JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2009, p. 872–873 Vol. 47, No. 3
0095-1137/09/$08.00+0 doi:10.1128/JCM.02425-08

Misidentification of a Variant Biotype of *Escherichia coli* O157:H7 as *Escherichia fergusonii* by Vitek 2 Compact▽

The source of contamination of leafy green produce with *Escherichia coli* O157:H7 has become a serious national concern. A state and federal collaborative investigation of watershed contamination was conducted in the Salinas Valley of California from October 2005 to September 2006. Surface water from creeks, agricultural drainage ditches, sloughs, and a river, modified Moore swabs placed in flowing surface water, and creek sediment and soil samples were obtained and examined for *E. coli* O157:H7 (3, 6).

Soil and sediments were suspended in modified buffered peptone water with sodium pyruvate (mBPWp). Surface water was mixed with an equal volume of 2× mBPWp. Modified Moore swabs were immersed in mBPWp. Pre-enrichment broths were subjected to recirculating immunomagnetic separation using the Pathatrix system (Matrix Microscience, Golden, CO) (6). Paramagnetic beads were washed, resuspended, and plated onto tellurite-cefixime-sorbitol-MacConkey agar (TC-SMAC) and CHROMagar O157 (CHROMagar, Paris, France). Up to eight colonies per sample were confirmed using eosin-methylene blue agar, motility, the 4-methylumbelliferyl-β-D-glucuronic acid (MUG) reaction, Vitek 2 Compact biochemical identification (bioMerieux, Inc., Durham, NC), O157:H7 serology (Denka Seiken, Japan), and real-time PCR (8).

Twenty-three strains of a variant saccharose/sucrose-negative (SAC) *E. coli* O157:H7 biotype were recovered from seven

TABLE 1. *E. coli* and *E. fergusonii* isolates and biochemical reactions from Vitek 2 Compact

Identification	Vitek identification	Isolate	Source	Biochemical reaction (GN card location no.)[a]						
				ADO (3)	PyrA (4)	D-CEL (7)	ProA (23)	SAC (33)	PHOS (45)	lMLTa (61)
O157	O157, low discrimination	262-C1	Water	−	−	−	+ A	−	+ A	+ A
		303-1	MS[b]	−	−	−	+ A	−	+ A	+ A
		308A	MS	−	−	−	+ A	−	(+) A	+ A
		421-1	Soil	−	−	−	+ A	−	(−) A	+ A
	E. fergusonii	304-2	MS	−	−	−	− A	−	− A	− A
		308C	MS	−	−	−	− A	−	− A	− A
		389D	Water	−	−	−	− A	−	− A	− A
		420-1	Soil	−	−	−	− A	−	− A	− A
		420-9	Soil	−	−	−	− A	−	− A	− A
		421-9	Soil	−	−	−	− A	−	− A	− A
	O157, 97–99% probability	162-P1	MS	−	−	−	−	+ B	(−)	−
		162-P7	MS	−	−	−	−	+ B	+	−
		166-P2	MS	−	−	−	−	+ B	(−)	−
		276-2	Water	−	−	−	+	+ B	+	−
		315D	MS	−	−	−	−	+ B	−	−
		319E	MS	−	−	−	−	+ B	−	−
		322D	MS	−	−	−	−	+ B	−	−
		43888	ATCC	−	−	−	+	+ B	+	−
		43889	ATCC	−	−	−	+	+ B	(+)	−
		43890	ATCC	−	−	−	−	+ B	+	−
		43894	ATCC	−	−	−	+	+ B	+	−
		700375	ATCC	−	−	−	+	+ B	(+)	−
E. fergusonii	*E. fergusonii*	85A5953	Human	+C	+C	+C	−	−	−	−
		87A1710	Human	+C	+C	+C	−	−	+	−
		87A3837	Human	+C	+C	+C	+	−	+	−
		50003-14a	Goose	+C	+C	+C	−	−	−	−
		50003-12a	Goose	+C	+C	+C	−	−	−	−
		50002-B	Pig	+C	+C	+C	+	−	(−)	−
		35469	ATCC	+C	+C	+C	+	−	+	−
		35471	ATCC	+C	+C	+C	−	−	+	−
		35472	ATCC	+C	+C	+C	−	−	+	−
		35473	ATCC	+C	+C	+C	−	−	+	−

[a] Significant characteristics establishing biotype are highlighted by letters: A, SAC negative; B, SAC-positive and variable or negative for ProA, PHOS, and I-MLTa; C, ADO, PyrA, and D-CEL positive (see the text).
[b] MS, modified Moore swab.

samples of water, soil, and modified Moore swabs. The 23 isolates were MUG negative and O157:H7 serology positive and contained the *stx1*, *stx2*, and *uidA*m genes. Of the 23 isolates, 15 were misidentified by Vitek 2 Compact version 01.02 0.01.04 as *Escherichia fergusonii* with 99% probability and no contraindications, requiring 5 h to complete analysis. Eight were identified as "low discrimination," with "analysis organisms and tests to separate" given as *Escherichia coli* O157 and *Escherichia coli*. The "contraindicating typical biopattern(s)" listed negative SAC at 99% probability. Results required an average of 10.25 h to completion. Multiple isolates from the same sample fell into both categories.

Many factors influence the results provided by an automated biochemical identification system: age of the culture, the medium, saline diluent concentration, pH, cell suspension density, card lots, and the database and algorithm of the machine (Vitek 2 user manual; bioMerieux, Inc., Durham, NC). Subsequently, 32 strains, including *E. coli* O157 of both high and low probability, previously misidentified *E. coli* O157, and *E. fergusonii* from human and animal sources were run on Vitek 2 Compact on the same day, controlling other factors. The 47 biochemical reactions on the gram-negative (GN) card were analyzed. Relevant biochemical reactions are given in Table 1.

All misidentified *E. coli* O157:H7 strains and the "low-discrimination" strains shared the SAC-negative phenotype. The "low-discrimination" strains were proline arylamidase (ProA) positive, phosphatase (PHOS) positive or variable, and L-malate assimilation (lMLTa) positive. The misidentified strains were negative for these three biochemicals. The *E. coli* O157 strains with 97 to 99% probabilities were all SAC positive and were variable or negative in the above three reactions. All *E. fergusonii* strains were positive for adonitol (ADO) and D-cellobiose (D-CEL) fermentation, the reactions used to characterize this species (4, 5). None of the misidentified strains was ADO or D-CEL positive.

E. fergusonii has been associated with pathogenesis in humans and animals (2, 9), so this identification cannot be discounted. Confirmation that the organism is ADO and D-CEL positive is critical.

A biotype is defined as a group of strains that have a common biochemical reaction pattern that is unusual for the particular species (4, 7). Even within a given biotype there appears to be some diversity. Several authors have noted the pitfalls of automated biochemical identification systems with increasing biodiversity (1, 10, 11). The consequences of misidentifying *E. coli* O157:H7 are severe. Multiple identification tools, such as serology and molecular methods, should be used for confirmation of identifications made by automated systems.

REFERENCES

1. **Abbott, S. L., L. S. Sell, M. Catino, Jr., M. A. Hartley, and J. M. Janda.** 1998. Misidentification of unusual *Aeromonas* species as members of the genus *Vibrio*: a continuing problem. J. Clin. Microbiol. **36:**1103–1104.
2. **Bain, M. S., and C. C. Green.** 1999. Isolation of *Escherichia fergusonii* in cases clinically suggestive of Salmonellosis. Vet. Rec. **144:**511.
3. **Cooley, M., D. Carychao, L. Crawford-Miksza, M. T. Jay, C. Myers, C. Rose, C Keys, J. Farrar, and R. E. Mandrell.** 2007. Incidence and tracking of Escherichia coli O157:H7 in a major produce production region in California. PLoS ONE **2(11):**e1159.
4. **Farmer, J. J., III, B. R. Davis, F. W. Hickman-Brenner, A. McWhorter, G. P. Huntley-Carter, M. A. Asbury, C. Riddle, H. G. Wathen-Grady, C. Elias, G. R. Fanning, A. G. Steigerwalt, C. M. O'Hara, G. K. Morris, P. B. Smith, and D. J. Brenner.** 1985. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. J. Clin. Microbiol. **21:**46–76.
5. **Farmer, J. J., III, G. R. Fanning, B. R. Davis, C. M. O'Hara, C. Riddle, F. W. Hickman-Brenner, M. A. Asbury, V. A. Lowery III, and D. J. Brenner.** 1985. *Escherichia fergusonii* and *Enterobacter taylorae*, two new species of *Enterobacteriaceae* isolated from clinical specimens. J. Clin. Microbiol. **21:**77–81.
6. **Himathongkham, S., M. L. Dodd, J. K. Yee, D. K. Lau, R. G. Bryant, A. S. Badoiu, H. K. Lau, L. S. Guthertz, L. K. Crawford-Miksza, and M. S. Soliman.** 2007. Recirculating immunomagnetic separation and optimal enrichment conditions for enhanced detection and recovery of low levels of *Escherichia coli* O157:H7 from fresh leafy produce and surface water. J. Food Prot. **70:**2717–2724.
7. **Janda, J. M., and S. L. Abbott.** 1998. The Enterobacteria. Lippencott-Raven Publishers, Philadelphia, PA.
8. **Jinneman, K. C., K. J. Yoshitomi, and S. D. Weagant.** 2003. Multiplex real-time PCR method to identify Shiga toxin genes *stx1* and *stx2* and *Escherichia coli* O157:H7/H$^-$ serotype. Appl. Environ. Microbiol. **69:**6327–6333.
9. **Mahapatra, A., S. Mahapatra, and A. Mahapatra.** 2005. *Escherichia fergusonii*: an emerging pathogen in South Orissa. Indian J. Med. Microbiol. **23:**204–205.
10. **Wang, T. K. F., W.-C. Yam, K.-Y. Yuen, and S. S. Y. Wong.** 2006. Misidentification of a mucoid strain of *Salmonella enterica* serotype Cholerasuis as *Hafnia alvei* by the Vitek GNI+ card system. J. Clin. Microbiol. **44:**4605–4606.
11. **Ware, J. M., S. L. Abbott, and J. M. Janda.** 2000. A new diagnostic problem: isolation of *Escherichia coli* O157:H7 strains with aberrant biochemical properties. Diagn. Microbiol. Infect. Dis. **38:**185–187.

Leta K. Crawford-Miksza*
Sunee Himathongkham†
Mary L. Dodd‡
Alexandru S. Badoiu
Olivia M. Badoiu
Linda S. Guthertz‡
Food and Drug Laboratory Branch
California Department of Public Health
850 Marina Bay Parkway
Richmond, California 94804

*Phone: (510) 412–6233
Fax: (510) 412–6280
E-mail: Leta.crawford-miksza@cdph.ca.gov

† Present address: U.S. FDA San Francisco District Office Laboratory, Alameda, CA 94502.

‡ Present address: Microbial Diseases Laboratory, California Department of Public Health, Richmond, CA 94804.

▽ Published ahead of print on 30 December 2008.