any of the antimicrobial agents was observed among the strain collections examined during the study. Only one strain from each of the 1972-to-1976, 1980, and 1985 collections was not susceptible to tetracycline, but five (2.0%) of the strains isolated in 1990 were not susceptible to this compound. All strains not susceptible to tetracycline, regardless of isolation date, belonged to serotype O:5,27. One strain isolated in 1980 and serotyped as O:3 was nonsusceptible to cefamandole, cefotaxime, aztreonam, kanamycin, gentamicin, tobramycin, amikacin, and sulfamethoxazole. Among the collection of strains isolated in 1985, three O:3 strains were nonsusceptible to cefamandole, aztreonam, and chloramphenicol. In addition, two O:3 strains from 1990 were not susceptible to cefamandole, cefotaxime, aztreonam, kanamycin, gentamicin, tobramycin, amikacin, and sulfamethoxazole and one O:3 strain was not susceptible to sulfamethoxazole, trimethoprim, cotrimoxazole and chloramphenicol.

Specific patterns of susceptibility to certain β-lactam antibiotics were identified among human *Y. enterocolitica* strains belonging to serotypes O:3, O:5,27, and O:8 (Table 2). More than 99% of serotype O:3 strains examined in this study were nonsusceptible to ampicillin, carbenicillin, ticarcillin, and cephalothin, but only 26% of these strains were nonsusceptible to amoxicillin-clavulanic acid. In comparison, most strains of serotype O:5,27 were not susceptible to ampicillin, cephalothin, and amoxicillin-clavulanic acid, but, overall, only 20.7% of these strains were not susceptible to both carbenicillin and ticarcillin. On the other hand, O:8 strains had high rates of nonsusceptibility to carbenicillin, ticarcillin, and cephalothin, but only 1.8 and 13% of these strains were nonsusceptible to amoxicillin-clavulanic acid and ampicillin, respectively. The correlations between serotype and β-lactam susceptibility patterns were observed consistently among strains isolated in each of the four sampling periods; however, the rates of susceptibility to both carbenicillin and ticarcillin decreased from 86.7% in 1985 to 30.8% in 1990 for O:5,27 strains (data not shown).

TABLE 3. Comparative number of susceptible *Y. enterocolitica* strains from animals

Antimicrobial agent	No. (%) of susceptible strains isolated in:			
	1972–1976 (n = 20)	1980 (n = 3)	1985 (n = 12)	1990 (n = 10)
Ampicillin	1 (5.0)	0 (0)	0 (0)	0 (0)
Carbenicillin	4 (20.0)	1 (33.3)	2 (16.7)	0 (0)
Piperacillin	20 (100)	3 (100)	12 (100)	10 (100)
Ticarcillin	4 (20.0)	1 (33.3)	2 (16.7)	0 (0)
Amoxicillin-clavulanic acid	13 (65.0)	2 (66.7)	9 (75.0)	7 (70.0)
Cephalothin	0 (0)	0 (0)	0 (0)	0 (0)
Cefamandole	20 (100)	3 (100)	12 (100)	10 (100)
Cefotaxime	20 (100)	3 (100)	12 (100)	10 (100)
Aztreonam	20 (100)	3 (100)	12 (100)	10 (100)
Kanamycin	20 (100)	3 (100)	12 (100)	10 (100)
Gentamicin	20 (100)	3 (100)	12 (100)	10 (100)
Tobramycin	20 (100)	3 (100)	12 (100)	10 (100)
Amikacin	20 (100)	3 (100)	12 (100)	10 (100)
Sulfamethoxazole	20 (100)	3 (100)	12 (100)	10 (100)
Trimethoprim	20 (100)	3 (100)	12 (100)	10 (100)
Trimethoprim-sulfamethoxazole	20 (100)	3 (100)	12 (100)	10 (100)
Clindamycin	0 (0)	0 (0)	0 (0)	0 (0)
Erythromycin	0 (0)	0 (0)	0 (0)	0 (0)
Furazolidone	0 (0)	0 (0)	0 (0)	0 (0)
Chloramphenicol	20 (100)	3 (100)	12 (100)	10 (100)
Tetracycline	20 (100)	3 (100)	12 (100)	10 (100)
Ciprofloxacin	20 (100)	3 (100)	12 (100)	10 (100)

Y. enterocolitica strains isolated from animals during the same periods displayed susceptibility patterns similar to those of the human strains (Table 3). All of these strains were not susceptible to clindamycin, erythromycin, furazolidone, and cephalothin. In addition, high percentages of the strains from each period were not susceptible to ampicillin, carbenicillin, and ticarcillin. The rate of susceptibility to amoxicillin-clavulanic acid varied from a minimum of 65% of the strains isolated in the 1972-to-1976 period to a maximum of 75% of strains isolated in the 1985 period. In contrast to the various susceptibility patterns identified among the human strains, none of the strains isolated from animals were nonsusceptible to cefamandole, cefotaxime, aztreonam, kanamycin, gentamicin, tobramycin, amikacin, sulfamethoxazole, trimethoprim, cotrimoxazole, chloramphenicol, or tetracycline.

DISCUSSION

During the past two decades, four serotypes of *Y. enterocolitica* (O:3, O:5,27, O:8, and O:9) have remained the serotypes most commonly associated with human infections in Canada (unpublished data). The present study has provided new insights into the current antimicrobial susceptibilities of these serotypes, the stability of susceptibility patterns between 1972 and 1990, and the relationships between susceptibility patterns and serotypes in this important human pathogen.

High percentages of strains from each group were nonsusceptible to the β-lactamase-sensitive penicillins (ampicillin, carbenicillin, and ticarcillin) and to cephalothin, a narrow-spectrum cephalosporin, but some serotype-specific differences were observed. In contrast, most strains from each group were susceptible to cefamandole, an extended-spectrum cephalosporin, cefotaxime, a broad-spectrum cephalosporin, and aztreonam, a monobactam. In addition, all 1,105 strains tested were susceptible to piperacillin at the breakpoint concentration. These results are in general agreement with those of previous investigations of the susceptibilities of *Y. enterocolitica* to β-lactam antibiotics conducted in various countries (8, 10, 12, 15). In contrast, Kwaga and Iversen (11) reported that nearly 50% of *Yersinia* strains isolated from slaughtered pigs and pork products in Canada were resistant to piperacillin, but they also reported discrepancies between the results generated by agar dilution and disc diffusion methods for this antibiotic.

When tested against the non-β-lactam compounds used in our study, all 1,105 strains were not susceptible to clindamycin, erythromycin, and furazolidone. These results are in agreement with those of previous investigations (4, 10, 11, 19, 20). Erythromycin, furazolidone, and lincomycin are used for growth promotion and disease prophylaxis in pigs in North America (18), and erythromycin and lincomycin have been used in human medicine. These factors could contribute to the decreased susceptibility to clindamycin, erythromycin, and furazolidone, or, alternatively, *Y. enterocolitica* may be intrinsically nonsusceptible to these compounds. On the other hand, all strains tested were susceptible to ciprofloxacin at the concentration of 1 μg/ml, and these results confirmed the excellent in vitro activity of fluoroquinolones against this species reported previously (7). High percentages of strains from each period were also susceptible to chloramphenicol, tetracycline, sulfamethoxazole, trimethoprim, cotrimoxazole, and the four aminoglycosides kanamycin, gentamicin, tobramycin, and amikacin. Previous investigators of *Y. enterocolitica* susceptibilities also found that most isolates were susceptible to these various classes of antibiotics (6, 10, 15, 19, 20).

Correlations between the susceptibilities to certain β-lactam antibiotics (ampicillin, carbenicillin, ticarcillin, amoxicillin-clavulanic acid, and cephalothin) and serotypes O:3, O:5,27, and O:8 were demonstrated clearly in our study. Strains of serotype O:3, the most common serotype isolated in Canada, displayed high rates of nonsusceptibility to each of these agents except amoxicillin-clavulanic acid. In a previous study, Pham and coworkers (15) reported that *Y. enterocolitica* clinical strains isolated in Australia belonging to biotypes 1A, 3, and 4 displayed patterns of resistance to certain β-lactam agents specific for each biotype. Hornstein et al. (8) showed that each of the four most common serotypes (O:3, O:5,27, O:8, and O:9) in France had specific patterns of susceptibility to ampicillin, carbenicillin, cephalothin, cephaloridine, cephalexin, and cefoxitin. Similarly, Ahmedy et al. (1) identified differences in susceptibility to ampicillin and carbenicillin among food-isolated strains of biogroups 1 and 3 in France. Recent findings indicate that differences in the distribution of β-lactamase enzymes among *Y. enterocolitica* serogroups and biogroups may underly the different patterns of resistance to these agents (16, 23). In 1974, Toma and Lafleur (21) reported that all serotype O:3 strains isolated in Canada were resistant to ampicillin, while all O:8 strains were susceptible. The results of the present study indicate that this serotype-specific pattern of resistance has continued during the last two decades.

Between 1972 and 1990, no major decrease in susceptibility to any of the antibiotics tested was identified among human or animal strains. However, the percentage of human strains not susceptible to tetracycline increased from 0.4% in 1985 to 2.0% in 1990. In addition, a total of seven human strains isolated in 1980, 1985, and 1990 displayed nonsusceptibility to various combinations of antibiotics, including the aminoglycosides, chloramphenicol and cotrimoxazole. Multiply resistant *Y. enterocolitica* strains have been identified infrequently by investigators in other countries (10, 12, 22). Recently, Trallero and coworkers (22) reported the emergence of resistance to chloramphenicol, sulfonamide, and streptomycin in serotype O:3

(biotype 4) strains isolated from humans and pork products in Spain and they proposed that overuse of antibiotics in veterinary medicine was the primary cause of the multiple resistance encountered in human strains. In the present investigation, no strain isolated from animal sources was identified as having any of the additional nonsusceptibility patterns observed in the human strains; however, the overall number of animal isolates available for study was relatively small. In agreement with our findings, Kwaga and Iversen (11) reported that yersiniae isolated from slaughtered pigs and pork products in Canada in 1990 displayed little or no resistance to the aminoglycosides, chloramphenicol, tetracycline, and cotrimoxazole.

Although our data show that pathogenic *Y. enterocolitica* strains were uniformly susceptible to both ciprofloxacin and piperacillin, they also indicate that the agents used traditionally to treat human infections, including cotrimoxazole, tetracycline, chloramphenicol, and the aminoglycosides, retained their high levels of in vitro activities during the past two decades. In addition, our results illustrate the potential for this species, like other members of the family *Enterobacteriaceae*, to acquire decreased susceptibility to multiple antimicrobial agents and emphasize the need for continued surveillance of the susceptibility patterns of *Y. enterocolitica* from both human and animal sources.

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