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Identification of *Yersinia* Species by the Vitek GNI Card

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The Vitek GNI card was used to identify 212 isolates of 10 *Yersinia* species. Identification was correct for 96.3% of the isolates (156 of 162) to the genus level and for 57.4% of the isolates (93 of 162) to the species level for *Yersinia* spp. listed in the Vitek database. We recommend additional identification methods for isolates assigned to the genus *Yersinia* by the Vitek system.

The genus *Yersinia* belongs to the family *Enterobacteriaceae* and contains 11 species, three of which are pathogenic in humans. *Yersinia pestis* is the bacterial agent of plague and was not included in this study. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* can cause gastroenteritis and mesenteric lymphadenitis mimicking appendicitis, but the bacteria may also cause infections at other sites, such as wounds, joints, and the urinary tract, or invoke postinfectious sequelae such as reactive arthritis, urethritis, pleurisy, vasculitis, cholecystitis, and erythema nodosum. *Y. enterocolitica* and *Y. pseudotuberculosis* are the most important causes of gastroenteritis after *Salmonella* spp. and *Campylobacter* spp. (9). Other species include *Yersinia aldovae*, *Yersinia bercovieri*, *Yersinia frederiksenii*, *Yersinia intermedia*, *Yersinia kristensenii*, *Yersinia mollaretii*, and *Yersinia rohdei*, all of which were formerly considered to be biovars of *Y. enterocolitica* and can act as opportunistic pathogens (2, 5, 6, 8, 15, 16). *Yersinia ruckeri* causes redmouth disease in salmonids (10).

Yersinia species are relatively slow growers among the *Enterobacteriaceae* and display their biochemical characteristics most reliably at temperatures between 25 and 32°C (3). Traditional identification is based on a number of biochemical reactions often not incorporated in commercially available tests. In *Y. enterocolitica*, enteropathogenicity is restricted to members of some serovar-biovar combinations harboring the 64-kDa *Yersinia* virulence plasmid. Its presence is demonstrated by a positive autoagglutination test (1).

The Vitek system (bioMérieux Vitek, Inc., Hazelwood, Mo.) is an automated miniaturized biochemical test system operated at a fixed incubation temperature of 37°C. The GNI card is designed to identify members of the *Enterobacteriaceae* family and a select group of nonfermenting gram-negative bacteria.

TABLE 1. Performance of the Vitek GNI card in the identification of *Yersinia* spp.

Yersinia spp.	No. of isolates	% Of isolates correctly identified		% Of isolates identified with additional tests	Time to identification (h [mean ± SD])	
		Genus level	Species level		Without additional tests	With additional tests
All species	212	90			10.7 ± 2.8	
In Vitek database						
All	162	96	57	32	9.7 ± 2.7	32.4 ± 24.4
Y. enterocolitica	98	98	45	35	10.5 ± 1.9	46.5 ± 19.9
Y. pseudotuberculosis	22	100	96	5	7.2 ± 2.7	7.2 ± 2.7
Y. frederiksenii	18	100	78	56	10 ± 2.5	40.6 ± 23.8
Y. intermedia	12	92	42	50	9 ± 0.0	28.2 ± 26.3
Y. kristensenii	12	75	75	0	11.3 ± 3.3	11.3 ± 3.3
Not in Vitek database[a]						
All	50	72		66	10.7 ± 3.0	44.3 ± 22.8
Y. aldovae	12	75		41	10.6 ± 3.5	38.6 ± 25.1
Y. bercovieri	12	75		92	10.6 ± 3.0	54.6 ± 13.4
Y. mollaretii	11	82		54	9.9 ± 3.5	36.1 ± 26.7
Y. rohdei	10	80		70	11.1 ± 2.6	44.7 ± 23.6
Y. ruckeri	5	20		80	12.2 ± 1.8	50.6 ± 21.1

[a] For *Yersinia* spp. not listed in the Vitek database, the correct identification is evaluated only to the genus level.

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TABLE 2. Misidentification of *Yersinia* spp. by Vitek, biopattern, and absolute calculated likelihood

Yersinia spp.	Total no. of isolates	GNI identification		Biopattern	Absolute calculated likelihood (%)
		Species	No. of isolates		
In Vitek database					
Y. enterocolitica	54	*Y. enterocolitica*, *Y. frederiksenii*, *Y. intermedia*	1	7010364032	99
		Y. frederiksenii	1	7341735260	96
		Y. intermedia	2	7340634262	96
		Y. frederiksenii	1	7341731262	95
		Y. frederiksenii	1	7361735062	95
		Hafnia alvei	1	3000700176	92
		Y. frederiksenii	8	7341735262	92
		Y. intermedia	2	7340630262	92
		Y. frederiksenii	1	7741735262	91
		Y. frederiksenii	1	7340774360	89
		Y. frederiksenii	5	7341775262	79
		Y. frederiksenii	1	7340735262	79
		Y. intermedia	1	7341624262	73
		Y. intermedia	1	7341674262	73
		Y. intermedia	2	7341670262	67
		Y. frederiksenii	20	7341735062	66
		Y. frederiksenii	1	4370730362	61
		Y. frederiksenii	1	7341770262	55
		Y. intermedia	1	7341734262	55
		Y. frederiksenii	1	7341675262	47
		Unidentified	1	3341275262	
Y. pseudotuberculosis	1	*Y. enterocolitica*	1	7010260030	99
Y. frederiksenii	4	*Y. enterocolitica*	1	7010240020	97
		Y. intermedia	1	7610360232	92
		Y. intermedia	1	7341670262	67
		Y. enterocolitica	1	7341670062	39
Y. intermedia	7	*Y. frederiksenii*	1	7610360032	99
		Y. frederiksenii	1	7610360030	99
		Y. frederiksenii	1	7210360232	99
		Y. frederiksenii	1	7210360032	99
		Y. frederiksenii	1	7610764432	92
		Y. frederiksenii	1	7634370230	76
		Unidentified	1	7672364073	
Y. kristensenii	3	*Actinobacillus ureae*	1	30406200000	99
		Hafnia alvei	1	6002700033	68
		Unidentified	1	6436324012	
Not in Vitek database					
Y. aldovae	12	*Y. kristensenii*	2	7010324022	99
		Y. intermedia	1	7030274030	99
		Hafnia alvei	1	6040600433	99
		Actinobacillus ureae	1	60102000000	99
		Actinobacillus ureae	1	6010200000	99
		Y. enterocolitica, *Y. frederiksenii*, *Y. intermedia*	1	7014360032	98
		Y. enterocolitica, *Y. frederiksenii*, *Y. intermedia*	1	3041735262	97
		Y. kristensenii	1	7014304020	95
		Y. kristensenii	1	7014220032	89
		Y. kristensenii	1	7010621032	77
		Y. kristensenii	1	7014224032	76
Y. bercovieri	12	*Y. enterocolitica*	1	7014360032	99
		Y. enterocolitica	1	7010360032	99
		Actinobacillus ureae	1	6010300000	99
		Y. enterocolitica	1	7410360072	98
		Y. enterocolitica	1	7010364030	98
		Y. enterocolitica	1	7010360032	98
		Y. enterocolitica, *Y. frederiksenii*, *Y. intermedia*	3	7010360030	95
		Y. enterocolitica	1	7040735260	93
		Pasteurella haemolytica	1	6000360000	92
		Unidentified	1	66143642101	
Y. mollaretii	11	*Y. enterocolitica*, *Y. frederiksenii*, *Y. intermedia*	1	7014364032	99
		Y. enterocolitica	1	7610260032	99
		Salmonella spp.	1	6020724533	99
		Y. enterocolitica, *Y. frederiksenii*, *Y. intermedia*	1	7410360032	98
		Y. enterocolitica	1	7000360032	97
		Y. kristensenii	1	7000721062	95
		Y. kristensenii	1	7010320032	94
		Y. enterocolitica	1	7014764232	91
		Y. enterocolitica, *Y. frederiksenii*, *Y. intermedia*	1	7000340032	74
		Acinetobacter lwoffi	1	4052000000	73
		Y. frederiksenii	1	7614365232	67

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TABLE 2—*Continued*

Yersinia spp.	Total no. of isolates	GNI identification		Biopattern	% Identification
		Species	No. of isolates		
Y. rohdei	10	*Y. intermedia*	1	7010370032	99
		Y. intermedia	1	7010274020	99
		Y. intermedia	1	7010260032	99
		Y. enterocolitica, *Y. frederiksenii*, *Y. intermedia*	1	7030270230	99
		Y. enterocolitica, *Y. frederiksenii*, *Y. intermedia*	1	7030260230	99
		Y. enterocolitica, *Y. frederiksenii*, *Y. intermedia*	1	7010370032	99
		Y. enterocolitica	1	7030260030	99
		Actinobacillus ureae	1	60102000000	99
		Y. enterocolitica	1	7010374232	92
		Unidentified	1	7060771260	
Y. ruckeri	5	*Y. intermedia*	1	7010370032	99
		Hafnia alvei	1	6040200022	63
		Shigella spp.	1	3000600022	23
		Unidentified	1	6042200022	
		Unidentified	1	2000200022	

Six species of the genus *Yersinia* are listed in the Vitek database: *Y. enterocolitica*, *Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. pestis*, and *Y. pseudotuberculosis*. The Vitek system is routinely used for identification of *Enterobacteriaceae* in our laboratory. Rapid commercial identification systems generally favor the faster-growing and biochemically more active *Enterobacteriaceae*, which might result in a higher probability of misidentification for *Yersinia* spp.

The present study evaluated the ability of the Vitek Gram-Negative Identification (GNI) card—an automated rapid miniaturized biochemical identification system—to identify members of the genus *Yersinia*. Evaluation of the Vitek system has already been reported for various genera of the *Enterobacteriaceae* family, including *Yersinia*. The present study contains the largest collection of strains belonging to the genus *Yersinia* tested by the Vitek system.

A total of 212 strains belonging to the genus *Yersinia* was included in this study. Enteropathogenic and nonenteropathogenic strains of *Y. enterocolitica* and *Y. pseudotuberculosis* were isolated from humans. Other isolates included one reference strain of each species and field strains from human, animal, and environmental sources. Strains were provided by the Institute of Hygiene (Hamburg, Germany), G. Wolf (Munich, Germany), the Central Institute of FAF (Munich and Berlin, Germany), B. Niederwöhrmeier (Munster, Germany), and by the Institute for Medical Microbiology (Regensburg, Germany). Type strains (except *Y. pestis*) were obtained from the American Type Culture Collection (Rockville, Md.). Strains were maintained at 4°C on nutrient agar and subcultured twice on Columbia blood agar before testing (subculture on cefsulodin-Irgasan-novobiocin-agar [Oxoid, Wesel, Germany] did not influence identification results [data not shown]). Conventional biochemical tests were done as described by Bockemühl (7) by using the differentiation scheme of Aleksic and Bockemühl (1), including testing of enteropathogenicity by autoagglutination. Identification by the GNI card was carried out in accordance with the instructions of the manufacturer, including additional tests for melibiose, rhamnose, raffinose, and salicin (read after 48 h) when necessary. Manual reading of Vitek reactions was done in accordance with the table of color reactions provided by the manufacturer.

We investigated a total of 212 phenotypically characterized strains belonging to the genus *Yersinia*. For the species listed in the Vitek database, the GNI card correctly identified 96.3% (156 of 162) of all strains to the genus level and 57.4% (93 of 162 strains) to the species level. Correct identification to the species level was 44.9% (44 of 98 strains) for *Y. enterocolitica*, 95.5% (21 of 22 strains) for *Y. pseudotuberculosis*, 77.8% (14 of 18 strains) for *Y. frederiksenii*, 41.7% (5 of 12 strains) for *Y. intermedia*, and 75% (9 of 12 strains) for *Y. kristensenii*. The time to identification was 9.7 ± 2.7 h (mean ± standard deviation) without and 32.4 ± 24.4 h with the additional biochemical tests recommended in the Vitek manual. Ninety-two percent of pathogenic strains of *Y. enterocolitica* (24 of 26) were identified correctly to the species level, compared to 27% (17 of 63) of nonpathogenic strains ($P < 0.001$, chi-square test according to Pearson). Eighty-four percent of *Y. enterocolitica* strains (42 of 51) were misidentified as *Y. frederiksenii*, and 17.6% (9 of 51) were misidentified as *Y. intermedia*. For 18 isolates of *Y. enterocolitica* misidentified as *Y. frederiksenii*, *Y. intermedia*, or *Hafnia alvei*, the absolute calculated likelihood was ≥85%. A detailed analysis of the parameters associated with the identification is shown in Tables 1 and 2.

A number of authors have discussed the shortcomings associated with commercially available miniaturized tests for identifying members of the genus *Yersinia* (see overview in Table 3). Because of their slow growth, growth optimum at 25 to 32°C, and high biochemical similarity, *Yersinia* spp. present a special challenge to standard biochemical test systems. However, conventional biochemical testing, which was used as the "gold standard" in this study, might fail to correctly identify some isolates (11). From a clinical point of view, correct identification of *Y. enterocolitica* and *Y. pseudotuberculosis* (*Y. pestis* not investigated) and separation of nonenteropathogenic strains from *Y. enterocolitica* and *Y. pseudotuberculosis* are most important. In the present study, correct identification of *Y. enterocolitica* to the species level was achieved in only 44.9% of

TABLE 3. Results of studies evaluating identification of *Yersinia* spp. by commercial systems

Test	Organism	No. of isolates	% Of isolates correctly identified		Reference
			Genus level	Species level	
API 20E	*Y. enterocolitica*	15	100	100	13
Crystal	*Y. enterocoliticay*	14	100	100	13
Crystal	*Yersinia* spp.	24		88	12
Vitek GNI+	*Yersinia* spp.			75	12
API 20E	*Yersinia* spp.	105		93	4
API 20E	*Yersinia* spp.	183		90	14
Vitek GNI	*Yersinia* spp.	196	96	57	Present study

strains and in 18 of 98 (18.4%) cases, the absolute calculated likelihood was >85%. When the absolute calculated likelihood is ≥85%, the result is not likely to be questioned by the reader. Two of four isolates of *Y. frederiksenii* were misidentified as *Y. enterocolitica*, as were 1 of 12 *Y. bercovieri* isolates, 1 of 11 *Y. mollaretii* isolates, and 1 of 10 *Y. rohdei* isolates. However, pathogenic strains of *Y. enterocolitica* were correctly identified to the species level significantly more often than were nonpathogenic strains, presumably because of their higher metabolic activity. We made several attempts to improve inconclusive results by modifying the test procedure. Using an inoculum equivalent to a McFarland turbidity standard of no. 2 instead of no. 1 failed to influence profile generation (data not shown).

Unfortunately, incubation of Vitek cards in an external incubator at 28°C is not possible. We therefore stored cards with inconclusive results at room temperature for another 24 h and read the reactions manually, but these readings were no more conclusive (data not shown). Since the Vitek GNI card attributed 96.3% of all strains to the genus *Yersinia* within 9.7 ± 2.7 h, we conclude that the system can be used effectively to screen for *Yersinia* spp. among *Enterobacteriaceae*. Other methods such as traditional macroscale biochemical testing or sequencing of 16S rRNA should be used for definitive species identification.

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