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Natural antibiotic susceptibility and biochemical profiles of *Yersinia enterocolitica*-like strains: *Y. bercovieri, Y. mollaretii, Y. aldovae* and '*Y. ruckeri*'

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The natural susceptibility of 54 *Yersinia enterocolitica*-like strains of *Y. bercovieri* (formerly *Y. enterocolitica* biovar 3B, n = 17), *Y. mollaretii* (formerly *Y. enterocolitica* biovar 3A, n = 12), *Y. aldovae* (formerly *Y. enterocolitica*-like group X2, n = 10) and '*Y. ruckeri*' (n = 15) was tested to 69 antibiotics. MIC values were determined with a microdilution procedure in IsoSensitest broth for all strains and in cation-adjusted Mueller Hinton broth for some strains. All yersiniae tested showed uniform MIC distributions to most antibiotics and were naturally sensitive or intermediate to aminoglycosides, several cephalosporins, and penicillins, carbapenems, aztreonam, quinolones, tetracyclines, antifolates, chloramphenicol and nitrofurantoin, and naturally resistant to benzylpenicillin, oxacillin, all macrolides except azithromycin, lincosamides, streptogramins, glycopeptides, rifampicin and fusidic acid. Significant differences in susceptibility affecting clinical assessment criteria were seen with aminopenicillins (in the presence and absence of β-lactamase inhibitors), some cephalosporins (e.g., cefoxitin) and fosfomycin. Whereas strains of *Y. aldovae* and '*Y. ruckeri*' were naturally sensitive or intermediate to amoxicillin and amoxicillin/clavulanate, strains of *Y. bercovieri* and *Y. mollaretii* were naturally resistant or naturally resistant or intermediate, respectively. Strains of the two latter species were also highly susceptible to fosfomycin. These data can be valuable for the validation of routine susceptibility test results. β-Lactam MICs suggest that *Y. bercovieri* and *Y. mollaretii* strains express chromosomally encoded AmpC β-lactamases and that most *Y. aldovae* and '*Y. ruckeri*' strains express no, or only small amounts, of enzyme. An evaluation of 30 biochemical tests that determined phenotypic identification to the *Yersinia* species level is presented.

Introduction

Yersinia enterocolitica is a well-known human pathogen that causes various gastrointestinal and systemic syndromes [1]. It has been shown previously that several strains previously labelled as *Y. enterocolitica* or *Y. enterocolitica*-like were distinct species. Today eight 'new' *Yersinia* species are known which were primarily assigned to the *Y. enterocolitica* complex. Although the clinical significance of these species has been discussed controversially, there is strong evidence that they are implicated in human disease. Most of the 'new' species can be found in water, soil and different animals, but all have been isolated from human clinical samples [2, 3]. Moreover, in recent years virulence markers have been found in these 'non-pathogenic' *Yersinia* spp. [4–9] and some strains of several species cause human disease [2, 10, 11]. There is evidence that failure to detect 'environmental' strains acting as agents of human infections is because of their biochemical similarity. Most commercially available identification systems are unable to identify 'non-pathogenic' *Yersinia* spp. to the species level; in most cases they are misidentified as *Y. enterocolitica* [12]. Because there is only little information on the antibiotic susceptibility patterns of the 'new' *Yersinia* spp., this study focused on the antibiotic susceptibility of four of them. *Y. mollaretii* and *Y. bercovieri* have been described most recently [13] and were formerly called *Y. enterocolitica* biovars 3A and 3B, respectively. Strains of the latter species produce a novel heat-stable enterotoxin [8], and some *Y. mollaretii* strains also display enterotoxin activity [7]. *Y. aldovae* strains were formerly designated *Y. enterocolitica*-like group X2 [14]. They, like *Y. mollaretii* and *Y. bercovieri* have been isolated from human faeces [3, 13]. Some isolates of *Y. aldovae* also

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produce enterotoxin [5]. '*Y. ruckeri*', one of the 'classical' agents of red mouth disease in salmon and trout, was shown to be taxonomically distinct from *Yersinia* spp. [15–17]. Strains of this species have been found occasionally in human patients [2, 3].

The aim of the study was to establish a database for the natural susceptibility to a wide range of antibiotics of *Y. bercovieri*, *Y. mollaretii*, *Y. aldovae* and '*Y. ruckeri*' strains and determine whether there are differences in natural susceptibility, which could be valuable for the validation of routine susceptibility test results and might contribute to the identification of these bacteria.

Material and methods

Bacterial strains

A total of 54 *Yersinia* strains was examined, of which 33 strains – *Y. aldovae* (8), *Y. bercovieri* (10), *Y. mollaretii* (11) and '*Y. ruckeri*' (4) were kindly provided by Heinrich Neubauer, Munich, and came from the Hygiene-Institut of Hamburg, Germany and included isolates from clinical specimens, mammals, aquatic sources and different soils. Three further '*Y. ruckeri*' strains from the culture collection of A. Rodloff, Leipzig, were isolated from clinical sources. Five strains of *Y. bercovieri* from human specimens were kindly provided by Marisa Dolina, Lugano, Switzerland (3) and Gerda Stempfel, Weingarten, Germany (2). *Y. aldovae* ATCC 35236 (isolated from water, Czech Republic), *Y. bercovieri* ATCC 43970 (isolated from human faeces, USA) and *Y. mollaretii* ATCC 43969 (isolated from soil, France) were obtained from the American Type Culture Collection (Rockville, MD, USA). *Y. aldovae* ATCC 35237 (isolated from fish, USA), *Y. bercovieri* CCUG 26330 (isolated from food, France), '*Y. ruckeri*' ATCC 29473 and CCUG 21537 (isolated from rainbow trout in France and the Czech Republic) were provided by the Culture Collection of the University of Göteborg, Sweden. Most of six further '*Y. ruckeri*' strains from the USA were from rainbow trout; they were obtained from the Bacteria Culture Collection in Gent, Belgium. *Escherichia coli* ATCC 25922 and *Y. pseudotuberculosis* ATCC 29833 served as controls for antibiotic susceptibility testing.

Identification

Strains were identified to the genus level with a commercial identification system for Enterobacteriaceae (Micronaut-E, Merlin-Diagnostika, Bornheim, Germany). The inoculum for the test reactions was a suspension in physiological saline solution at 1×10^6 cfu/ml from an overnight culture on IsoSensitest Agar (Oxoid). Identification to species level was by conventional tests with discriminating features of *Yersinia* spp. [2, 18, 19]. Sugar fermentation tests were performed in plates on bromcresol-purple-agar (Difco Laboratories, Detroit, MI, USA) supplemented to 0.5% with one of the following sugars: cellobiose, fucose, α-methyl-D-glucoside, lactose, maltose, melibiose, raffinose, and sorbose (all Fluka Chemie, Buchs, Switzerland). Salicin fermentation was tested in Salicin broth (Fluka Chemie) with salicin 0.5%. All tests were incubated at 28°C and read after 24 h. Tube and plate tests were also read after 48 h and 7 days.

Antibiotics and antibiotic susceptibility testing

Antibiotic susceptibility was tested with a microdilution procedure in IsoSensitest Broth (Oxoid). Five strains of each species were also tested in cation-adjusted Mueller Hinton Broth (CAMHB; Difco). After inoculation of antibiotic-containing microtitration plates (Merlin-Diagnostika) with 100 μl of bacterial suspension, $(3–7) \times 10^5$ cfu/ml, and incubation for 22 h at 37°C, MIC values were determined with a photometer for microtitration plates (Labystems Multiscan Multisoft, Helsinki, Finland). MIC data were evaluated with EXCEL (Microsoft). All antibiotics were kindly provided by the manufacturers to Merlin-Diagnostika who produced the antibiotic-containing plates.

Evaluation of natural antibiotic susceptibility

The evaluation of natural antibiotic susceptibility was performed as described previously [20–22]. Clinical breakpoints for apramycin, lividomycin A and ribostamycin were defined as proposed recently [23].

Results

Identification

The identification of all the strains submitted was confirmed to both the genus and the species level. In most cases the data were in agreement with the literature. However, for several tests (β-galactosidase, ornithine decarboxylase, urease, citrate assimilation, fermentation of myo-inositol, rhamnose and xylose), and some species, the percentages of positive reactions were significantly higher than the data of Farmer [19] (Table 1). Surprisingly, the test results of the Voges Proskauer reaction and the fermentation results of cellobiose, melibiose and lactose for strains of *Y. aldovae* disagreed with the data of Neubauer *et al.* [18], but were similar to the data of Farmer [19] (Table 1).

Antibiotic susceptibility, natural sensitivities and resistances

An overview of the antibiotic susceptibilities of the strains is shown in Table 2. Natural antibiotic sensitivities and resistances are summarised in Table 3.

Although there were some species-related differences in susceptibility, all species were naturally sensitive or

Table 1. Biochemical features of the *Yersinia* strains tested

Biochemical test	Test procedure	Positive reactions (% of strains)													
		Y. bercovieri (n = 17)			*Y. mollaretii* (n = 12)			*Y. aldovae* (n = 10)			'*Y. ruckeri*' (n = 15)			*Y. enterocolitica*	
		I	II	III	**I**	II	III	**I**	II	III	**I**	II	III	II	III
Amino acid deaminase	MCN-E	0	0	0	0	0	0	0	0	0	0	0	0	0	0[b]
Arginine dihydrolase	MCN-E	0	0	0	0	0	0	10	0	0	6	0	5	0	0[b]
β-Galactosidase[1]	MCN-E	88	73	80	100	100	20	30	0	0	100	100	50	57–99[a]	95[b]
β-Glucuronidase[2]	MCN-E	0	0	NS	0	0	NS	0	0	NS	0	0	NS	0	NS
β-Glucosidase[3]	MCN-E	24	18	20	25	25	0	0	0	0	0	0	0	0–100[a]	25[b]
H_2S production	MCN-E	0	0	0	0	0	0	0	0	0	0	0	0	0	0[b]
Lysine decarboxylase	MCN-E	0	9	0	0	0	0	10	0	0	19	5	50	0–8[a]	0[b]
Ornithine decarboxylase	MCN-E	94	100	80	92	88	80	90	100	40	100	100	100	0–100[a]	95[b]
Tryptophanase[4]	MCN-E	0	0	0	0	0	0	0	0	0	0	0	0	0–100[a]	50[b]
Urease	MCN-E	**100**	100	60	**100**	100	20	**100**	100	60	**0**	0	0	100	75[b]
Voges Proskauer reaction	MCN-E	0	0	0	0	13	0	0	67	0	0	0	10	0–46[a]	2[b]
β-Xylosidase[5]	MCN-E	0	0	NS	0	0	NS	0	0	NS	0	0	NS	0	NS
Assimilation of															
citrate	MCN-E	0	0	0	42	25	0	80	33	0	13	0	0	0–13[a]	0[b]
malonate	MCN-E	0	0	0	0	0	0	0	0	0	0	0	0	4–50[a]	0[b]
Fermentation of															
adonitol	MCN-E	0	0	0	0	0	0	0	0	0	0	0	0	0	0[b]
cellobiose	Plate test	**94**[c]	99	100	**83**	75	100	0	100	0	**0**	0	5	70–100[a]	75[b]
fucose	Plate test	**82**[c]	NS	⩾90	**0**	NS	⩽10	**60**[c]	NS	V	0	NS	NS	NS	V[b]
glucose	MCN-E	100	100	100	100	100	100	100	100	100	100	100	100	100	100[b]
α-methyl-D-glucoside	Plate test	0	NS	0	0	NS	0	0	NS	0	0	NS	0	NS	0[b]
(myo)-inositol	MCN-E	0	0	0	67	38	0	60	67	0	0	0	0	20–92[a]	30[b]
lactose	Plate test	6	27	20	0[d]	0	40	0[e]	100	0	0	0	0	0–71[a]	5[b]
maltose	Plate test	**100**	NS	100	**83**[c]	NS	60	0	NS	0	**100**	NS	95	NS	75[b]
melibiose	Plate test	0	0	0	0	25	0	10	100	0	0	0	0	0–10[a]	1[b]
raffinose	Plate test	0	0	0	0	0	0	0	0	0	0	0	5	0–8[a]	5[b]
rhamnose	MCN-E	0	0	0	0	0	0	60	100	0	0	0	0	0–8[a]	1[b]
salicin	Tube test	12[f]	18	20	0	38	20	0	0	0	0	0	0	0–100[a]	20[b]
sorbitol	MCN-E	**100**	100	100	**100**	100	100	**100**	100	60	**0**	0	50	29–100[a]	100[b]
sorbose	Plate test	**0**	0	⩽10	**100**	99	⩾90	**0**	0	⩽10	**0**	0	NS	57–100[a]	V[b]
sucrose	MCN-E	**100**	100	100	**100**	100	100	**40**	33	20	**0**	0	0	43–100[a]	95[b]
D-xylose	MCN-E	**100**	100	100	**83**	75	60	**80**	100	40	**0**	0	0	10–100[a]	70[b]

[a]Results depend on the biovar; [b]data without consideration of the respective biovars; [c]100, [d]92, [e]10, [f]18% positive strains after 48 h; V, variable (percentages not given). The results (I) were read after 24 h at 28°C and are contrasted to the data of Neubauer *et al.* (II) [18] and Farmer (III) [19]; fucose and sorbose reactions are compared to the data of Brenner (III) [2]. Key discriminating reactions are given in bold print. Cleavage of [1]ortho-nitro-phenyl-β-galactopyranoside (ONPG); [2]para-nitrophenyl-β-glucuronide (PGUR), [5]ortho-nitrophenyl-β-D-xylo-pyranoside (ONPX); [3]hydrolysis of aesculin; [4]indole production; NS, not stated. As reference, identification results of *Y. enterocolitica* were included. Please note differences in testing conditions (see *Discussion*).

Table 2. Antibiotic susceptibility of *Y. bercovieri, Y. mollaretii, Y. aldovae* and '*Y. ruckeri*' strains

Antibiotic	Concentrations examined (mg/L)	Species	Number of strains with MIC (mg/L) of																
			0.01	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Tetracyclines																			
Tetracycline	0.03–64	*Y. bercovieri*						2	12	3									
		Y. mollaretii						1	5	6									
		Y. aldovae						3	3	3	1								
		'*Y. ruckeri*'						2	9	4									
Doxycycline	0.03–64	*Y. bercovieri*					6	11											
		Y. mollaretii					2	9	1										
		Y. aldovae					3	4	2	1									
		'*Y. ruckeri*'					3	12											
Minocycline	0.03–64	*Y. bercovieri*				8	9												
		Y. mollareti				3	7	2											
		Y. aldovae			1	1	6	1		1									
		'*Y. ruckeri*'				1	11	3											
Aminoglycosides																			
Amikacin	0.13–256	*Y. bercovieri*					15	2											
		Y. mollaretii				3	5	4											
		Y. aldovae				1	4	2	2	1									
		'*Y. ruckeri*'					1	6	6	2									
Gentamicin	0.06–128	*Y. bercovieri*			9	8													
		Y. mollareti			5	5	2												
		Y. aldovae			3	3	2	1	1										
		'*Y. ruckeri*'			1	3	7	4											
Netilmicin	0.06–128	*Y. bercovieri*			9	5	3												
		Y. mollaretii			5	5	2												
		Y. aldovae			3	2	3	2											
		'*Y. ruckeri*'			1	3	6	4	1										
Tobramycin	0.06–128	*Y. bercovieri*			11	6													
		Y. mollaretii			6	4	2												
		Y. aldovae			3	2	3	2											
		'*Y. ruckeri*'				5	7	2	1										
Streptomycin	0.13–256	*Y. bercovieri*					2	11	4										
		Y. mollaretii				1	2	4	3	2									
		Y. aldovae					3	2	2	2		1							
		'*Y. ruckeri*'						1	6	7	1								
Kanamycin	0.13–256	*Y. bercovieri*				1	10	5	1										
		Y. mollaretii				3	4	4	1										
		Y. aldovae				1	2	4	2	1									
		'*Y. ruckeri*'						5	7	3									
Neomycin	0.13–256	*Y. bercovieri*				11	6												
		Y. mollaretii				10		2											
		Y. aldovae				3	2	4	1										
		'*Y. ruckeri*'				1	9	5											
Spectinomycin	0.13–256	*Y. bercovieri*								3	13	1							
		Y. mollaretii								3	8	1							
		Y. aldovae							1	2	4	2		1					
		'*Y. ruckeri*'								1	9	5							

Antibiotic	Concentrations examined (mg/L)	Species	Number of strains with MIC (mg/L) of: 0.01	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Apramycin	0.06–128	*Y. bercovieri*					5	11	1										
		Y. mollaretii				1	3	7	1										
		Y. aldovae					1	2	5	2									
		'*Y. ruckeri*'						1	5	8	1								
Ribostamycin	0.06–128	*Y. bercovieri*					1	15	1										
		Y. mollaretii				2	2	3	1	2	2								
		Y. aldovae					1	4	1	3		1							
		'*Y. ruckeri*'						4	2	7	2								
Lividomycin A	0.06–128	*Y. bercovieri*						6	11										
		Y. mollaretii				1	2	3	4	2									
		Y. aldovae						2	3	3	2								
		'*Y. ruckeri*'							2	6	6	1							
***β*-Lactams: Penicillins**																			
Benzylpenicillin	0.01–32	*Y. bercovieri*											1	6	10				
		Y. mollaretii											4	7	1				
		Y. aldovae									3	3	3	1					
		'*Y. ruckeri*'									7	8							
Oxacillin	0.03–64	*Y. bercovieri*								1	1	2	13						
		Y. mollaretii									5		7						
		Y. aldovae		4							1	3	1	1					
		'*Y. ruckeri*'		2								1	12						
Amoxicillin	0.06–128	*Y. bercovieri*											3	5	7	2			
		Y. mollaretii										2	3	7					
		Y. aldovae							2	4	3		1						
		'*Y. ruckeri*'						2	6	7									
Amoxicillin/clavulanic acid	0.06–128	*Y. bercovieri*											3	5	5	4			
		Y. mollaretii										4	8						
		Y. aldovae						1	4	2	2	1							
		'*Y. ruckeri*'					1	2	7	5									
Ampicillin/sulbactam	0.06–128	*Y. bercovieri*								2	4	8	3						
		Y. mollaretii								4	8								
		Y. aldovae					1	4	3	2									
		'*Y. ruckeri*'					1	11	3										
Piperacillin	0.13-256	*Y. bercovieri*							5	6	6								
		Y. mollaretii						2	3	6	1								
		Y. aldovae				5	3	1	1										
		'*Y. ruckeri*'				4	10	1											
Piperacillin/tazobactam	0.13–256	*Y. bercovieri*				1	4	12											
		Y. mollaretii				4	5	3											
		Y. aldovae				7	3												
		'*Y. ruckeri*'				8	6	1											
Ticarcillin	0.13–256	*Y. bercovieri*							4	6	5	2							
		Y. mollaretii							6	5	1								
		Y. aldovae				1	1	4	3	1									
		'*Y. ruckeri*'				4	9	2											

continued overleaf

Table 2. (*continued*)

Antibiotic	Concentrations examined (mg/L)	Species	Number of strains with MIC (mg/L) of																
			0.01	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Mezlocillin	0.13–256	*Y. bercovieri*						2	6	5	4								
		Y. mollaretii					1	4	3	4									
		Y. aldovae				3	3	2	2										
		'*Y. ruckeri*'					1	4	6	3	1								
Azlocillin	0.25–512	*Y. bercovieri*								3	5	5	4						
		Y. mollaretii							1	3	2	6							
		Y. aldovae						2	6	1	1								
		'*Y. ruckeri*'					2	3	10										
***β*-Lactams: Cephalosporins**																			
Cefaclor	0.13–256	*Y. bercovieri*								3	7	4	3						
		Y. mollaretii							1	4	4	3							
		Y. aldovae						2	2	4	1		1						
		'*Y. ruckeri*'						4	10	1									
Cefazoline	0.13–256	*Y. bercovieri*							1	7	8		1						
		Y. mollaretii							4	8									
		Y. aldovae				1	2	2	4			1							
		'*Y. ruckeri*'						1	12	2									
Loracarbef	0.13–256	*Y. bercovieri*							2	4	7	4							
		Y. mollaretii						1	2	3	5	1							
		Y. aldovae				1		2	5	1		1							
		'*Y. ruckeri*'					4	10	1										
Cefuroxime	0.03–64	*Y. bercovieri*							9	8									
		Y. mollaretii						3	3	6									
		Y. aldovae				1	3	4		2									
		'*Y. ruckeri*'						4	10	1									
Cefotiam	0.03–64	*Y. bercovieri*			4	11	2												
		Y. mollaretii		2	4	6													
		Y. aldovae			4	5			1										
		'*Y. ruckeri*'			1	3	6	5											
Cefoxitin	0.03–64	*Y. bercovieri*										4	7	6					
		Y. mollaretii							6	5	1								
		Y aldovae					1	5	2	2									
		'*Y. ruckeri*'						2	11	2									
Cefixim	0.03–64	*Y. bercovieri*						2	4	7	4								
		Y. mollaretii						3	1	4	3		1						
		Y aldovae				2	3	4		1									
		'*Y. ruckeri*'	/			1	1	7	6										
Cefpodoxime	0.03–64	*Y. bercovieri*						3	6	8									
		Y. mollaretii						2	3	5		2							
		Y aldovae				3	3	3	1										
		'*Y. ruckeri*'			1	7	6	1											
Cefdinir	0.03–64	*Y. bercovieri*					4	5	8										
		Y. mollaretii				3	5	3			1								
		Y aldovae			2	5	2		1										
		'*Y. ruckeri*'				5	7	3											

Antibiotic	Concentrations examined (mg/L)	Species	Number of strains with MIC (mg/L) of																
			0.01	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Cefoperazone	0.03–64	*Y. bercovieri*					3	5	9										
		Y. mollaretii				1	3	3	5										
		Y aldovae				2	7	1											
		'*Y. ruckeri*'		1	1	5	7	1											
Cefotaxime	0.03–64	*Y. bercovieri*		1	5	9	2												
		Y. mollaretii		2	3	6	1												
		Y aldovae			2	5	2		1										
		'*Y. ruckeri*'		15															
Ceftibutene	0.03–64	*Y. bercovieri*				1	6	8	2										
		Y. mollaretii				3	2	5	1		1								
		Y aldovae		1	1	1	2	4		1									
		'*Y. ruckeri*'		1	6	6	2												
Ceftriaxone	0.03–64	*Y. bercovieri*		16	1														
		Y. mollaretii		12															
		Y aldovae		9	1														
		'*Y. ruckeri*'		15															
Ceftazidime	0.03–64	*Y. bercovieri*				3	12	2											
		Y. mollaretii			1	3	3	5											
		Y aldovae		1	4	4	1												
		'*Y. ruckeri*'		15															
Cefepime	0.03–64	*Y.bercovieri*		16	1														
		Y. mollaretii		12															
		Y. aldovae		10															
		'*Y. ruckeri*		15															
***β*-Lactams: Carbapenems**																			
Imipenem	0.03–64	*Y. bercovieri*			6	10	1												
		Y. mollaretii			4	8													
		Y aldovae		2	2	6													
		'*Y. ruckeri*'			13	2													
Meropenem	0.03–64	*Y. bercovieri*		17															
		Y. mollaretii		12															
		Y aldovae		10															
		'*Y. ruckeri*'		10	2	1	2												
***β*-Lactams: Monobactams**																			
Aztreonam	0.03–64	*Y. bercovieri*	1		9	6	1												
		Y. mollaretii		4	3	5													
		Y aldovae		7	2		1												
		'*Y. ruckeri*'		15															
Quinolones																			
Ciprofloxacin	0.01–32	*Y. bercovieri*	13	4															
		Y. mollaretii	11	1															
		Y aldovae	10																
		'*Y. ruckeri*'	15																
Sparfloxacin	0.01–32	*Y. bercovieri*	15	2															
		Y. mollaretii	11	1															
		Y aldovae	10																
		'*Y. ruckeri*'	15																

continued overleaf

Table 2. (*continued*)

Antibiotic	Concentrations examined (mg/L)	Species	Number of strains with MIC (mg/L) of																
			0.01	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Norfloxacin	0.03–64	*Y. bercovieri*		9	8														
		Y. mollaretii		6	5		1												
		Y aldovae		6	2	1	1												
		'*Y. ruckeri*'		14	1														
Ofloxacin	0.01–32	*Y. bercovieri*	2	8	7														
		Y. mollaretii	2	8	1	1													
		Y aldovae	3	5	2														
		'*Y. ruckeri*'	13	2															
Enoxacin	0.01–32	*Y. bercovieri*	1	1	6	9													
		Y. mollaretii			4	7		1											
		Y aldovae	1	1	3	5													
		'*Y. ruckeri*'	3	10	2														
Fleroxacin	0.01–32	*Y. bercovieri*	1	10	6														
		Y. mollaretii	1	6	4		1												
		Y aldovae	2	6	1	1													
		'*Y. ruckeri*'	12	3															
Pefloxacin	0.01–32	*Y. bercovieri*	2	11	4														
		Y. mollaretii	1	5	5	1													
		Y aldovae	2	4	3	1													
		'*Y. ruckeri*'	10	5															
Pipemidic acid	0.06–128	*Y. bercovieri*						4	11	2									
		Y. mollaretii						1	9	1			1						
		Y aldovae						1	7	2									
		'*Y. ruckeri*'						12	2	1									
Macrolides																			
Erythromycin	0.03–64	*Y. bercovieri*										2	9	6					
		Y. mollaretii											1	9	2				
		Y aldovae										1	3	5	1				
		'*Y. ruckeri*'										1	6	5	3				
Roxithromycin	0.03–64	*Y. bercovieri*										2		2	12	1			
		Y. mollaretii												1	4	7			
		Y aldovae											1	3	3	3			
		'*Y. ruckeri*'												1	3	11			
Clarithromycin	0.03–64	*Y. bercovieri*									3	4	7	3					
		Y. mollaretii										2	2	8					
		Y aldovae									2	2	5		1				
		'*Y. ruckeri*'										1	3	8	3				
Azithromycin	0.03–64	*Y. bercovieri*							2	8	7								
		Y. mollaretii							1	3	7	1							
		Y aldovae						1	2	3	3	1							
		'*Y. ruckeri*'						1	5	5	4								
Lincosamides																			
Lincomycin	0.01–32	*Y. bercovieri*													17				
		Y. mollaretii													12				
		Y aldovae											1		9				
		'*Y. ruckeri*'												2	13				

Antibiotic	Concentrations examined (mg/L)	Species	Number of strains with MIC (mg/L) of																
			0.01	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Clindamycin	0.01–32	*Y. bercovieri*									2	14	1						
		Y. mollaretii										1	3	6	2				
		Y aldovae							1		2	5	1	1					
		'*Y. ruckeri*'										5	8	2					
Streptogramins																			
Dalfopristin	0.03–64	*Y. bercovieri*											1	1	11	4			
		Y. mollaretii													1	11			
		Y aldovae													6	3			
		'*Y. ruckeri*'											1		1	14			
Quinupristin	0.03–64	*Y. bercovieri*														17			
		Y. mollaretii														12			
		Y aldovae														10			
		'*Y. ruckeri*'														15			
Dalfopristin/quinupristin (Synercid)	0.03–64	*Y. bercovieri*											2	3	11	1			
		Y. mollaretii													3	9			
		Y aldovae										1		2	7				
		'*Y. ruckeri*'													9	6			
Antifolates																			
Sulfamethoxazole	0.25–512	*Y. bercovieri*										2	6	9					
		Y. mollaretii									3	4	5						
		Y aldovae										1	1	3	4	1			
		'*Y. ruckeri*'								2	4	5	3	1					
Trimethoprim	0.03–64	*Y. bercovieri*				1	9	7											
		Y. mollaretii				2	4	5	1										
		Y aldovae				2	4	3	1										
		'*Y. ruckeri*'			2	9	4												
Trimethoprim/sulfamethoxazole (Co-trimoxazole)	0.13–256	*Y. bercovieri*						1	6	10									
		Y. mollaretii						5	6	1									
		Y aldovae						1	5	4									
		'*Y. ruckeri*'					4	7	4										
Glycopeptides																			
Teicoplanin	0.06–128	*Y. bercovieri*															17		
		Y. mollaretii															12		
		Y aldovae														2	8		
		'*Y. ruckeri*'															15		
Vancomycin	0.03–64	*Y. bercovieri*														17			
		Y. mollaretii														12			
		Y aldovae													2	8			
		'*Y. ruckeri*'														15			
Other antibiotics																			
Chloramphenicol	0.06–128	*Y. bercovieri*							1	2	6	8							
		Y. mollaretii								3	3	5	1						
		Y aldovae							4	3	3								
		'*Y. ruckeri*'						1	8	6									

continued overleaf

Table 2. (*continued*)

Antibiotic	Concentrations examined (mg/L)	Species	Number of strains with MIC (mg/L) of																
			0.01	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Nitrofurantoin	0.13–256	*Y. bercovieri*											8	9					
		Y. mollaretii											1	10	1				
		Y aldovae										2	6	2					
		'*Y. ruckeri*'									1	2	12						
Rifampicin	0.01–32	*Y. bercovieri*									5	11	1						
		Y. mollaretii							1		3	8							
		Y aldovae									1	5	4						
		'*Y. ruckeri*'								1	1	12	1						
Fosfomycin	0.13–256	*Y. bercovieri*					5	7	5										
		Y. mollaretii					3	6	3										
		Y aldovae									2	5	3						
		'*Y. ruckeri*'								4	3	4	3	1					
Fusidic acid	0.01–32	*Y. bercovieri*							1				2	11	3				
		Y. mollaretii													12				
		Y aldovae											4	3	3				
		'*Y. ruckeri*'					1						1	6	7				

The number of strains with the corresponding MIC value is cited. Strains in the column for the lowest concentration of the antibiotic (c_{min}) have MICs $\leqslant$ this lowest concentration (MIC $= c_{min} \rightarrow$ MIC $\leqslant c_{min}$). MICs higher than the highest concentration tested were assigned to two times the highest concentration which was tested. MIC values in shaded areas indicate the clinically intermediate area according to the German standard (DIN). A thick black line indicates the breakpoint between clinically sensitive and clinically resistant strains, if the 'intermediate' does not apply. If the DIN criteria for an antibiotic are not applicable, other standards were employed. NCCLS breakpoints were used for spectinomycin, cefdinir, dalfopristin, quinupristin, dalfopristin/ quinupristin, sulfamethoxazole and teicoplanin; French standards were utilised for streptomycin, kanamycin, neomycin, pefloxacin, lincomycin and fosfomycin and Swedish criteria for roxithromycin, clarithromycin, rifampicin and fusidic acid. UK breakpoints were used for trimethoprim. Breakpoints for apramycin, ribostamycin and lividomycin A were defined as proposed recently [23].

Table 3. The natural susceptibility of *Y. bercovieri*, *Y. mollaretii*, *Y. aldovae* and '*Y. ruckeri*' strains to antibiotics according to the standards in Table 2

Antibiotic		Species	Naturally sensitive	Naturally intermediate	Naturally resistant
Tetracyclines	Tetracycline	All species			
	Doxycycline, minocycline	All species			
Aminoglycosides	All tested aminoglycosides	All species			
Penicillins	Benzylpenicillin, oxacillin	All species			
	Amoxicillin	*Y. bercovieri*			
		Y. mollaretii			
		Y. aldovae			
		'*Y. ruckeri*'			
	Amoxicillin/clavulanate	*Y. bercovieri*			
		Y. mollaretii			
		Y. aldovae			
		'*Y. ruckeri*'			
	Ampicillin/sulbactam	*Y. bercovieri*			
		Y. mollaretii			
		Y. aldovae, 'Y. ruckeri'			
	Azlocillin	*Y. bercovieri*			
		All species except *Y. bercovieri*			
	All further tested penicillins	All species			
Cephalosporins	Cefoxitin	*Y. bercovieri*			
		All species except *Y. bercovieri*			
	Cefaclor	*Y. bercovieri, Y. mollaretii*			
		Y. aldovae			
		'*Y. ruckeri*'			
	Cefixim	*Y. bercovieri, Y. mollaretii*			
		Y. aldovae, 'Y. ruckeri'			
	Loracarbef	*Y. bercovieri, Y. mollaretii*			
		Y. aldovae, 'Y. ruckeri'			
	Cefpodoxime	*Y. bercovieri, Y. mollaretii*			
		Y. aldovae, 'Y. ruckeri'			
	All further tested cephalosporins	All species			
Carbapenems	Imipenem, meropenem	All species			
Monobactams	Aztreonam	All species			
Quinolones	All tested quinolones	All species			
Macrolides	Erythromycin, roxithromycin	All species			
	Clarithromycin	*Y. mollaretii, 'Y. ruckeri'*			
		Y. bercovieri, Y. aldovae			
	Azithromycin	All species			
Lincosamides	Lincomycin	All species			
	Clindamycin	*Y. mollaretii, 'Y. ruckeri'*			
		Y. bercovieri, Y. aldovae			
Streptogramins	All tested streptogramins	All species			
Glycopeptides	Teicoplanin, vancomycin	All species			
Antifolates	All tested antifolates	All species			
Other antibiotics	Fusidic acid	All species			
	Rifampicin	All species			
	Chloramphenicol	All species			
	Nitrofurantoin	All species			
	Fosfomycin	All species			

Note: If ⩽10% of the strains belonging to a natural population were of a different clinical category, they were disregarded.

of intermediate susceptibility to tetracyclines, aminoglycosides, numerous cephalosporins, several penicillins, carbapenems, aztreonam, quinolones, antifolates, azithromycin, chloramphenicol, nitrofurantoin and fosfomycin, and naturally resistant or of intermediate susceptibility to benzylpenicillin, oxacillin (although four strains of *Y. aldovae* and two of '*Y. ruckeri* ' seemed to be highly susceptible, see Discussion), macrolides (except azithromycin), lincosamides, streptogramins, glycopeptides, rifampicin and fusidic acid. Significant differences in susceptibility affecting clinical assessment criteria from resistant to sensitive (or *vice versa*) were seen for aminopenicillins (with and without β-lactamase inhibitors), and the cephalosporins cefaclor and cefoxitin. *Y. bercovieri* was the least susceptible species to β-lactams and was naturally resistant to amoxicillin, amoxicillin/clavulanate and naturally resistant or intermediate to cefaclor and cefoxitin. Whereas MIC values of aminopenicillins and cefaclor for strains of *Y. mollaretii* were slightly decreased in comparison with the strains of *Y. bercovieri* (clinical assessment of the respective natural populations led to

similar results), the MICs of cefoxitin for *Y. mollaretii* strains were three or four doubling dilution steps lower, indicating natural sensitivity to this cephalosporin. Strains of *Y. aldovae* and '*Y. ruckeri*' were uniformally sensitive to cefoxitin and naturally sensitive ('*Y. ruckeri*') or naturally sensitive or intermediate (*Y. aldovae*) to amoxicillin, amoxicillin/clavulanate and cefaclor. Significant differences in susceptibility not affecting clinical categorisations were found for several antibiotics and comprised numerous β-lactams and fosfomycin.

Quality control, medium dependency in susceptibility testing

The MIC data of all antibiotics were reproducible for *E. coli* ATCC 25922 and *Y. pseudotuberculosis* ATCC 29833. The MIC values for *E. coli* ATCC 25922 were within the control limits for susceptibility testing according to DIN criteria (data not shown). The MICs for *Y. pseudotuberculosis* ATCC 29833 were in agreement with the data of our previous study [24]. For most of the antibiotics tested, there were either no or only minor differences in susceptibility dependent on the medium. For all the species, the MICs of tetracyclines were generally two doubling dilution steps higher in IsoSensitest broth than in CAMHB (data not shown). This led to the absence of strains of intermediate susceptibility to tetracylines in the presence of CAMHB and all the species tested showed natural sensitivity to tetracyclines (Table 2).

Discussion

In recent years it has been shown that strains of 'non-pathogenic' and 'environmental' *Yersinia* spp. are probably associated with human disease. Because of the phenotypic similarity between these species and *Y. enterocolitica*, there is strong evidence that several infections due to these organisms have been attributed wrongly to *Y. enterocolitica*. In most cases, the databases of commercially available identification systems either contain only the 'classical' *Yersinia* spp. or identification of *Y. enterocolitica*-like species is based on only a few discriminating features. Because *Y. enterocolitica* is a phenotypically heterogenous species consisting of six biovars [25] as well as strains with atypical features [26], several tests are necessary for a definitive identification of *Y. enterocolitica*-like strains to the species level. In this study it was shown that a combination of the key reactions proposed by Neubauer *et al*. [18], Farmer [19] and Brenner [2] was sufficient for a reliable assignment of the strains tested. In particular, the urease test and fermentation of cellobiose, fucose, maltose, sorbitol, sorbose, sucrose and D-xylose were key identifying reactions (Table 1). It is not surprising that in this study, for some tests and species, the percentages of positive reactions were significantly higher than the data of Farmer [19] (Table 1), but in agreement with the data of Neubauer *et al*. [18]. These tests and those of Neubauer *et al*. were made at 28°C and evaluated after 24 h, whereas the tests by Farmer were made at 36°C and read after 48 h [19]. Numerous phenotypic properties of *Yersinia* spp. are highly dependent on temperature and it is known that many metabolic reactions of several *Yersinia* spp. are increased at lower temperatures [27]. In general, a prolonged incubation time increases the proportion of positive results in slow-growing organisms at lower temperatures, as shown for the fermentation tests with cellobiose, fucose, lactose, maltose and salicin (Table 1). When tests were made at 36°C, the results of this study were the same or similar to the data of Farmer (data not shown). Varying the incubation times at this temperature did not significantly influence the results. However, the discrepancy between some of the results for *Y. aldovae* (Table 1) and the data of Neubauer *et al*. [18] was not clarified. The results of the Voges Proskauer reaction and fermentation of cellobiose, lactose and melibiose for *Y. aldovae* strains were uniformally negative at 28°C (Table 1) and 36°C (data not shown). An extension of the incubation time to 48 h and 7 days for the fermentation tests had only little effect. The discrepancies seen might be due to differences of methodology and might indicate a higher sensitivity of the system applied by Neubauer *et al*. [18].

Apart from '*Y. ruckeri*', there are no published data on the antibiotic susceptibilities of the other *Yersinia* spp. tested in this study. Moreover, the studies of the antibiotic susceptibility of '*Y. ruckeri*' provided no information on the natural sensitivities or resistances of this species [28–30].

The aim of the present study was to establish a database for the natural susceptibility of *Y. bercovieri*, *Y. mollaretii*, *Y. aldovae* and '*Y. ruckeri*' strains to a range of antibiotics. The MIC values of all β-lactams (except oxacillin) were unimodally distributed and characteristic for the species, pointing to the presence (or absence) of specific mechanisms of resistance. Uniform MIC distributions of β-lactams in strains of *Y. enterocolitica* are uncommon and restricted to a few β-lactams, e.g., some modern cephalosporins and carbapenems [21]. The MIC values of numerous β-lactams depend on the biovar of *Y. enterocolitica* and there are also significant differences in susceptibility to some agents within the same biovar [21]. Resistance of *Y. enterocolitica* to numerous β-lactams is predominantly attributed to the expression of two different chromosomally encoded β-lactamases called BlaA (a class A enzyme) and BlaB (a class C β-lactamase) [31]. Recently it was shown that although all *Y. enterocolitica* strains were likely to possess genes for both BlaA- and BlaB-related enzymes, the differences in β-lactam susceptibility were predominantly due to differences in *BlaA* and *BlaB* expression which depended on the biovar and, in some cases, on the

individual strain [32, 33]. It is unlikely that the data found here for *Y. enterocolitica* can be transferred to all *Y. enterocolitica*-like species. Uniform MIC distributions of all β-lactams indicate that the strains tested either expressed one enzyme alone or that they showed no or little β-lactamase activity. The natural resistance of *Y. bercovieri* to amoxicillin, amoxicillin/clavulanate and cefoxitin combined with a natural sensitivity to ticarcillin points to the presence of a distinct chromosomally encoded class C β-lactamase in this species. Similar phenotypes in *Yersinia* spp. have been documented for several strains of *Y. enterocolitica* biovar 2, but there were differences in the susceptibility profiles to several cephalosporins and most biovar 2 strains were naturally resistant to ticarcillin (indicating the presence of a second enzyme, i.e., BlaA). *Y. mollaretii* strains, although resistant or of intermediate susceptibility to amoxicillin and amoxicillin/clavulanate, were more susceptible to these agents than *Y. bercovieri*. The susceptibility patterns to cefoxitin indicate that *Y. mollaretii* possesses a further distinct AmpC enzyme and not a β-lactamase related to the enzyme of *Y. bercovieri*, expressed at lower levels. The β-lactam susceptibility patterns of *Y. aldovae* and '*Y. ruckeri*' strains point either to the absence of β-lactamases or to the expression of only small amounts of enzyme. Interestingly, the MICs of amoxicillin, amoxicillin/clavulanate and cefaclor for one of the *Y. aldovae* strains tested were 16, 8 and 16 mg/L, respectively (data not shown). These MIC values probably point to a higher expression of an AmpC-type enzyme, expressed naturally at low levels. Enterobacteriaceae that naturally express their β-lactamases at low levels are not uncommon and include several species, e.g., *Proteus mirabilis*, *E. coli*, *Shigella* spp. [34] and *Edwardsiella tarda* [35]. In general, β-lactam MICs for these species do not significantly differ from the MICs seen for some β-lactamase-negative Enterobacteriaceae (e.g., *Salmonella enterica*) [36]. However, it should be noted that *Y. pseudotuberculosis* and *Y. pestis* that are completely β-lactamase negative are highly susceptible to nearly all β-lactams including benzylpenicillin [24]. This might indicate that both *Y. aldovae* and '*Y. ruckeri*' express β-lactamases. Otherwise, it was shown that the high β-lactam susceptibility of *Y. psudotuberculosis* and *Y. pestis* strains is largely attributed to their unusual cell wall composition [37]. A direct comparison of the MIC values for strains of '*Y. ruckeri*' with the MICs for classical pathogenic *Yersinia* strains is impeded because of their evolutionary distance to *Yersinia* spp. [15–17].

It should be noted that some strains of *Y. aldovae* and '*Y. ruckeri*' seemed to be highly susceptible to oxacillin (Table 2). In a recent study it was shown that failure of *Ed. ictaluri* to grow in oxacillin-containing microtitration plates is attributable to the salt concentration used in microtitration plates containing dehydrated oxacillin [35] (the oxacillin wells in the present study contained NaCl 2%). Oxacillin susceptibility testing of both the oxacillin-'sensitive' yersiniae of this study and the oxacillin-'sensitive' strains of *Y. enterocolitica* and *Y. pseudotuberculosis* from [21] and [24] in NaCl-free oxacillin plates revealed susceptibilities similar to the oxacillin-resistant strains of the respective species (data not shown). Thus, it seems likely that some *Yersinia* strains of some species do not tolerate higher salt concentrations, but all strains of all species examined so far are resistant to oxacillin.

Apart from the susceptibility patterns to β-lactams, species-associated differences in susceptibility were restricted to a few antibiotics, in particular fosfomycin. Although all species were naturally sensitive to fosfomycin, strains of *Y. bercovieri* and *Y. mollaretii* were significantly more susceptible than strains of the other species. Because there was no naturally occurring resistance, it is likely that a reduced permeability of the cell membrane to this antibiotic rather than a fosfomycin:gluthathione-S-transferase contributed to the phenotypes observed, unless the transferase has low affinity to fosfomycin or there is a low-level expression of enzyme [38].

The natural resistance of all species to rifampicin, lincosamides, glycopeptides, streptogramins and fusidic acid was expected, as resistance to these agents is a typical feature of nearly all species of Enterobacteriaceae and has been largely attributed to the barrier of their outer membrane [39].

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