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# Antibiotic Susceptibility Patterns of *Yersinia enterocolitica*

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**ABSTRACT**

Antibiotic susceptibility patterns for *Yersinia enterocolitica* strains involving 10 different serotypes were analyzed and compared. All *Y. enterocolitica* were susceptible to colistin, gentamicin, kanamycin, neomycin and doxycycline, whereas all isolates displayed resistance to penicillin G, methicillin (derivative of penicillin), novobiocin, and clindamycin. The antibiograms for the *Y. enterocolitica* isolates were in some instances related to the somatic serotypes, especially serotype 0:8 for which the antimicrobial susceptibility pattern displayed the greatest disparity. By eliminating the antibiograms for the four serotype 0:8 strains, antimicrobial susceptibility patterns for atypical and typical strains were similar.

Numerous *Yersinia enterocolitica* serotypes and strains possessing a diversity of biochemical reactions have been isolated with increasing frequency from various foods (*11,19*), environmental sources (*12,14*) and humans (*3*). Different *Y. enterocolitica* enrichment procedures and plating media have been shown to isolate certain strains or serotypes at various frequencies, indicating differences in sensitivity to selective agents and environmental situations among strains and/or serotypes (*4,19*). In recent years, an increase in isolations have occurred of atypical (environmental) or *Y. enterocolitica*-like strains from human (*5,6*) and a variety of nonhuman sources including vacuum-packaged beef (*11*), raw and pasteurized milk (*19*) and water (*12*). These strains differ from the typical strains by their acid production from melibiose, rhamnose, raffinose or salicin, use of citrate and/or failure to ferment sucrose. Further, the rhamnose-positive atypical *Y. enterocolitica* strains possess a more extensive profile of temperature-dependent biochemical and physiological responses than the typical strains (*4*). Invasiveness of HeLa cells was demonstrated to occur for typical clinical strains of *Y. enterocolitica* while none of the esculin- and salicin-positive or food and water atypical isolates adhered to or invaded HeLa cells (*15*). In addition, enterotoxin production is much more prevalent in strains isolated from humans (including serotypes 0:3, 0:8, 0:9, 0:5, 27 and 0:6, 30) than isolates from animals, water, raw milk and food (*17*). Therefore, enterotoxigenicity could be another characteristic that may be common to a large majority of typical *Y. enterocolitica* strains but scarce in atypical strains. In addition, Bottone (*4*) has suggested that the atypical or *Y. enterocolitica*-like strains not be designated as *Y. enterocolitica* but placed in a new species such as *Yersinia rhamnophilica*. Further, according to deoxyribonucleic acid (DNA) homology studies, Brenner et al. (*7*) demonstrated that the rhamnose-positive atypical strains are not closely related to typical *Y. enterocolitica* strains and placed these atypical strains into separate DNA relatedness groups.

Some *Y. enterocolitica* serotypes display a dramatic geographical distribution. Serotype 0:8 is isolated from humans almost exclusively in the United States (*4*) while 0:3 (phage type 96), 0:5, 27 and 0:6, 30 are the predominant serotypes of *Y. enterocolitica* isolates from Canada (*20*). In Japan, serotypes 0:3 and 0:5 are the most common isolates from animal and clinical sources (*1,21*) while in European countries serotype 0:3 is the dominant *Y. enterocolitica* isolate, with 0:9 the next most commonly encountered strain from nonhuman and human sources (*16*). Similar to serotype distribution, indole production in *Y. enterocolitica* is variable, with most European and Japanese isolates reported as indole-negative as compared to the United States isolates where most are indole-positive (*4*). Further, Knapp and Thal (*13*) have proposed that indole-positive and -negative strains be placed in separate species, *Y. enterocolitica* and *Yersinia enteritidis*, respectively. Since *Y. enterocolitica* is not a homogenous species and comparisons of antibiotic sensitivity testing among species are scarce (*3,10,18*), antibiograms of various strains isolated from different geographical locations in the world were determined and compared.

## MATERIALS AND METHODS

### *Bacterial strains*

The antigen type, origin, contributor and Nilehn's biotype (*16*) of atypical or typical *Y. enterocolitica* strains used in this study are listed in Table 1. Stock cultures were maintained on Difco brain heart infusion (BHI) slants and stored at 4 C.

### *Antibiotics*

The following antibiotic disks were tested: ampicillin (10 μg), carbenicillin (100 μg), cephalothin (30 μg), cephaloglycin (30 μg), colistin (10 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), methicillin (5 μg), neomycin (30 μg), novobiocin (30 μg), oleandomycin (15 μg), penicillin (10 U), clindamycin (2 μg) and doxycycline (30 μg). All of the antibiotic disks were purchased from BBL, Baltimore, Maryland.

### *Susceptibility testing*

Antibiotic susceptibility tests were made by the disk diffusion

method of Bauer et al. (*2*). When testing the susceptibility of *Y. enterocolitica* strains to novobiocin, Müller-Hinton medium without the 5% blood was used. The antibiotic disks were speciously placed on the inoculated Müller-Hinton agar plates. The plates were incubated at 25 C for 18-24 h. Zones of inhibition to the nearest millimeter were interpreted as susceptible, intermediate or resistant based on the interpretative table recommended by the Food and Drug Administration (*12*).

## RESULTS AND DISCUSSION

Results of antibiotic susceptibility tests comparing 10 somatic serotypes isolated worldwide are presented in Table 2. As shown by other authors (*3,5,8,10,14,18*), all strains in this investigation were sensitive to colistin, gentamicin, kanamycin, neomycin and doxycycline (tetracycline derivative). In addition, all *Y. enterocolitica* strains were sensitive to the cephalosporin derivative, cephaloglycin, indicating the inability of these strains to produce a β-lactamase that degrades cephaloglycin. All isolates displayed resistance to penicillin G, methicillin (derivative of penicillin), novobiocin and clindamycin, as reported in previous investigations (*3,10,14,18*).

Bissett (*3*) showed that resistance to some antimicrobial agents was related to the *Y. enterocolitica* somatic serotypes. All of the serotype 0:8 strains were sensitive to ampicillin and cephalothin, whereas strains of the remaining serotypes were intermediate to highly resistant to these two antibiotics. For oleandomycin, 2 of the 4 serotype 0:8 strains were susceptible, whereas all the strains representing the other serotypes were resistant. The *Y. enterocolitica* isolates displayed a wide range of sensitivity for carbenicillin and erythromycin, among the 10 serotypes used in this investigation. All the strains for serotypes 0:8, 0:5, 27 and 0:5 and 1 of 4 strains for serotype 0:17 were susceptible to carbenicillin while the strains from the remaining serotypes were resistant or intermediate in resistance. Twenty-eight strains of *Y. enterocolitica* from human and animal sources have been shown to be slightly sensitive to resistant to erythromycin (*14*). In this investigation, the data further show that strains from 70% of the serotypes were either resistant or intermediate in resistance to erythromycin. However, all of the studies from serotypes 0:8, 0:16, and 0:20 and 50% of the 0:17 serotype strains were sensitive to this antibiotic.

Table 3 shows a comparison of antibiotic susceptibilities between typical and atypical *Y. enterocolitica* strains. In general, the typical strains displayed greater susceptibility than the atypical strains to antibiotics used in this investigation. However, this disparity in antibiograms was mainly caused by the susceptibility of all or some of serotype 0:8 strains to ampicillin,

TABLE 1. Yersinia enterocolitica *strains tested.*

| Antigen type | Origin | Contributor | Niléhn biotype | Atypical vs. typical[a] |
|---|---|---|---|---|
| 0:8 | Human, U.S. | E. J. Bottone | 2 | T[c] |
| 0:17 | Human, U.S. | " | —[d] | A |
| 0:3[b] | Human, Canada | S. Toma | 4 | T |
| 0:8 | Human, Canada | " | 2 | T |
| 0:9 | Unknown, Canada | " | 1 | A |
| 0:6,30 | Human, Canada | " | 1 | T |
| 0:4,32 | Human, Canada | " | 2 | T |
| 0:5,27 | Human, Canada | " | 2 | T |
| 0:17 | Human, U.S. | F. J. Bottone | — | A |
| 0:16 | Stream water, U.S. | S. Harvey | 1 | A |
| 0:3 | Swine, Japan | M. Tsubokura | 4 | T |
| 0:5 | Swine, Japan | " | 2 | T |
| 0:3 | Swine, Japan | " | 4 | T |
| 0:5 | Swine, Japan | " | 2 | T |
| 0:17 | Vacuum-packed meats, U.S. | C. Vanderzant | — | A |
| 0:17 | Vacuum-packed meats, U.S. | " | 1 | A |
| 0:20 | Vacuum-packaged meats, U.S. | " | — | A |
| Nontyped | Vacuum-packaged meats, U.S. | " | 1 | A |
| 0:3 | Human, Europe | H. H. Mollaret | 4 | T |
| 0:9 | Human, Europe | " | 2 | T |
| Unknown | Human (face, U.S.) | ATCC 9610 | 2 | T |
| Unknown | Human (blood, U.S.) | ATCC 23715 | 2 | T |
| 0:8 | Human (blood, U.S.) | ATCC 27729 | 2 | T |
| 0:8 | Stream Water, U.S. | ATCC 27739 | 2 | T |

[a]Atypical strains were classified on the basis of the utilization of rhamnose, raffinose, melibiose, citrate, or salicin or the failure of acid production from sucrose.

[b]Phage type 9b. Different from European 0:3 serotype.

[c]T, typical; A, atypical.

[d]—, does not conform to any of the five Niléhn's biotypes.

carbenicillin, cephalothin, erythromycin and oleandomycin. By eliminating the antibiograms for the four serotype 0:8 strains, antimicrobial susceptibility patterns for atypical and typical strains would be more similar.

Differences in atypical and typical strains of *Y. enterocolitica* have been documented by various authors (*3,18*). The atypical strains are encountered mainly from environmental and food sources, whereas the typical strains are isolated most often from human and animal sources (*4*). Also, the atypical strains differ from the typical strains in certain biochemical reactions (*3*), invasiveness of HeLa cells (*15*) and enterotoxin production (*17*). However, results of this investigation failed to show an overt difference between atypical and typical strains with respect to their antimicrobial susceptibility patterns. Instead, the antibiogram for serotype 0:8 was different from the antimicrobial susceptibility patterns of the other serotypes used in this investigation. Therefore, antibiograms of atypical and typical *Y. enterocolitica* fail to substantiate the view that these two groups of *Y. enterocolitica* should be separated into different relatedness groups or genera.

TABLE 3. *Comparisons of antibiotic susceptibility between typical versus atypical strains of* Yersinia enterocolitica.[a]

| Antimicrobial agents | Atypical | | Typical | |
|---|---|---|---|---|
| | No. sensitive | % Sensitive | No. sensitive | % Sensitive |
| Ampicillin | 0 | 0.0 | 6 | 37.5 |
| Carbenicillin | 1 | 12.5 | 9 | 56.3 |
| Cephalothin | 0 | 0.0 | 6 | 37.5 |
| Cephaloglycin | 7 | 87.5 | 16 | 100.0 |
| Colistin | 8 | 100.0 | 16 | 100.0 |
| Erythromycin | 2 | 25.0 | 7 | 43.8 |
| Gentamicin | 8 | 100.0 | 16 | 100.0 |
| Kanamycin | 8 | 100.0 | 16 | 100.0 |
| Methicillin | 0 | 0.0 | 0 | 0.0 |
| Neomycin | 8 | 100.0 | 16 | 100.0 |
| Novobiocin | 0 | 0.0 | 0 | 0.0 |
| Oleandomycin | 0 | 0.0 | 2 | 12.5 |
| Penicillin G | 0 | 0.0 | 0 | 0.0 |
| Clindamycin | 0 | 0.0 | 0 | 0.0 |
| Doxycycline | 8 | 100.0 | 16 | 100.0 |

[a]Refer to Table 1 for determination of atypical versus typical strains. Interpretations of antibiotic susceptibility testing were determined according to the rule and regulations in the *Federal Register* (*9*) and utilizing the single disk method (*2*).


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TABLE 2. *The relationship of antibiotic susceptibility to somatic serotypes of* Yersinia enterocolitica *strains isolated from various parts of the world*[a].

| Antimicrobial agents[b] | Somatic serotypes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0:8 4[c] | 0:17 4 | 0:3 4 | 0:9 2 | 0:6,30 1 | 0:4,32 1 | 0:5,27 1 | 0:16 1 | 0:5 2 | 0:20 1 |
| Ampicillin (14) | 18-19-S[d] | 10-12-R | 6-R | 6-R | 9-R | 9-R | 11-R | 8-R | 6-9-R | 13-I |
| Carbenicillin (23) | 24-26-S | 16-23-I | 6-12-R | 8-11-R | 16-R | 15-R | 28-S | 15-R | 27-30-S | 22-I |
| Cephalothin (18) | 18-21-S | 6-13-R | 8-9-R | 6-9-R | 6-R | 17-I | 6-R | 6-R | 6-R | 13-R |
| Cephaloglycin (15) | 25-28-S | 20-24-S | A-20-S | 15-19-S | 17-S | 25-S | 18-S | 18-S | 18-20-S | 25-S |
| Colistin (11) | 12-18-S | 14-15-S | 13-15-S | 13-14-S | 14-S | 15-S | 14-S | 13-S | 13-S | 15-S |
| Erythromycin (18) | 22-24-S | 13-17-I | 11-18-I | 11-15-R | 12-R | 16-I | 12-R | 18-S | 8-13-R | 18-S |
| Gentamicin (13) | 23-24-S | 20-25-S | 20-24-S | 20-22-S | 21-S | 20-S | 22-S | 24-S | 21-S | 25-S |
| Kanamycin (18) | 16-27-I | 23-27-S | 22-25-S | 21-22-S | 23-S | 20-S | 22-S | 23-S | 21-23-S | 28-S |
| Methicillin (14) | 6-R | 6-R | 6-R | 6-R | 6-R | 6-R | 6-R | 6-R | 6-R | 6-R |
| Neomycin (17) | 20-22-S | 17-23-S | 19-22-S | 19-20-S | 19-S | 18-S | 20-S | 20-S | 18-19-S | 20-S |
| Novobiocin (22)[e] | 10-12-R | 8-17-R | 6-8-R | 9-11-R | 7-R | 8-R | 10-R | 10-R | 6-8-R | 17-R |
| Oleandomycin (17) | 15-17-I | 6-9-R | 6-R | 6-R | 6-R | 10-R | 6-R | 6-R | 6-R | 6-R |
| Penicillin (22) | 8-13-R | 6-8-R | 6-R | 6-R | 6-R | 6-R | 6-R | 6-R | 6-R | 6-R |
| Clindamycin (17) | 6-R | 6-R | 6-R | 6-R | 6-R | 6-R | 6-R | 6-R | 6-R | 6-R |
| Doxycycline (19) | 24-27-S | 25-26-S | 20-22-S | 20-23-S | 21-S | 21-S | 25-S | 24-S | 21-24-5 | 27-S |

[a]The interpretations of the antibiotic susceptibility testing were determined according to the rules and regulations of antibiotics (Part 147) in the *Federal Register* (*9*) utilizing the single disk method (*2*).
[b]Numerical value of parentheses indicates minimum diameter in millimeters of inhibition zone for an interpretation of sensitive.
[c]Number of strains.
[d]The zone of inhibition was a range when more than one strain was used for a particular somatic serotype.
[e]Blood was omitted from the medium when testing the susceptibility of the strains to novobiocin.
S, sensitive; I, intermediate; R, resistant.

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