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Cronobacter Infections Not from Infant Formula, Taiwan

To the Editor: Species of the genus *Cronobacter* are relatively heterogeneous at the phenotypic and molecular levels (*1*). In 2012, the following 7 *Cronobacter* species had been defined: *C. sakazakii, C. malonaticus, C. turicensis, C. dublinensis, C. muytjensii*, *C. condimenti,* and *C. universalis* (*2*). These opportunistic pathogens cause bacteremia and meningitis in neonates and are associated with necrotizing enterocolitis (*3*); ≈30% of infants with *Cronobacter* bacteremia or meningitis have died (*4*). *Cronobacter* spp. primarily infect infants, but infections among immunocompromised patients, particularly elderly patients, have been reported (*5*). Although these organisms are ubiquitous in the environment and have been isolated from a variety of foods, *Cronobacter* spp. infections in infants have been epidemiologically associated with ingestion of contaminated powdered infant formula (*6*). Few reports of *C. sakazakii* infections in adults have been published.

During 2002–2011, a total of 5 *C. sakazakii* isolates, 1 from each of 5 patients, were identified at the National Taiwan University Hospital in northern Taiwan (Table). These isolates were identified as belonging to the *C. sakazakii* group by use of 2 systems: PMIC/ID-30 (Becton Dickinson Diagnostic Systems, Sparks, MD, USA) and the Vitek 2 System GN card (bioMérieux Inc., La Balme les Grottes, France). The phenotypic profiles that use 14 biochemical characteristics to differentiate 7 species and 3 subspecies of *C. dublinensis* within *Cronobacter* gen. nov. (*C. sakazakii* group) have been described (*2*). Although we did not apply the 14 biochemical tests to differentiate the 5 isolates (*2*), the isolates' lack of indole production and dulcitol utilization, obtained by use of Enterotube II (Becton Dickinson Diagnostic Systems), was compatible with identification of the following 3 species or subspecies: *C. sakazakii, C. malonaticus,* or *C. dublinensis* subsp. *lausannensis* (*2*). Results of partial 16S rRNA gene sequence analysis with primers 8FPL and 1492RPL indicated that the isolates were probably *C. sakazakii* (*7*), and results of a 2-step *rpoB*-based PCR that used 2 sets of primer pairs (*Csakf/Csakr* and *Cmalf/Cmalr*) confirmed that the isolates were *C. sakazakii* (*8*).

Serogroups of the 5 *C. sakazakii* isolates were determined by using 5 primer pairs specific to the *weh*C, *weh*I, and *wzx* genes (*9*). Of these 5 isolates, 3 were serogroup O1, and 2 were not typeable (not serogroups O1, O2, or O3).

Table. Characteristics of 5 patients with *Cronobacter sakazakii* infections, National Taiwan University Hospital, Taiwan, 2002–2011*

Patient age/sex	Year of diagnosis	Underlying medical conditions	Clinical presentation	Infection type	Outcome	Isolation site	Isolate GenBank accession no.	Isolate serogroup
77 y/M	2002	Laryngeal cancer, diabetes mellitus, pulmonary tuberculosis	Cardiac arrest at arrival at emergency department	Primary bacteremia	Died	Blood	FJ947061.1	O1
72 y/M	2002	Gastric cancer	Fever	Primary bacteremia	Survived	Blood	JF330153.1	NT
2 mo/M	2005	Congenital heart disease	Fever	Pneumonia	Survived	Sputum	F330133.1	O1
37 y/M	2008	None	Abdominal pain	Acute cholecystitis	Survived	Bile	JF330153.1	NT
64 y/F	2011	Breast cancer	Hemoptysis	Pneumonia	Survived	Sputum	GU727684.1	NT

*All isolates were identified as C. *sakazakii* I (99% identity) by 16S sequencing. NT, not typeable (not serogroups O1, O2, or O3).

Results of disk-diffusion susceptibility testing showed that all isolates were susceptible to cefotaxime, cefepime, piperacillin–tazobactam, ertapenem, imipenem, meropenem, ciprofloxacin, gentamicin, and amikacin and that all were resistant to cefazolin. The random amplified polymorphic DNA patterns generated by arbitrarily primed PCR that used 2 random oligonucleotide primers (M13 and ERIC1) differed among the 5 isolates, indicating that these 5 *C. sakazakii* strains were not clonally related (*10*).

The clinical and microbiological characteristics of the 5 patients (4 adult, 4 male) with *C. sakazakii* infection are summarized in the Table. Primary bacteremia was found in 2 patients, pneumonia in 2 (predominant growth of *C. sakazakii* from purulent sputum samples), and acute cholecystitis in 1.

The nonadult patient was a 2-month-old boy with congenital heart disease. Because of apnea and cyanosis, he was sent to an emergency department and later received assisted ventilation and supportive care in an intensive care unit. He was extubated on day 11 of hospitalization; however, fever and increased purulent sputum were noted on day 18. Bacterial culture of the suctioned sputum specimen yielded *C. sakazakii*. Before being hospitalized, the boy had been fed reconstituted powdered infant formula (Nestlé H.A.1, Gold; Nestlé Taiwan Ltd., Taipei, Taiwan) by mouth without other supplemental nutrition. During hospitalization, he received infant formula made by the hospital nutritional department through nasogastric tube. Although the powdered infant formula was not tested for *C. sakazakii,* initial sputum culture disclosed viridans group streptococci, and *C. sakazakii* was isolated from sputum obtained on day 18 of hospitalization. Thus, *C. sakazakii* from this infant might not have been associated with contaminated powdered infant formula.

Among the 4 adult patients, 3 had underlying solid organ malignancy and had received immunosuppressive drugs., and the other had bacteremia and died of cardiac arrest at arrival at the emergency department. The sources of *C. sakazakii* infection in the 1 nonimmunocompromised adult and the infant remain unclear; further research is needed to identify the source of *C. sakazakii* infections in Taiwan.

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Methicillin-Resistant *Staphylococcus pseudintermedius* in Rats

To the Editor: *Staphylococcus pseudintermedius* is a coagulase-positive species in the *S. intermedius* group. Previously misidentified as *S. intermedius*, *S. pseudintermedius* is now recognized as a leading cause of opportunistic infection in dogs (*1*) and a cause of sporadic infections in other species, including humans (*1*,*2*). Additionally, evidence of zoonotic transmission of *S. pseudintermedius* from dogs to humans has been reported (*3*,*4*). Although information regarding the pathogenic process of *S. pseudintermedius* is limited, the bacterium is known to possess virulence factors similar to those found in *S. aureus*, including a leukotoxin comparable to the Panton-Valentine leukocidase associated with community-acquired *S. aureus* infection (*1*).

Of concern is the emergence and widespread international recognition of methicillin-resistant *S. pseudintermedius* (MRSP) (*1*). One veterinary laboratory noted a 272% increase in MRSP cases from 2007–2008 through 2010–2011 (*5*). As with methicillin-resistant *S. aureus*, MRSP resistance is conferred by the *mecA* gene, making MRSP resistant to all β-lactam antimicrobial drugs and some other antimicrobial drug classes (*1*). Compared with methicillin-susceptible strains, MRSP seems better able to colonize humans (*3*).

The potential for zoonotic transmission and concerns that MRSP could be mistaken for other methicillin-resistant staphylococci (*1*,*2*) suggest the need for further investigation into the epidemiology of this pathogen. One question yet to be addressed is whether commensal pests, particularly rats (*Rattus* spp.), could serve as a source of MRSP because of their pervasiveness, their propensity toward close contact with humans, and the fact that they are the source of several other zoonotic diseases (*6*). We report MRSP carriage in wild Norway rats (*R. norvegicus*) in Vancouver, British Columbia, Canada.

During September–November 2011, Norway rats were trapped in a random sample of alleys in Vancouver's Downtown Eastside, an impoverished neighborhood with high levels of homelessness, intravenous drug use, and HIV infection. Immediately after the rats were euthanized, a sterile swab was used to sample the oropharynx and nares of each rat.

Swabs were placed in 2 mL of enrichment broth containing 10 g/L tryptone T, 75 g/L sodium chloride, 10 g/L mannitol, and 2.5 g/L yeast extract and incubated for 24 h at 35°C. Aliquots of 100 μL were streaked onto mannitol salt agar with 2 μg/mL oxacillin and incubated at 35°C for 48 h. Suspected staphylococcal isolates were subcultured onto Columbia blood agar and identified according to colony morphologic appearance, Gram staining, and catalase reaction. Tube coagulase-positive isolates were speciated by using a multiplex PCR specific for the thermonuclease (*nuc*) gene (*7*). Methicillin resistance was confirmed by demonstrating penicillin-binding protein 2a antigen with the latex-agglutination test (Oxoid Ltd., Basingstoke, UK). Isolates were typed by sequencing of the *mec*-associated direct repeat unit (*dru* typing) (*8*). Antimicrobial drug susceptibility was evaluated by broth microdilution (Sensititre; Trek Diagnostics, Cleveland, OH, USA), according to Clinical and Laboratory Standards Institute guidelines (www.clsi.org/). The study was approved by the University of British Columbia Animal Care Committee.

MRSP was isolated from 5 (2.1%) of 237 rats trapped. However, lack of standardized screening methods for MRSP could have resulted in underestimation of MRSP prevalence. Of the 5 isolates, 3 were *dru* type dt11a, a strain commonly found in dogs (*8*), and the other 2 were a novel *dru* type (assigned dt7ac). All isolates tested demonstrated resistance to multiple antimicrobial drug classes (Table).

Carriage of MRSP has not been identified in wild rats; therefore, the epidemiologic and public health implications of these findings are difficult to determine. However, the isolation of a common dog-associated *dru* type from rats suggests that MRSP might be transmissible between dogs and rats. This possibility is not surprising given the potential for direct and indirect contact between these species. Indeed, rat-to-dog transmission of other bacterial pathogens has been recognized (*9*). Detection of a *dru* type not previously detected in methicillin-resistant staphylococci suggests that these isolates might have evolved independently of methicillin-resistant staphylococci in other animal species.