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Virulence characteristics and epidemiology of *Yersinia enterocolitica* and Yersiniae other than *Y. pseudotuberculosis* and *Y. pestis* isolated from water and sewage

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ABSTRACT

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Aims: To determine the species, bio-sero-phagetypes, antimicrobial drug resistance and also the pathogenic potential of 144 strains of *Yersinia* spp. isolated from water sources and sewage in Brazil.

Methods and Results: The 144 *Yersinia* strains were characterized biochemically, serologically and had their antibiotic resistance and phenotypic virulence markers determined by microbiological and serological standard techniques. The *Y. enterocolitica* strains related to human diseases were also tested for the presence of virulence genes, by the PCR technique. The isolates were classified as *Y. enterocolitica*, *Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii* and *Yersinia* biochemically atypical. The 144 isolates belonged to various bio-serogroups. Half of the strains showed resistance to three or more drugs. The *Y. enterocolitica* strains related to human diseases exhibited phenotypic virulence characteristics and virulence genes.

Conclusions: Water from various sources and sewage are contaminated with *Yersinia* spp. in Brasil. Among these bacteria, virulent strains of *Y. enterocolitica* were found, with biotypes and serogroups related to human diseases.

Significance and Impact of the Study: This is the first documented description of the occurrence of pathogenic *Y. enterocolitica* in water sources and sewage in Brazil. The occurrence of virulence strains of *Y. enterocolitica* shows that the environment is a potential source of human infection by this species in this country.

Keywords: antimicrobial, isolation, pathogenic, pollution, sewage, water, *Yersinia*.

INTRODUCTION

Yersinia enterocolitica is a human pathogen, which causes a variety of intestinal and extraintestinal clinical symptoms of varying severity ranging from mild gastroenteritis to mesenteric lymphadenitis, which mimics appendicitis and septicaemia. Infection with *Y. enterocolitica* can also lead to postinfection immunological sequelae, including erythema nodosum, arthritis and glomerulonephritis (Bottone 1999; Robins-Browne 2001).

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Pathogenic *Y. enterocolitica* strains have traditionally been linked to specific biotypes and serogroups, with biochemical reactions such as pyrazinamidase production, esculin hydrolysis and salicin fermentation, and with a variety of virulence characteristics, including calcium dependence, autoagglutination, and Congo Red absorption (Bottone 1999). Molecular genetic studies have emphasized the importance of a virulence plasmid (pYV), which encodes various virulence genes as well as specific chromosomal genes that mediate cell invasion (genes *inv* and *ail*), capture of iron (genes of the high-pathogenicity-island) and the ability to produce the enterotoxin Yst (gene *yst*), among others (Cornelis 1994; Carniel 1995; Cornelis *et al.* 1998).

Human clinical infections with *Y. enterocolitica* most frequently occur after ingestion of food and/or water contaminated with the bacterium. After ingestion, the bacteria pass through the stomach, colonize the epithelial cell surface of the small intestinal mucosa, and cause diarrhoea possibly by producing Yst (Delor and Cornelis 1992). The bacteria can also invade the epithelial cells, binding to intestinal brush-border membranes, from where they penetrate M cells and gain access to and multiply in Peyer's patches. Bacteria taken up by M cells are usually phagocytosed and killed by macrophages of Peyer's patches; however, pathogenic *Y. enterocolitica* strains have several surface components which enable them to resist phagocytosis and escape from complement-mediated death. Continuing proliferation of the bacteria results in an inflammatory reaction, which leads to local microabscess formation and ulceration of the overlying epithelium. Finally, the bacteria may spread to the mesenteric lymph nodes and enter the bloodstream (Robins-Browne 2001; Salyers and Whitt 2002).

Besides *Y. enterocolitica*, two other *Yersinia* species, *Y. pseudotuberculosis* and *Y. pestis*, have long been known to cause human disease (Carniel and Mollaret 1990; Bottone 1999). The remaining eight species (*Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii*, *Y. aldovae*, *Y. rohdei*, *Y. bercovieri*, *Y. mollaretii* and *Y. ruckeri*), have not been studied extensively, and because of the absence of classical *Yersinia* virulence markers, they have been generally considered to be environmental and nonpathogenic species. However, an increasing number of these 'nonpathogenic' species have been isolated from sick humans, raising the question of their possible pathogenicity (Sulakvelidze 2000).

The objectives of this work were to study *Yersinia* strains isolated from fresh and salt water sources and from sewage in Brazil and to determine their species, biotype, serogroup and phagetype and evaluate their drug resistance profile and some other virulence characteristics.

MATERIALS AND METHODS

Bacterial strains

A total of 144 strains of *Yersinia* spp. were received at the *Yersinia* Reference Laboratory in Brazil, School of Pharmaceutical Sciences, Araraquara, UNESP, for complete identification. They had been isolated by Freitas *et al.* (1987) in the city of Rio de Janeiro (67 strains) and by M.T. Martins (personal communication) in the city of São Paulo (77 strains), Brazil, from sources recorded as fresh water (38), waterfall (six), ocean (nine), polluted river (29), polluted estuary (seven), polluted (salt water) lagoon (three), stream (two), spring (seven) and sewage (43). The classification of polluted waters was defined according to the faecal coliform concentration (Freitas *et al.* 1987).

Species and bio-sero-phagetype classification

The isolates were all assigned to species and biotypes as recommended in Aleksic and Bockemühl (1999). They were serotyped according to Wauters *et al.* (1991) and phagetyped according to Nicolle *et al.* (1976).

Drug resistance

All the isolates were tested for drug resistance using the disc diffusion technique (Bauer *et al.* 1966). The following antimicrobial drugs and concentrations were used: amikacin, 30 μg (Ami); chloramphenicol, 30 μg (Clo); cefoxitin, 30 μg (Cfo); cephalothin, 30 μg (Cfl); cefotaxime, 30 μg (Ctx); gentamicin, 10 μg (Gen); kanamycin, 30 μg (Kn); imipenem, 10 μg (Imp); tobramycin, 10 μg (Tob); cefazolin, 30 μg (Cfz); ampicillin, 10 μg (Amp); tetracycline, 30 μg (Tet); sulphamethoxazole-trimethropim, 25 μg (Sut).

Phenotypic virulence characteristics

The following tests were performed on all isolates, as described in the cited texts: temperature-dependent autoagglutination, salicin fermentation and esculin hydrolysis (Farmer *et al.* 1992), calcium-dependent growth and Congo-Red absorption on CR-Mox agar, both at 37°C (Riley and Toma 1989) and pyrazinamidase production (Kandolo and Wauters 1985).

Detection of the genes *inv*, *ail*, *yst* and *virF* by the PCR technique

These studies were performed on the isolates of types *Y. enterocolitica* 2/O:5,27/Xz (34 strains) and *Y. enterocolitica* 3/O:5,27/Xz (four strains). Genomic DNA was extracted as described by Harnett *et al.* (1996) and its concentration determined as in Sambrook *et al.* (1989). The general PCR procedure was performed according to the method of Saiki *et al.* (1988) using 2.0 U KlenTaq1^{TM} DNA polymerase (Ab Peptides, Inc., St Louis, MO, USA). The primers used and the number of base pairs in each product are displayed in Table 1. The PCR reactions conditions used for detection of the various genes were as described in the following: for *inv*, Rasmussen *et al.* (1994); for *ail*, Nakajima *et al.* (1992); for *yst*, Ibrahim *et al.* (1997); for *virF*, Wren and Tabaqchali (1990). The strains of *Y. enterocolitica* 7660 and 197, isolated from human diarrhoeic faeces and food respectively, contain all the tested genes and were used as positive controls. Reactions without DNA as a template or with genomic DNA of *Escherichia coli* were used as negative controls.

Table 1 Primers used to detect the *inv*, *ail*, *yst* and *virF* genes in *Y. enterocolitica*

Gene	Primer name	Sequence	bp
Inv	YC1	CTG TGG GGA GAG TGG GGA AGT TTG G	570
	YC2	GAA CTG CTT GAA TCC CTG AAA ACC G	
Ail	Ail1	ACT CGA TGA TAA CTG GGG AG	170
	Ail2	CCC CCA GTA ATC CAT AAA GG	
yst	Pr2a	A ATG CTG TCT TCA TTT GGA GCA	145
	Pr2c	ATC CCA ATC ACT ACT GAC TTC	
virF	VirF 1	TCA TGG CAG AAC AGC AGT CAG	590
	VirF 2	ACT CAT CTT ACC ATT AAG AAG	

Visualization of the amplified products

The PCR products were analysed by agarose gel electrophoresis and visualized by u.v. after staining with ethidium bromide (0·5 μg ml^{-1}).

RESULTS

The distribution of the 144 *Yersinia* strains in species, sources of isolation and origin is presented in Table 2. The *Yersinia* strains were classified as *Y. enterocolitica* (67 strains), *Y. intermedia* (64 strains), *Y. frederiksenii* (nine strains), *Y. kristensenii* (three strains) and untypable *Yersinia* (one strain).

Tables 3–5 present the strains of *Y. enterocolitica*, *Y. intermedia*, *Y. frederiksenii*, *Y kristenseii* and untyped *Yersinia*, respectively, grouped according to bio-sero-phagetype, resistance profile and source of isolation. All the *Yersinia* isolates were sensitive to amikacin, chloramphenicol, cefotaxime, tetracycline, gentamicin, kanamycin, tobramycin, imipenem, and sulphamethoxazole-trimethropim. In relation to the other drugs (cefoxitin, cephalothin, cefazolin and ampicillin), the sensitivity/resistance results were variable. None of the strains was sensitive or resistant to all the drugs tested.

The results of the phenotypic virulence tests show that four isolates, all of *Y. enterocolitica* biotype 3, serogroup O:5,27 and phagetype Xz, showed positive virulence in all these tests; 34 isolates, of *Y. enterocolitica* biotype 2, serogroup O:5,27 and phagetype Xz, were positive only for the biochemical pathogenic profile of pyrazinamidase production, esculin hydrolysis and salicin fermentation, while none of the other 112 strains showed virulence characteristics in any of these tests. The four 3/O:5,27/Xz *Y. enterocolitica* strains were also positive for the presence of *inv*, *ail* and *virF*, but only two of these strains were *yst* positive. All 34 *Y. enterocolitica* 2/O:5,27/Xz strains possessed *inv*, *ail* and *yst* but the *virF* gene was not identified in any of these strains. Table 6 presents the phenotypic virulence characteristics and the presence of the virulence genes in these 38 strains.

Table 2 Distribution of the 144 *Yersinia* isolates according to the species, source and origin

Yersinia species	No. of samples per species	Source	No. of Samples per source	Origin
Y. enterocolitica	67	Ocean	4	RJ
		Waterfall	3	RJ
		Polluted river	2	RJ
		Sewage	26	SP
		Fresh water	32	SP
Y. intermedia	64	Ocean	5	RJ
		Waterfall	2	RJ
		Polluted river	26	RJ
		Polluted estuary	6	RJ
		Polluted lagoon	2	RJ
		Fresh water	4	RJ
		Fresh water	2	SP
		Sewage	17	SP
Y. frederiksenii	9	Polluted lagoon	1	RJ
		Stream	2	RJ
		Spring	6	RJ
Y. kristensenii	3	Waterfall	1	RJ
		Polluted river	1	RJ
		Polluted estuary	1	RJ
Yersinia NT	1	Spring	1	RJ

RJ, Rio de Janeiro; SP, São Paulo; NT, not typed.

Table 3 Distribution of the 67 *Y. enterocolitica* strains isolated from water and sewage according to the source of isolation, bio-sero-phagetypes and resistance profiles

No. of strains	Source	Bio-sero-phagetypes	Resistance profiles
02	Polluted river	Ye 1A/O:5/Xz	Cfl, Amp
02	Water fall	Ye 1A/O:5/Xz	Cfl, Amp
20	Fresh water	Ye 2/O:5,27/Xz	Cfl, Amp
02	Sewage	Ye 2/O:5,27/Xz	Cfl, Amp
01	Fresh water	Ye 2/O:5,27/Xz	Amp
04	Fresh water	Ye 2/O:5,27/Xz	Cfo, Cfl, Amp
06	Fresh water	Ye 2/O:5,27/Xz	Cfo, Cfl, Cfz, Amp
01	Fresh water	Ye 2/O:5,27/Xz	Cfl, Cfz, Amp
01	Ocean	Ye 3/O:5,27/Xz	Cfl
01	Sewage	Ye 3/O:5,27/Xz	Cfl
02	Ocean	Ye 3/O:5,27/Xz	Cfo, Cfl
01	Waterfall	Ye 1A/O:10/Xo	Cfl
01	Ocean	Ye 1A/O:16/Xz	Amp
16	Sewage	Ye 1A/O:27/Xz	Cfl, Amp
04	Sewage	Ye 1A/O:27/Xz	Cfo, Cfl, Amp
02	Sewage	Ye 1A/O:27/Xz	Cfo, Cfl, Cfz, Amp
01	Sewage	Ye 1A/O:27/Xz	Cfo, Amp

Ye, *Y. enterocolitica*; Amp, ampicillin; Cfl, cephalothin; Cfo, cefoxitin; Cfz, cefazolin.

Table 4 Distribution of the 64 *Y. intermedia* strains isolated from water and sewage according to the source of isolation, bio-sero-phagetypes and resistance profiles

No. of strains	Source	Bio-sero-phagetypes	Resistance profiles
01	Fresh water	Yi 1/O:4,32/Xz	Cfl, Amp
01	Sewage	Yi 1/O:4,32/Xo	Cfo, Cfl, Cfz, Amp
01	Sewage	Yi 1/O:4,32/Xz	Cfo, Cfl, Amp
03	Sewage	Yi 4/O:4,32/Xz	Cfo, Cfl, Cfz, Amp
04	Sewage	Yi 4/O:4,32/Xz	Cfo, Cfl, Amp
01	Sewage	Yi 4/O:4,32/Xz	Cfl, Amp
01	Sewage	Yi 4/O:4,32/Xz	Cfo, Cfl
01	Polluted river	Yi 1/O:4,33/Xz	Cfo, Cfl, Cfz, Amp
01	Polluted lagoon	Yi 1/O:4,33/Xz	Cfo, Cfl, Cfz, Amp
04	Polluted river	Yi 1/O:4,33/Xz	Cfo, Cfl, Amp
01	Polluted lagoon	Yi 1/O:4,33/Xz	Cfo, Cfl, Amp
01	Ocean	Yi 2/O:4,33/Xz	Cfo, Cfl, Amp
01	Fresh water	Yi 4/O:7,8/Xz	Cfl, Amp
01	Ocean	Yi 1/O:10 Xz	Cfo, Cfl, Amp
01	Polluted river	Yi 1/O:10/Xo	Cfo, Cfl, Amp
01	Polluted river	Yi 1/O:10/Xo	Cfo, Cfl, Cfz, Amp
01	Fresh water	Yi 1/0:10/Xz	Cfo, Cfl, Cfz, Amp
01	Water fall	Yi 1/O:10/Xo	Cfo, Cfl, Cfz, Amp
01	Ocean	Yi 1/O:10/Xz	Cfo, Cfl, Cfz, Amp
01	Fresh water	Yi 1/O:13,7/Xo	Cfo, Cfl, Cfz, Amp
01	Fresh water	Yi 1/O:14/Xo	Cfo, Cfl
02	Polluted river	Yi 1/O:14/Xo	Cfo, Cfl, Cfz
03	Polluted river	Yi 1/O:14/Xo or Xz	Cfo, Cfl, Cfz, Amp
01	Polluted river	Yi 1/O:14/Xz	Cfl, Amp
01	Polluted river	Yi 1/O:15/Xz	Cfl, Cfz
02	Ocean	Yi 4/O:16/Xz	Cfo, Cfl, Amp
01	Polluted estuary	Yi 1/O:17/Xz	Cfl, Amp
01	Polluted estuary	Yi 1/O:18/Xz	Cfl, Amp
01	Fresh water	Yi 1/O:25/Xo	Cfl, Amp
01	Polluted river	Yi 1/O:25/Xo	Cfo, Cfl, Amp
01	Polluted estuary	Yi 1/O:33/Xz	Cfl
01	Waterfall	Yi 1/O:37/Xz	Cfo, Cfl, Cfz, Amp
02	Sewage	Yi 1/O:40/Xz	Cfl, Amp
03	Sewage	Yi 1/0:40/Xz or Xo	Cfo, Cfl, Cfz, Amp
01	Sewage	Yi 1/0:40/Xz	Cfl, Cfz, Amp
01	Polluted river	Yi 4/O:40/Xo	Cfo, Cfl, Cfz, Amp
02	Polluted river	Yi 1/O:48/Xo	Cfo, Cfl
01	Polluted estuary	Yi 1/O:48/Xz	Cfo, Cfl
03	Polluted river	Yi 1/O:48/Xz or Xo	Cfo, Cfl, Cfz, Amp
01	Polluted river	Yi 1/O:48/Xo	Cfl
03	Polluted river	Yi 1/O:48/Xz or Xo	Cfo, Cfl, Amp
01	Poluted river	Yi 1/O:66/Xz	Cfl
01	Estuary	Yi 1/NAG/Xz	Cfl, Cfz
01	Polluted estuary	Yi 1/NAG/Xz	Cfo, Cfl, Amp

Yi, *Y. intermedia*; Amp, ampicillin; Cfl, cephalothin; Cfo, cefoxitin; Cfz, cefazolin.

Table 5 Distribution of the nine *Y. frederiksenii*, three *Y. kristensenii* and one not typable *Yersinia* strains, isolated from water, according to the source of isolation, sero-phagetypes and resistance profiles

Yersinia species	No. of strains/source	Sero-phagetypes	Resistance profiles
Y. frederiksenii	01 Polluted lagoon	Yf O:2,3/Xz	Cfo, Cfl, Cfz, Amp
	02 Strean	Yf O:10/Xo	Cfo, Cfl, Cfz, Amp
	01 Spring	Yf O:10/Xz	Cfo, Cfl, Cfz, Amp
	03 Spring	Yf O:16/Xo	Cfo, Cfl, Amp
	01 Spring	Yf O:16/Xz	Cfo, Cfl, Cfz, Amp
	01 Polluted estuary	Yf O:16/Xz	Cfl, Amp
Y. kristensenii	01 Polluted river	Yk O:11,24/Xo	Cfl
	01 Waterfall	Yk O:61/Xz	Cfl
	01 Polluted estuary	Yk O:61/Xo	Cfl
Yersinia NT	01 Spring	Y NT O:10/Xz	Cfo, Cfl, Cfz, Amp

NT, not typable; Amp, ampicillin; Cfl, cephalothin; Cfo, cefoxitin; Cfz, cefazolin.

Table 6 Phenotypic virulence characteristics and PCR results for the presence of *inv*, *ail*, *yst* and *virF* virulence genes in the 144 *Yersinia* strains isolated from water and sewage

	Bacterial species/positive strains		
	Y. enterocolitica		Other *Yersinia*
Phenotypic tests/virulence genes	2/O:5,27 *3/O:5,27*	Others*	(Yi, Yf, Yk, Ya, YNT)
Expression of plasmidial genes			
Autoagglutination at 37°C	4	–	–
Ca^{++} dependency at 37°C	4	–	–
Congo Red absorption	4	–	–
Other phenotypic tests			
Esculin hydrolysis	38	–	–
Salicin fermentation	38	–	–
Pyrazinamidase production	38	–	–
Presence of virulence genes			
inv	38	nt	nt
ail	38	nt	nt
yst	36	nt	nt
virF	04	nt	nt

Yi, *Y. intermedia*; Yf, *Y. frederiksenii*; Yk, *Y. kristensenii*; YNT, *Yersinia* not typable; nt, not tested.
*Others, bioserogroups of *Y. enterocolitica* other than 2/O:5,27 and 3/O:5,27.

DISCUSSION

Nonpathogenic bio-serogroups of *Y. enterocolitica*, *Y. intermedia*, *Y. kristensenii* and *Y. frederiksenii* have not yet been clearly demonstrated to cause human diseases although they have been isolated from humans worldwide, including persons with gastrointestinal disorders, which may suggest that these species and *Y. enterocolitica* 1A may not be as

harmless as they were previously thought to be (Sulakvelidze 2000). The majority of these species are found in the environment. They have been isolated from freshwater sources such as rivers, lakes (Massa *et al.* 1988) and drinking water (Langeland 1983; Kuznetsov and Timchenko 1998). They have also been isolated from sewage (Ruhle *et al.* 1990; Ziegert and Diesterweg 1990).

It is interesting to note that, of the 11 known species of *Yersinia*, only four (*Y. enterocolitica*, *Y. intermedia*, *Y. frederiksenii* and *Y. kristensenii*) were isolated from water sources and sewage in Brazil (Table 2). The relatedness between the untypable strain, found in spring water, and the other species of *Yersinia* will be the subject of further study.

It is important to emphasize that 56·7% (38 strains) of the *Y. enterocolitica* isolates in this study belong to biotypes and serogroups with variable degrees of clinical and epidemiological significance: 34 of these isolates were *Y. enterocolitica* 2/O:5,27 and four *Y. enterocolitica* 3/O:5,27 (Table 3). Most primary pathogenic strains of human and domestic animals occur within biotypes 1B serogroup O:4,32, O:8, O:13a, O:13b, O:18, O:20, O:21, O:41, 42; biotype 2 serogroups, O:5,27, O:9; biotype 3 serogroups O:1,2,3, O:5,27; biotype 4 serogroup O:3 and biotype 5 serogroup O:2,3 (Bottone 1999). The other 43·3% (29 strains) of the *Y. enterocolitica* isolates were classified as *Y. enterocolitica* biotype 1A with various serogroups (O:5, O:10, O:16, O:27) that are not related to human diseases but are commonly obtained from terrestrial and freshwater ecosystems (Robins-Browne 2001). However, there is growing epidemiological and experimental evidence to suggest that some biotype 1A of *Y. enterocolitica* isolated from humans can cause disease (Tennant *et al.* 2003). These authors suggest that there may be two subgroups of biotype 1A of *Y. enterocolitica*: pathogenic strains of clinical origin and nonpathogenic strains that occur in the environment.

All of the 144 *Yersinia* isolates, except 38 strains of *Y. enterocolitica*, showed negative virulence behaviour in the phenotypic virulence tests. Four positive isolates were *Y. enterocolitica* biotype 3, serogroup O:5,27 and phagetype Xz and 34 strains were classified as biotype 2, serogroup O:5,27 and phagetype Xz, these serotypes being related to human and animal diseases (Bottone 1999).

The four *Y. enterocolitica* 3/O:5,27/Xz strains were positive for the phenotypic virulence tests of autoagglutination, calcium dependency at 37°C and Congo-Red absorption and had a pathogenic biochemical profile when tested for pyrazinamidase production, esculin hydrolysis and salicin fermentation (Farmer *et al.* 1992). These strains were also positive for the presence of the chromosomal genes *inv* and *ail*, related to invasion, and for *virF*, located on the virulence plasmid, pYV (Salyers and Whitt 2002). However, only two of these four strains were *yst* positive. The presence of the gene *inv*, which occurs in all *Yersinia* (but expresses invasin only in pathogenic strains), confirms the genus *Yersinia*. The gene *ail* shows an isolate to be virulent, as it occurs only in pathogenic strains (Carniel 1995). The presence of the gene *virF* confirms that of the virulence plasmid, which is essential for bacterial pathogenesis (Cornelis 1994). Three of these strains were isolated from ocean water in the city of Rio de Janeiro and the fourth from sewage, in the city of São Paulo, at different times. Additionally, two of those from the ocean, were also positive for the presence of the gene *yst* related to the production of the ST enterotoxin (Yst), which is largely restricted to the classical pathogenic biotypes of *Y. enterocolitica* (Delor *et al.* 1990). Other toxins that resemble Yst in terms of heat stability and reactivity in infant mice, such as Yst-b and Yst-c, have been described in *Yersinia* species, but their role in the pathogenic process is unknown (Robins-Browne 2001). Because of this, the presence of *yst*-b and -c genes were not investigated in this study.

The 34 strains of *Y. enterocolitica* biotype 2, serogroup O:5,27 and phagetype Xz, were negative for auto-agglutination, Ca^{+2}-dependence at 37°C and did not absorb the Congo Red dye, but were positive for the other phenotypic virulence markers tested. All were isolated in São Paulo from water and sewage. These strains were also positive for the presence of *inv*, *ail* and *yst* genes and negative for the *virF* gene when tested by PCR. These results confirm the phenotypic tests and suggest that probably these strains lost the plasmid in collection.

It is difficult to make a correlation between the strains isolated in São Paulo and Rio de Janeiro and their different sources, because they were sent in by different groups (Freitas *et al.* 1987; Martins, personal communication) to our Reference Lab for complete characterization. However, they are representative of water quality in Brazil.

The presence of strains carrying important virulence markers is totally undesirable in the ocean water used as a recreational area by a large number of people, including poor, malnourished slum-dwellers and occasionally people with suppressed immunity. In addition, the presence of these strains in the sewage shows unsatisfactory treatment of sewage, which may lead, for instance, to future contamination of other water reservoirs used for human consumption or in crop irrigation. It is also possible that the sewage may contaminate the ocean. This should be a warning that sanitary control measures have to be taken.

The absence of classical virulence markers in strains of *Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii* and *Y. enterocolitica* biovar 1A led to them being considered possibly nonpathogenic (Robins-Browne 2001; Floccari *et al.* 2003). However, these strains may be using other virulence factors not yet characterized, as these so-called 'nonpathogenic' species have been isolated from people with gastrointestinal disorders (Morris *et al.* 1991; Burnens *et al.*

1996; Sulakvelidze 2000; Robins-Browne 2001) and exhibited multiple drug resistance in the present work (Tables 3–5). Regarding the epidemiology of these so-called environmental strains, this study produced some interesting results. The predominating serogroups of *Y. frederiksenii* are said to be O:16, O:1 and O:2, while those of *Y. kristensenii* are O:12, O:28 and O:11 (Sulakvelidze 2000). Finally, for *Y. intermedia*, serogroups O:4 and O:17 appear to predominate; however, as most *Y. intermedia* strains are not typable by Wauter's serotyping scheme, the actual predominant serogroup is unclear (Sulakvelidze 2000). In our research the serogroup O:4,32 was the most prevalent among the 64 *Y. intermedia* strains studied, corresponding to 18·75% (12 strains) of the total (Table 4). The next most prevalent serogroups among *Y. intermedia* in this work were O:10 (five strains), O:48 (five strains), O:14 (four strains) and O:40 (four strains). Just one O:17 *Y. intermedia* was isolated, and no O:4 (Table 4). Among the nine *Y. frederiksenii* studied, five were O:16, said to be one of the most prevalent *Y. frederiksenii* serogroups (Sulakvelidze 2000). Regarding the three *Y. kristensenii* studied, none possessed the supposedly most common serogroups; however, the number of these strains was too low to draw any conclusions from this. The question whether Brazil has a different epidemiology from other countries, in respect of the most prevalent serogroups of *Y. intermedia*, *Y. frederiksenii* and *Y. kristensenii*, should be further studied. Furthermore, the genetic relatedness between these strains and the same species isolated from sick humans, as well as its correlation with pathogenic bioserogroups of *Y. enterocolitica*, would be an interesting topic for research.

The results of antimicrobial resistance showed that 50% of strains presented multiple drug resistance: 27% of the *Y. enterocolitica*, 70·3% of *Y. intermedia*, 88·9% of *Y. frederiksenii* and the untypable *Yersinia* strain were resistant to three or more drugs. The other strains and the *Y. kristensenii* isolates were sensitive to one or two drugs. We would like to point out that the four 3/O:5,27/Xz *Y. enterocolitica* strains, positive for the virulence markers tested, did not show multiple drug resistance, while a large number of the other strains did show multiple drug resistance, including some 2/O:5,27/Xz *Y. enterocolitica*.

The resistance profile of the strains analysed here is in agreement with others studies (Stock and Wiedemann 1999, 2003; Tzelepi *et al.* 1999; White *et al.* 2002). All strains were sensitive to amikacin, chloramphenicol, cefotaxime, tetracycline, gentamicin, kanamycin, tobramycin, a carbapenen (imipenem), and to sulphamethoxazole-trimethropim. Resistance to ampicillin and numerous cephalosporins was common to the strains of *Y. enterocolitica*, *Y. intermedia* and *Y. frederiksenii*. In fact, the expression of A and B types of β-lactamase enzymes is characteristically associated with these *Yersinia* species (Stock *et al.* 1999, 2000; Stock and Wiedemann 1999, 2003; Tzelepi *et al.* 1999). Thus, we can speculate that the *Yersinia* strains analysed here also express these enzymes. However, further studies are necessary to confirm this hypothesis.

The three strains of *Y. kristensenii* exhibited resistance only to cephalothin. In fact, in a recent study, the only aminopenicillin-sensitive strains detected belong to the *Y. kristensenii* group (Stock and Wiedemann 2003).

The occurrence of virulence markers in strains of *Y. enterocolitica* that belong to biotypes and serogroups related to human diseases, isolated from water and sewage, shows that the environment can be responsible for human infection with *Y. enterocolitica* in Brazil. In addition, the existence of environmental *Yersinia* strains resistant to drugs used in human therapy suggests that these strains could be a source of resistance markers that may be transmitted to pathogenic strains present in the environment, by lateral gene transfer. Finally, currently available data suggest that the role of *Y. enterocolitica* 1A, as well as *Y. intermedia*, *Y. frederiksenii* and *Y. kristensenii*, as possible disease-causing agents, should not be disregarded.

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