Isolation of a Nonpathogenic Strain of Citrobacter sedlakii Which Expresses Escherichia coli O157 Antigen

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A nonpathogenic strain of Citrobacter sedlakii which expresses the Escherichia coli O157 antigen is described. The discovery of this strain emphasizes the necessity of additional biochemical and/or toxigenicity testing when isolates react with E. coli O157 latex reagents.

Since the discovery of Escherichia coli O157:H7 as an important human pathogen in 1982, numerous food-associated outbreaks of diarrheal disease have been attributed to this organism (4, 6, 10). Symptoms have ranged from mild, bloody diarrhea to hemorrhagic colitis and, in some instances, hemolytic-uremic syndrome. The organism’s ability to elaborate Shiga-like toxins is believed to explain its pathogenic potential. Currently many laboratories use sorbitol-MacConkey agar to screen for colorless, sorbitol-negative colonies which are then tested with a latex reagent containing antibodies to E. coli O157. A positive latex test is considered a presumptive result since other non-E. coli organisms which express the O157 antigen have been identified. Therefore, further biochemical or toxigenicity testing must be performed to confirm the positive results of the screening test.

In 1993 Bettelheim et al. (1) reported a typical Citrobacter freundii strain isolated from an infant who had died of sudden infant death syndrome. This nonpathogenic strain exhibited the O157 antigen and had a positive reaction in an E. coli O157 latex test (Oxoid, Hampshire, England). Additionally, other investigators reported strains of Escherichia hermannii and Salmonella group N which were found to cross-react strongly with E. coli antiserum and O157 latex reagents (8).

This report details the isolation of Citrobacter sedlakii from stool of a patient who had developed a diarrheal illness after traveling in Southeast Asia. The isolate was a sorbitol fermenter and thus produced pink colonies on sorbitol-MacConkey agar. This organism produced typical positive agglutination reactions with E. coli O157 latex reagents in the Oxoid E. coli O157, RIM E. coli O157:H7 (Remel, Lenexa, Kans.), and Wellcolex E. coli O157 (Murex Diagnostics, Dartford, England) test kits. This organism also agglutinated with E. coli O157 antiserum (BDMS/Dilco, Sparks, Md.) and reacted strongly with fluorescein-conjugated antibody to E. coli O157 (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) (7). It also had a positive reaction in an E. coli O157 enzyme-linked immunosorbent assay (LMD Laboratories, Carlsbad, Calif.). The isolate was determined to be negative for Shiga-like toxins I and II by the Premier EHEC test (Meridian Diagnostics, Cincinnati, Ohio) and negative for H7 by a flagellar-immobilization test and thus was not considered to be the etiologic agent of the patient’s gastroenteritis.

There was no agreement among commercial identification systems as to the identity of this organism. The Vitek system with the GNI card (bioMérieux Vitek, Inc., Hazelwood, Mo.) identified the isolate as Enterobacter amnigenus, while the RadID onE system (Remel Atlanta [formerly Innovative Diagnostic Systems], Norcross, Ga.) and the Walk/Away Neg Combo X panel (Dade Microscan, Inc., West Sacramento, Calif.) identified it as Citrobacter amalonaticus. Because of this discrepancy, the isolate was submitted to the Centers for Disease Control and Prevention, Atlanta, Ga., where it was classified as C. sedlakii by using conventional biochemicals. C. sedlakii, one of the new genomospecies of Citrobacter formerly called C. freundii, has been isolated from human blood, wounds, and stool and was recently isolated from cerebrospinal fluid from a neonate (2, 3). C. sedlakii is not currently in the databases of any of these three systems, but they should have rendered an identification of C. freundii. The C. freundii organism isolated by Sowers et al. (9) may have belonged to this new genomospecies of Citrobacter; however, this speculation has not been confirmed.

Conventional biochemical tests which yielded positive reactions included nitrate, indole production, citrate utilization, ornithine decarboxylase, arginine dihydrolase, o-nitrophenyl-β-D-galactopyranoside (ONPG), malonate, and fermentation of glucose, dulcitol, lactose, melibiose, arabinose, and maltose. Negative reactions were obtained with tests for oxidase, lysine decarboxylase, H2S production, and fermentation of raffinose, sucrose, and α-methyl-β-glucoside. C. sedlakii can generally be differentiated from C. freundii by its ability to decarboxylate ornithine and utilize malonate (5). We have tested 10 C. freundii and 2 Citrobacter diversus clinical isolates, and none were found to express the O157 antigen. Additionally, we tested three American Type Culture Collection (ATCC) strains of C. sedlakii (ATCC 51115, ATCC 51493, and ATCC 51494) and two ATCC strains of C. amalonaticus (ATCC 25405 and ATCC 25406), and all were negative for the O157 antigen. Citrobacter species are normally present in stool and are not considered to be enteric pathogens. To the best of our knowledge, this is the first report of a strain of C. sedlakii which expresses the O157 antigen. This discovery of false-positive results and those previously described by other investigators (1, 8, 9) further emphasize the necessity of additional biochemical and/or toxigenicity testing of isolates when they produce positive reactions with O157 reagents.

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REFERENCES


