J. Med. Microbiol. — Vol. 48 (1999), 157–160

ANTIMICROBIAL SUSCEPTIBILITY

Antibiotic susceptibilities of *Yersinia enterocolitica* and *Y. intermedia* isolates from aquatic environments

E. TZELEPI, M. ARVANITIDOU*, A. MAVROIDI and A. TSAKRIS†

*Department of Bacteriology, Hellenic Institute Pasteur, Athens and Departments of *Hygiene and †Microbiology, Medical School, Aristotelian University of Thessaloniki, Greece*

Of 37 *Yersinia* isolates from various aquatic environments, seven were *Y. enterocolitica* and 30 *Y. intermedia*. These isolates were biotyped, serotyped and tested for their susceptibility to 20 antibiotics. All *Y. enterocolitica* isolates were of biovar 1; those of *Y. intermedia* were distributed amongst four biovars (1, 2, 4 and 6). On the basis of combined biotyping and serotyping results, *Y. enterocolitica* isolates were distributed in five and *Y. intermedia* in 17 groups. With the exception of one *Y. enterocolitica* isolate which was resistant to tetracycline and streptomycin, the isolates were sensitive to the non-β-lactam antibiotics. In contrast, various patterns of β-lactam insensitivity were detected, including ampicillin and ticarcillin (35 isolates), cephalothin (33 isolates), carbenicillin (32 isolates), amoxycillin/clavulanate (23 isolates) and cefoxitin (22 isolates). No correlation between biotype or serotype and the susceptibility pattern of the isolates was apparent. Both inducible cephalosporinase activity against third-generation cephalosporins and inhibition of resistance to penicillins were detected in all *Y. enterocolitica* and *Y. intermedia* isolates by double-disk tests.

Introduction

Yersiniosis is related to various clinical syndromes, mainly caused by *Yersinia enterocolitica*, although all *Yersinia* species have been isolated from clinical specimens [1]. The micro-organism is usually transmitted by ingestion of contaminated food. Water supplies may also contribute to the dissemination of the disease. *Yersinia* strains are highly adaptable to aquatic environments, having minimal nutrient requirements and being capable of surviving in cold water for long periods [2]. Thus, surface water contaminated by sewage discharges may be a significant vehicle for transmission of human yersiniosis.

Antimicrobial susceptibilities have been documented sporadically for *Yersinia* strains of human, animal or food origin [3–6]. In these reports, yersiniae were usually found to be susceptible to most antibiotics, although a considerable number of strains showed decreased susceptibility to some older β-lactams. Published studies on β-lactamases focus on *Y. enterocolitica*, where two distinct chromosomal enzymes (named A and B) have been characterised [7–9]. However, there is a paucity of data concerning antibiotic susceptibility among strains of *Yersinia* isolated from aquatic environments. Furthermore, reports on susceptibility profiles of the previously named '*Y. enterocolitica*-like' species, and in particular of *Y. intermedia*, are rare.

This study reports the occurrence of *Y. enterocolitica* and *Y. intermedia* in aquatic environments of Greece. Data on the susceptibility of the isolates to a wide range of antibiotics are also presented.

Materials and methods

Samples and isolation procedures

Of 37 *Yersinia* isolates from samples taken from various aquatic environments in Northern Greece, nine came from three distinct bathing areas (Thessaloniki and Platamon in the Thermaikos Gulf, and Marmaras in the Toroneos Gulf, Chalkidiki), seven from river water samples (from the rivers Axios, Aliakmon and Strimon), 18 from drinking water samples in various areas and three were from mussels harvested from sea-water.

Yersinia spp. were isolated from water samples as

Received 16 Feb. 1998; revised version received 6 May 1998; accepted 22 June 1998.
Corresponding author: Dr A. Tsakris.

described previously [10]. Isolation of yersiniae from mussels involved homogenisation of mussels (30–40 g) in sterile distilled water (120–160 ml) in a Vir Tis blender at *c.* 50 000 rpm for 1 min. The blended mussel suspension (50 ml) was mixed with an equal volume of double-strength *Yersinia* enrichment medium and incubated at 4°C for 21 days. One loopful of this cold mixture was inoculated on to Cefsulodin-Irgasan-Novobiocin (CIN) Agar (Oxoid) and incubated at 32°C overnight. Examination of the suspected colonies and identification of the *Yersinia* isolates was performed as for the water samples. Biotyping and serotyping based on somatic antigens were performed by H. H. Mollaret (National Centre of Yersiniae, Institute Pasteur, Paris, France).

Antibiotic sensitivity testing

Yersinia isolates were tested for their susceptibility to 20 antimicrobial agents: ampicillin, ticarcillin, amoxycillin/clavulanate, piperacillin, carbenicillin, cephalothin, cefoxitin, cefuroxime, ceftazidime, cefotaxime, aztreonam, imipenem, streptomycin, spectinomycin, gentamicin, tobramycin, amikacin, tetracycline, chloramphenicol and co-trimoxazole. Susceptibility tests were performed at 28°C by a standard disk diffusion method, with Mueller-Hinton agar and antibiotic disks from Diagnostics Pasteur (Marnes La Coquette, France). Results were recorded by measuring the inhibition zones and scored as sensitive, intermediate susceptibility and resistant according to the NCCLS recommendations [11]. *Escherichia coli* ATCC 25922 was used as a reference strain.

Screening for induction and inhibition of β-lactamase expression

To detect inducible cephalosporinase activity, a double-disk antagonism test was performed as described by Sanders *et al.* [12]. A disk of imipenem (30 μg) was placed near to disks containing ceftazidime (30 μg) and cefotaxime (30 μg). Cephalosporinase activity was reflected by a decrease in inhibition radius around the disks of third-generation cephalosporins placed next to the disk of imipenem, which is a stronger inducer. Inhibition of penicillinase activity was detected by a double-disk synergy test, as described by Jarlier *et al.* [13]. A disk of amoxycillin + clavulanate (20 μg + 10 μg) was placed near disks of carbenicillin (100 μg), ampicillin (10 μg) and ticarcillin (75 μg) [13]. Synergy between the disk containing the β-lactamase inhibitor and those containing penicillins revealed penicillinase activity.

Results

Of 37 *Yersinia* isolates from the aquatic samples examined, 30 were identified as *Y. intermedia* and seven as *Y. enterocolitica*. Of the nine isolates from sea-water, seven were identified as *Y. intermedia* and two as *Y. enterocolitica*. All seven isolates from rivers were *Y. intermedia*. Of the isolates from drinking water, 15 were *Y. intermedia* and three *Y. enterocolitica*, while two *Y. enterocolitica* and one *Y. intermedia* were isolated from mussels. All *Y. enterocolitica* isolates belonged to biovar 1, whereas *Y. intermedia* isolates belonged to four different biovars with biovar 2 being the most common (12 isolates). When biotyping and serotyping were considered in combination, *Y. enterocolitica* isolates were distributed in five groups and *Y. intermedia* isolates in 17 groups (Table 1).

All the isolates were susceptible to the non-β-lactam antibiotics tested, with the exception of one *Y.*

Table 1. Characteristics of seven *Y. enterocolitica* and 30 *Y. intermedia* isolates

Species	Insensitivity to antibiotics*	Number of strains	Biovars/serotypes
Y. enterocolitica	Amp Tic Amc Cb Cth Fox Sm Tc	1	1/6,31
	Amp Tic Amc Cb Cth Fox	3	1/41,42,43
			1/5
			1/5
	Amp Tic Cb Cth	1	1/5
	Amp Tic Amc Cth Fox	1	1/na
	Cb Cth Fox	1	1/47
Y. intermedia	Amp Tic Cb	3	2/na; 4/40; 4/na
	Amp Tic Cb Cth	8	1/17; 1/na†; 2/4, 16; 2/37†; 2/pa; 4/4
	Amp Tic Amc Cb Cth Fox	15	1/4; 1/4, 32, 16-16; 1/17; 1/37; 1/na†; 2/3; 2/35, 36; 2/37†; 2/49, 51; 4/4†; 4/28-49, 51; 6/17
	Amp Tic Amc Cth	1	2/37
	Amp Tic Amc Cth Fox	1	4/4
	Amp Tic Amc	1	2/35, 36
	Cth	1	1/37

*The term insensitivity was used to include resistance and intermediate susceptibility. Antibiotics: Amp, ampicillin; Tic, ticarcillin; Amc, amoxycillin+clavulanate; Cb, carbenicillin, Cth, cephalothin; Fox, cefoxitin; Sm, streptomycin; Tc, tetracycline; na, non-agglutinable; pa, polyagglutinable.

†Type exhibited by two strains in this group.

Table 2. Susceptibility* of seven *Y. enterocolitica* and 30 *Y. intermedia* isolates to 12 β-lactam antibiotics

	Y. enterocolitica			*Y. intermedia*		
Antimicrobial agent	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Ampicillin	1	0	6	1	18	11
Amoxycillin/clavulanate	2	0	5	12	16	2
Ticarcillin	1	0	6	1	18	11
Piperacillin	7	0	0	30	0	0
Carbenicillin	1	2	4	4	1	25
Cephalothin	0	2	5	4	6	20
Cefoxitin	1	5	1	14	14	2
Cefuroxime	7	0	0	30	0	0
Ceftazidime	7	0	0	30	0	0
Cefotaxime	7	0	0	30	0	0
Aztreonam	7	0	0	30	0	0
Imipenem	7	0	0	30	0	0

*Susceptibility status was defined according to NCCLS criteria [11].

enterocolitica isolate which was resistant to tetracycline and streptomycin. The results of susceptibility testing for β-lactam agents are presented in Tables 1 and 2. Marked antibacterial activity was shown with piperacillin, as well as with third generation cephalosporins (ceftazidime and cefotaxime), aztreonam and imipenem. Of the second-generation cephalosporins tested, cefuroxime was active against all the isolates, whereas cefoxitin exhibited mostly moderate activity. The majority of the isolates were either resistant or intermediately susceptible to the older β-lactam antibiotics (ampicillin, ticarcillin, carbenicillin and cephalothin); most of them were also insensitive to the amoxycillin/clavulanate combination. No correlation between biotype or serotype and the susceptibility pattern of the isolates was apparent. However, in double-disk tests, inhibition of penicillinase activity between amoxycillin/clavulanate and ampicillin or carbencillin or ticarcillin disks was visible in all cases, even in one isolate that was sensitive to all penicillins. Also, induction of resistance to third-generation cephalosporins by imipenem was always present.

Discussion

Yersinia spp. have emerged as pathogens associated with various infections in man, but little is known about the occurrence and antimicrobial susceptibility of strains encountered in aquatic environments. It appears that *Y. intermedia* is the predominant species isolated from aquatic samples in Northern Greece; *Y. enterocolitica* was found less frequently. This is in contrast to previous findings from Italy, reporting *Y. enterocolitica* as the prevalent species isolated from river water [14]. Furthermore, unlike the latter study, other neighbouring species, such as *Y. frederiksenii* and *Y. kristensenii*, were not identified in the study region. It is of interest that none of the isolates was in a biogroup or serogroup that has traditionally been associated with human disease. However, *Y. enterocolitica* biotype 1, serotype O:5, one of the prevalent subgroups in the present study, has previously been associated with gastrointestinal infection [15], while there is an increasing body of data suggesting that strains outside standard pathogenic groups or species can cause illness in man [1, 16].

Susceptibility results showed a very low prevalence of resistance to non-β-lactam antibiotics among *Yersinia* isolates. This finding is in agreement with those of other authors [4, 6, 17]. It has been proposed that low resistance rates reflect the poor ability of *Yersinia* spp. to act as recipients in naturally occurring bacterial conjugation [4]. With respect to β-lactam antibiotics, most of the isolates were insensitive to the older β-lactam agents, including carbenicillin, and considerable numbers of them were also insensitive to the amoxycillin/clavulanate combination and to cefoxitin. It has been stated that strains of less human-related serotypes may be more frequently susceptible to ampicillin and cephalothin [3, 5]. Nevertheless, the results of the present study did not differ essentially from those reported by other authors, who examined yersiniae isolated from foods as well as from human and animal infections. In those studies, almost all *Y. enterocolitica* biovar 1 and *Y. intermedia* strains were resistant to ampicillin, cephalothin and cefoxitin [4, 6].

In *Y. enterocolitica*, resistance to penicillins and older cephalosporins has been attributed to the production of β-lactamases [5]. Cornelis and Abraham [7] studied the β-lactamases produced by a number of *Y. enterocolitica* strains of human and animal origin extensively and characterised two chromosomally mediated enzymes – a constitutive penicillinase named enzyme A and an inducible cephalosporinase they named enzyme B. Pham *et al.* [8] also studied β-lactamases of *Y. enterocolitica* isolates from Australia and found that biotype 1 strains produced both β-lactamases, in contrast with strains of biotypes 3 and 4 which were found to possess only one type of β-lactamase. However, there are no reports describing β-lactamases in *Y. intermedia*. Results from the double-disk tests in the present study indicate that strains in

this species also produce β-lactamases of both types. Induction of resistance to third-generation cephalosporins by imipenem indicated the presence of inducible cephalosporinases in all the *Y. intermedia* and *Y. enterocolitica* isolates in the present study. Similarly, inhibition of growth between clavulanate-containing disks and disks of penicillins indicated that all strains might also produce penicillinases.

In a previous report, differences in susceptibility to β-lactam agents within the same *Y. enterocolitica* biotype were found, when isolates came from distinct countries [9]. Isolates belonging to the same biovar and serotype but differing in β-lactam susceptibility phenotypes were also found in the present study in both *Yersinia* species examined. The majority of the isolates exhibited broad patterns of insensitivity, including resistance to both penicillins and cephalosporins. This is in agreement with previous studies, in which the simultaneous presence of a penicillinase and a cephalosporinase has been related to cross-resistance to penicillins, amoxycillin/clavulanate, cephalothin and cefoxitin [7, 8]. Resistance to cefoxitin has previously been found only in clinical *Y. enterocolitica* strains of biovar 1. [17]. Interestingly, all but one of the *Y. enterocolitica* and more than half of the *Y. intermedia* isolates in this study were found to be insensitive to cefoxitin. These observations suggest that the aquatic environment may contribute to the spread of yersiniae expressing resistance to various β-lactam antibiotics.

References

1. Sulakvelidze A, Dalakishvili K, Barry E *et al.* Analysis of clinical and environmental *Yersinia* isolates in the republic of Georgia. *J Clin Microbiol* 1996; **34**: 2325–2327.
2. Schiemann DA. *Yersinia enterocolitica* in drinking water. In: McFeters GA (ed) Drinking water microbiology. Brock/Springer Series in Contemporary Bioscience. New York, Springer Verlag. 1990: 322–339.
3. Juhlin I, Winblad S. Susceptibility to mecillinam and other antibiotics of 28 O-serotypes of *Yersinia enterocolitica*. *J Antimicrob Chemother* 1981; **8**: 291–297.
4. Ahmedy A, Vidon DJ-M, Delmas CL, Lett M-C. Antimicrobial susceptibilities of food-isolated strains of *Yersinia enterocolitca*, *Y. intermedia*, *Y. frederiksenii*, and *Y. kristensenii*. *Antimicrob Agents Chemother* 1985; **28**: 351–353.
5. Hornstein MJ, Jupeau AM, Scavizzi MR, Philippon AM, Grimont PAD. In-vitro susceptibilities of 126 clinical isolates of *Yersinia enterocolitica* to 21 β-lactam antibiotics. *Antimicrob Agents Chemother* 1985; **27**: 806–811.
6. Preston MA, Brown S, Borczyk AA, Riley G, Krishnan C. Antimicrobial susceptibility of pathogenic *Yersinia enterocolitica* isolated in Canada from 1972 to 1990. *Antimicrob Agents Chemother* 1994: **38**: 2121–2124.
7. Cornelis G, Abraham EP. β-Lactamases from *Yersinia enterocolitica*. *J Gen Microbiol* 1975; **87**: 273–284.
8. Pham JN, Bell SM, Lanzarone JYM. A study of the β-lactamases of 100 clinical isolates of *Yersinia enterocolitica*. *J Antimicrob Chemother* 1991; **28**: 19–24.
9. Pham JN, Bell SM, Hardy MJ, Martin L, Guiyoule A, Carniel E. Susceptibility to β-lactam agents of *Yersinia enterocolitica* biotype 4, serotype O:3 isolated in various parts of the world. *J Med Microbiol* 1995; **43**: 9–13.
10. Arvanitidou M, Stathopoulos GA, Constantinidis TC, Katsouyannopoulos V. The occurrence of *Salmonella, Campylobacter* and *Yersinia* spp. in river and lake waters. *Microbiol Res* 1995; **150**: 153–158.
11. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disc Susceptibility Tests; M2A2. Villanova, PA, NCCLS. 1992.
12. Sanders CC, Sanders WE, Goering RV. In vitro antagonism of beta-lactam antibiotics by cefoxitin. *Antimicrob Agents Chemother* 1982; **21**: 968–975.
13. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; **10**: 867–878.
14. Massa S, Cesaroni D, Poda G, Trovatelli LD. Isolation of *Yersinia enterocolitica* and related species from river water. *Zentralbl Microbiol* 1988; **143**: 575–581.
15. Ratnam S, Mercer E, Picco B, Parsons S, Butler R. A nosocomial outbreak of diarrheal disease due to *Yersinia enterocolitica* serotype O:5, biotype 1. *Infect Dis* 1982; **145**: 242–247.
16. Bissett ML, Powers C, Abbot SL, Janda JM. Epidemiologic investigations of *Yersinia enterocolitica* and related species: sources, frequency, and serogroup distribution. *J Clin Microbiol* 1990; **28**: 910–912.
17. Pham JN, Bell SM, Lanzarone JYM. Biotype and antibiotic sensitivity of 100 clinical isolates of *Yersinia enterocolitica*. *J Antimicrob Chemother* 1991; **28**: 13–18.