habitat to meet the genetic, population and ecosystem level objectives. Further, it has been emphasized that this practice should be at a scale large enough to buffer the disturbances because the undercanopy plants of old growth forests (like Cedar) require a considerably long disturbance-free period (as in the case of *Taxus*). It can be concluded that the Himalayan *Taxus* is under threat not only because of excessive harvesting but also due to degradation of forest sites due to other reasons. Biotic disturbances are apparent at the present study site but other factors like chilling temperatures, direct sunlight, warm and dry summer may also contribute to poor regeneration and subsequent recruitment processes.

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In vitro antibiotic susceptibilities of *Yersinia enterocolitica* and *Yersinia intermedia* isolated from sewage effluents

Indrajit Sinha and J. S. Virdi*

Department of Microbiology, University of Delhi South Campus, Benito Juarez Road, New Delhi 110 021, India

Of the 31 strains comprising nineteen *Yersinia enterocolitica* (biotype 1A) and twelve *Yersinia intermedia* (biotypes 1, 2 and 4) isolated from sewage effluents, most (94-97%) were resistant to β-lactam antibiotics. The β-lactam antibiotic to which maximum number of isolates were sensitive was cefotaxime. With regard to susceptibility of isolates to non-β-lactam antibiotics, none was found resistant to amikacin, ciprofloxacin, cotrimoxazole and tetracycline. However, a varying pattern of sensitivity was observed with other non-β-lactams such as chloramphenicol, erythromycin, sulphafurazole, clindamycin, tobramycin and trimethoprim. In comparison to earlier reports, differences were observed in the antimicrobial susceptibilities of these isolates to ceftazidime, cefuroxime and piperacillin.

Yersinia enterocolitica, an important food- and water-borne enteric pathogen, causes acute gastroenteritis, enterocolitis and mesenteric lymphadenitis as well as a variety of extraintestinal disorders[1]. *Y. enterocolitica* has been isolated from a variety of zoonotic and environmental sources. However, pigs and water have generally been regarded as the most important reservoirs of this organism. *Y. enterocolitica* is highly adaptable to aquatic environment and is capable of surviving in water for longer periods[2]. Many water-borne outbreaks of yersiniosis have been documented[2]. A number of studies have reported the antimicrobial susceptibilities of *Yersinia* strains isolated from humans[3–5], animals[6,7] and food[6,8]. There is paucity of information about the antibiotic susceptibilities of *Y. enterocolitica* of aquatic origin. Hausnerova *et al.*[9] studied 64 strains of *Y. enterocolitica* isolated from river water and fish, and found these to be sensitive to chloramphenicol, tetracycline, neomycin and colistin. Recently, Tzelepi *et al.*[10] reported antibiotic susceptibilities of seven *Y. enterocolitica* and thirty *Y. intermedia* strains isolated from river and drinking water, and mussels harvested from sea water; these strains were found to be resistant to β-lactam antibiotics, viz. ampicillin, carbenicillin, ticarcillin, cephalothin, amoxycillin/clavulanate and cefoxitin. All strains, however, were sensitive to non-β-lactam antibiotics such as amikacin, tetracycline, chloramphenicol, gentamycin, tobramycin and cotrimoxazole. Further studies on isolates obtained from other aquatic sources and from different parts of the world would help in judiciously assessing the over-all susceptibilities of these isolates. Among the various aquatic sources, sewage and sewage effluents constitute an important source of *Y. enterocolitica* and other related species like *Y. intermedia*[11,12]. Their discharge in surface waters may play a significant role in the transmission of human yersiniosis. To the best of our knowledge, there is no report on the antibiotic susceptibilities of *Y. enterocolitica* and *Y. intermedia* isolated from the Indian subcontinent. Here we present the results of a study concerning *in vitro* antibiotic suscepti-

*For correspondence. (e-mail: micro@dusc.ernet.in)

Table 1. *In vitro* antibiotic susceptibilities of 31 isolates of *Yersinia enterocolitica* and *Yersinia intermedia* isolated from sewage effluents

Antibiotic	Disk content (μg)	Number (percentage)		
		Sensitive	Intermediate	Resistant
β-lactam				
Amoxycillin	10	2(6)	–	29(94)
Ampicillin	10	2(6)	–	29(94)
Carbenicillin	100	0(0)	1(3)	30(97)
Ceftazidime	10	0(0)	5(16)	26(84)
Cefuroxime	30	0(0)	3(10)	28(90)
Cephadroxil	30	0(0)	1(3)	30(97)
Cephalexin	30	0(0)	2(6)	29(94)
Cephalothin	30	1(3)	–	30(97)
Cefotaxime	10	20(64)	2(6)	9(30)
Cephradine	25	0(0)	4(13)	27(87)
Methicillin	5	4(13)	–	27(87)
Piperacillin	30	4(13)	7(23)	20(64)
Ticarcillin	75	6(20)	2(6)	23(74)
Non-β-lactam				
Amikacin	30	16(52)	15(48)	0(0)
Chloramphenicol	30	25(80)	3(10)	3(10)
Ciprofloxacin	1	25(80)	6(20)	0(0)
Clindamycin	2	0(0)	–	31(100)
Colistin	10	12(39)	7(23)	12(39)
Cotrimoxazole	25	28(90)	3(10)	0(0)
Erythromycin	5	3(10)	8(26)	20(64)
Gentamycin	10	22(70)	6(20)	3(10)
Nalidixic acid	30	13(42)	9(29)	9(29)
Neomycin	30	8(26)	15(48)	8(26)
Netillin	10	14(45)	13(42)	4(13)
Nitrofurantoin	50	13(42)	15(48)	3(7)
Rifamycin	5	7(23)	–	24(77)
Sulphafurazole	300	8(26)	–	23(74)
Sulphamethoxazole	25	8(26)	5(16)	18(58)
Tetracycline	10	24(77)	7(23)	0(0)
Tobramycin	10	12(39)	8(26)	11(35)
Trimethoprim	2.5	16(52)	10(32)	5(16)

Yersinia enterocolitica (All biotype-1A): Serotype (no.) – O:6,30-6,31(7); O:6,31(1); O:10-34(5); O:15(1); O:41,42(1); O:41,43(1); NAG(3).
Yersinia intermedia: Biotype (no.) – 4(9); 2(1); 1(2).

bilities of *Y. enterocolitica* (19 isolates) and *Y. intermedia* (12 isolates) isolated from sewage effluents in New Delhi.

Yersinia strains were isolated from sewage effluents as follows. Effluent samples were collected from five sewage-treatment plants located at different sites covering the entire metropolitan area of Delhi. Effluent samples of 25–30 ml collected from the final settling tank was inoculated to 10-fold volume of phosphate-buffered saline containing sorbitol and bile salts[13] and kept at 4°C for 3 weeks. After this period, a loopful (0.04 ml) of the sample was treated with 0.1 ml of 0.5% KOH in 0.5% sodium chloride for 30 s (ref. 14). Cold-enriched and KOH-treated samples were plated on CIN (Cefsulodin–Irgasan–Novobiocin) and MacConkey agar medium (Himedia Laboratories, India). The plates were incubated at 25–26°C for 24–48 h. Colonies, which had combined features of characteristic bull's eye morphology on CIN agar and flat NLF (non-lactose fermenting) growth on MacConkey agar, were subjected to detailed biochemical characterization[15]. Isolates identified as belonging to genus *Yersinia* were biotyped and serotyped at Institut Pasteur, Paris or Central Public Health Laboratory Service (CPHL), Colindale (London) and have been provided with accession numbers of the respective laboratories.

Table 2. Multiple resistance of thirty one isolates of *Yersinia* spp.

Group	Number of isolates resistant (%)	
	Five or more antibiotics	Seven or more antibiotics
β-lactam	31 (100)	31 (100)
Non-*β*-lactam	19 (61)	11 (35)

Antibiotic susceptibility testing of bacterial strains was performed at 35°C by the agar diffusion method according to the procedure of Bauer *et al.*[16]. The antibiotic concentrations were chosen according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS)[17] and manufacturers of disks. The zone of inhibition was measured with slipping calipers. NCCLS susceptibility criteria were used to interpret the strains as resistant, sensitive or intermediate. The anti-

biotic-impregnated disks and Mueller–Hinton medium were obtained from Himedia Laboratories, India.

A total of 31 strains of *Yersinia* spp. were isolated from June 1997 to November 1998. These were identified as *Y. enterocolitica* biotype 1A (19 isolates) and *Y. intermedia* biotypes 1, 2 and 4 (12 isolates). When analysed separately, no difference in antibiotic susceptibilities of *Y. enterocolitica* and *Y. intermedia* was observed. Thus, the combined results are shown in Table 1. Most of the isolates (94–97%) were resistant to older β-lactam antibiotics such as ampicillin, carbenicillin and cephalothin. Except for two isolates of *Y. intermedia*, all were resistant to amoxycillin too. The β-lactam antibiotic to which maximum number of isolates were sensitive was cefotaxime. Each isolate was analysed for multiple resistance and the results are shown in Table 2. Each isolate was resistant to several (≥ 7) β-lactam antibiotics studied indicating a high degree of resistance to these antibiotics. Contrary to this, fewer isolates showed multiple resistance to non-β-lactam antibiotics. Though, in general, the results are similar to those reported by other investigators[10,18], an unexpected finding of our study was the resistance of several isolates to ceftazidime, cefuroxime and piperacillin. These results are different from those reported by Tzelepi *et al.*[10] who found all their aquatic isolates uniformly sensitive to these antibiotics. This may be due to differences in the type of aquatic sources from which *Yersinia* were obtained in the two studies. Isolates in our study were obtained from sewage effluents while those of Tzelepi *et al.*[10] were from various aquatic environments consisting of river, drinking and sea water. It has been reported that chlorination of sewage effluents may modulate the antibiotic susceptibility profile of bacteria including *Y. enterocolitica*[19]. Although sewage effluents in India are not subjected to chlorination, the presence in effluents, of residual amounts of chlorine used for disinfection of water before distribution cannot be ruled out. In this respect, it would also be of interest to study the production of inducible cephalosporinases and constitutive β-lactamases by our isolates as changes in their expression may account for the observed differences. With regards to non-β-lactams, no isolate showed resistance to amikacin, ciprofloxacin, cotrimoxazole and tetracycline although some isolates did show intermediate zone of inhibition against these antibiotics. In addition, varying number of isolates showed insensitivity against other non-β-lactam antibiotics tested, viz. chloramphenicol, erythromycin, sulphafurazole, clindamycin, tobramycin and trimethoprim. In this regard, our results are broadly similar to those reported earlier[10,18].

Antibiotic sensitivity pattern, which appeared to be biotype-specific, has been reported for clinical isolates of *Y. enterocolitica*[3]. In the present study, although all isolates of *Y. enterocolitica* were of biotype 1A, these were represented by several serotypes. Apparently, no relationship between serotype and antibiotic susceptibility could be discerned. This is in agreement with findings about *Yersinia* spp. isolated from other aquatic sources[10].

This study shows antibiotic susceptibilities of *Y. enterocolitica* and *Y. intermedia* isolated from sewage effluents in India. Barring a few differences such as those seen with ceftazidime, piperacillin and cefuroxime, the findings are similar to those reported from other parts of the world. Nevertheless, it would be of interest to understand the reasons for the observed resistance of our isolates to ceftazidime, a third generation cephalosporin and also to cefuroxime and piperacillin. It may highlight the effect of the nature of the aquatic environment (sewage vs surface waters) or the geographical origin of the isolates on the antibiotic susceptibilities of yersiniae.

The occurrence of resistance to antibiotics in aquatic isolates that represent environmental reservoirs may have direct consequences for the treatment of disease in humans[20].

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