

## Antimicrobial Susceptibilities of Food-Isolated Strains of *Yersinia enterocolitica*, *Y. intermedia*, *Y. frederiksenii*, and *Y. kristensenii*

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**The in vitro antimicrobial susceptibility of *Yersinia enterocolitica* and newly related species isolated from foods was examined. Only 4 of 375 isolates displayed resistance to non- $\beta$ -lactam antibiotics. MICs of ampicillin and carbenicillin determined by agar dilution with respect to 125 isolates showed the high susceptibility of *Y. kristensenii* and biovar 3 of *Y. enterocolitica* to carbenicillin (MIC for 90% of the strains,  $\leq 8$   $\mu$ g/ml).**

In contrast with data available concerning classical enterobacteria such as *Escherichia coli* and *Salmonella* spp., which are well documented, comparatively little is known about the antibiotic susceptibility of *Yersinia* spp. Cornelis et al. (3, 4, 7, 12) studied extensively the  $\beta$ -lactamases produced by a number of strains of *Yersinia enterocolitica* of human or animal origin and characterized two chromosomally mediated  $\beta$ -lactamases, types A and B. On the other hand, recent reports on in vitro activities of new  $\beta$ -lactam antibiotics have shown that clinical isolates of *Y. enterocolitica* were susceptible to these newer antimicrobial agents (10, 18). With respect to non- $\beta$ -lactam antibiotics, resistant *Yersinia* strains seem to be very uncommon. Some resistance plasmids have been found in strains of *Y. enterocolitica* isolated from human beings (6, 11, 20), but two recent studies of in vitro antimicrobial susceptibility of isolates from human, environmental, and animal sources have shown that they displayed essentially equal susceptibility patterns (9, 15).

Recently, three new species of *Yersinia*, previously called *Y. enterocolitica*-like and now named *Y. intermedia*, *Y. frederiksenii*, and *Y. kristensenii* (2), have been described. To our knowledge no data is yet available concerning the antimicrobial susceptibility of these new species. In this report we present the results of a study concerning in vitro antibiotic susceptibility of a number of bacteria of this group isolated from various foods in Alsace, a region of eastern France.

*Yersinia* strains were isolated from foods as follows. A 10-g sample of homogenized food was preenriched in 100 ml of phosphate-sorbitol-bile medium (13) at 4°C for 9 days. The preenriched mixture (1 ml) was transferred to 100 ml of bile-oxalate-sorbose selective enrichment broth (17), which was incubated at 22°C for 5 days. The enrichment broth (0.5 ml) was mixed with 4.5 ml of a 0.25% KOH solution and held for 2 min. (8). One loopful of the alkali-treated broth was finally streaked onto cefsulodin-irgasan-novobiocin agar (16), which was incubated at 28°C for 48 h. Presumptive colonies were identified by using the API-20E system (Montalieu-Vercieu), and then serogrouping, biovar, and phagovar determinations were carried out by H. H. Mollaret (Institut Pasteur, Paris).

Foods analyzed were pork, sausages, salads, cakes, ice creams, and raw milk, originating from pork butchers, cafeterias, and retailers in the Strasbourg area and neighbor-

ing regions of Alsace. Isolation of *Yersinia* spp. from raw milk was performed as previously described (19).

Susceptibility testing of bacterial strains was achieved at 28°C by the agar diffusion method according to the procedure of Bauer et al. (1). Disks of antibiotics and their concentrations in micrograms per disk were: ampicillin, 10; carbenicillin, 100; cephaloridine, 30; tetracycline, 30; chloramphenicol, 30; streptomycin, 10; and sulfadiazine, 200. The disks and Mueller-Hinton medium were obtained from Bio-Merieux or Institut Pasteur Production.

The MICs were determined at 28°C for 24 h by the dilution method in solid medium by using Trypticase soy agar (Bio-Merieux). The MICs of the following antibacterial agents, ampicillin (Serva), carbenicillin (Beecham-Sévigé), chloramphenicol (Roussel Uclaf), tetracycline (Pfizer), streptomycin (Specia), and HgCl<sub>2</sub> (Prolabo), were determined. Serially diluted agar plates were inoculated by streaking a loop (1  $\mu$ l) of 1/100-diluted overnight cultures in Trypticase soy broth (Bio-Merieux) at 28°C, delivering a total of ca. 10<sup>4</sup> CFU of bacterial cells.

A total of 375 strains of *Yersinia* spp. were isolated from foods from June 1983 to July 1984. These strains were identified as *Y. enterocolitica* biovars 1 (220 isolates) and 3 (12 isolates), plus one atypical strain; *Y. intermedia* biovars 1, 2, 4, and 5 (74 isolates); *Y. frederiksenii* (43 isolates); and *Y. kristensenii* (25 isolates).

All but 4 strains (98.9%) were susceptible to non- $\beta$ -lactam antibiotics. Eighty-seven percent of the strains were ampicillin and carbenicillin resistant.

The MICs for ampicillin and carbenicillin were determined for 125 strains. The strains of *Y. kristensenii* and *Y. enterocolitica* biovar 3 were the most susceptible, whereas isolates of *Y. enterocolitica* biovar 1 and *Y. frederiksenii* were the most resistant. MIC ranges and MICs that inhibited 50 and 90% of the strains (MIC<sub>50</sub> and MIC<sub>90</sub>, respectively) are detailed in Table 1.

The MICs with respect to other antibiotics tested were done for 26 strains representing all the biovars found in each species. The range of susceptibility to these agents was 4 to 8  $\mu$ g/ml for streptomycin, 2 to 8  $\mu$ g/ml for chloramphenicol, 1 to 6  $\mu$ g/ml for tetracycline, and 6 to 9  $\mu$ g/ml for mercuric chloride.

Four isolates of *Y. enterocolitica*, designated 274YH, 307PBK, 747G, and 748G, were streptomycin resistant; in addition, 307PBK, 747G, and 748G were resistant to tetracycline, sulfadiazine, and mercuric chloride. The three tetracycline-resistant strains remained susceptible to minocy-

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TABLE 1. In vitro susceptibility to ampicillin and carbenicillin of 125 strains of *Yersinia* spp. isolated from foods

Organism (no. of strains)	Biovar	Serogroups <sup>a</sup> (no.)	Antibiotic	MIC ( $\mu$ g/ml)		
				Range	50	90
<i>Y. enterocolitica</i> (49)	1	O:5 (11); O:7,8 (8); O:6 (5); O:10,34,K <sub>1</sub> (5); O:10,K <sub>1</sub> (2); O:14 (3); O:4 (1); O:25,35 (2); O:30,47 (2); O:30 (1); O:27 (1); O:7,8,19 (1); O:39,41 (4); O:48 (2); N(1).	Ampicillin	8–128	64	128
			Carbenicillin	2–>256	128	256
<i>Y. enterocolitica</i> (12)	3	O:7,8 (2); O:7 (1); O:7,19 (1); N (8)	Ampicillin	4–64	4	32
			Carbenicillin	2–8	2	4
<i>Y. intermedia</i> (27)		O:3 (1); O:4 (1); O:17 (1); O:40 (1); O:48 (7); O:50 (1); O:52 (2); O:14 (2); O:36 (1); N (9); AA (1)	Ampicillin	1–128	16	32
			Carbenicillin	1–256	64	256
<i>Y. frederiksenii</i> (12)		O:5 (1); O:35 (3); O:52, 53 (1); N (3); AA (4)	Ampicillin	32–128	64	64
			Carbenicillin	128–>256	256	>256
<i>Y. kristensenii</i> (25)		O:11 (9); O:12 (2); O:12, 25 (4); O:26, 35 (3); O:28 (1); O:35 (1); O:28,35 (1); O:46 (1); O:16,34,46 (1); N (2)	Ampicillin	4–15	8	16
			Carbenicillin	2–32	4	8

<sup>a</sup> N, Nonagglutinable by antisera; AA, autoagglutinable.

cline. All four strains were spectinomycin resistant also, but remained susceptible to the other aminoglycoside antibiotics kanamycin, gentamicin, tobramycin, neomycin, and amikacin.

We attempted to transfer the resistance markers from 274YH, 307PBK, 747G, and 748G to different recipient strains (*E. coli* K-12 PA505 Nal<sup>r</sup> [Nal4], *E. coli* K-12 C600 Rif<sup>r</sup>, and *Y. enterocolitica* 195A14J Rif<sup>r</sup> [195JR]) by mating on Trypticase soy agar. None of these experiments succeeded at 37°C or at 28°C for 24 h.

By using the conjugative plasmid F'lac, the resistance markers of 307PBK, 747G, and 748G (Tc, Sm, Su, Hg<sup>2+</sup>) were mobilized as a unit to Nal4 and retransferred from Nal4 to 195JR. The streptomycin resistance of 274YH was mobilized also, with a higher frequency in comparison with the resistance markers of the three other strains.

The four resistant strains and their F'lac exconjugants were screened for plasmid content by agarose gel electrophoresis of crude lysates prepared by the method of Meyers et al. (14). Only the streptomycin-resistant strain 274YH displayed the existence of plasmid DNA (ca. 45 kilobases of size), whereas no extrachromosomal DNA was detectable in the three other resistant strains. In addition, *E. coli* transconjugants from the four strains showed only one band of plasmid DNA, probably corresponding to the F'lac plasmid (data not shown).

Our results show a strikingly low incidence of drug resistance in food-isolated strains of *Y. enterocolitica* and neighboring species recently referred to as *Y. intermedia*, *Y. frederiksenii*, and *Y. kristensenii*. These results are in agreement with those reported by other authors concerning antimicrobial susceptibility of human or animal isolates of *Y. enterocolitica* and seem to reflect the low ability of this species to act as a recipient (5) as well as that of the three newly recognized species. This assumption is reinforced by the observation of the nontransferability of the resistance markers harbored by the four resistant wild-type strains, without the help of F'lac.

With respect to  $\beta$ -lactam antibiotics, the most salient feature of our results lies in the difference in susceptibility to carbenicillin, and to a lesser degree, to ampicillin, of the different species or biovars of *Yersinia* isolates. Indeed, carbenicillin-susceptible strains (MIC<sub>90</sub>,  $\leq 8$   $\mu$ g/ml) either belong to biovar 3 of *Y. enterocolitica* or are *Y. kristensenii*

strains. On the other hand, *Y. enterocolitica* biovar 1, *Y. intermedia*, and *Y. frederiksenii* are always resistant to carbenicillin (MIC<sub>90</sub>, >256  $\mu$ g/ml). Twelve years ago, Cornelis et al. found an homogeneous susceptibility to  $\beta$ -lactams within each serological group of isolates of *Y. enterocolitica* from different origins (7). Two groups displayed high susceptibility to carbenicillin (MIC,  $\leq 8$   $\mu$ g/ml): serogroup 5, 27 (or 5b) and a group including serogroups 11, 12, 23, 24, 25, and 26. Interestingly, the latter corresponds to strains belonging to *Y. kristensenii* in our study. Our observations on carbenicillin susceptibility of *Yersinia* spp. could be of interest in the taxonomy of these bacteria.

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