Research Note

Prevalence and Antimicrobial Resistance of Cronobacter sakazakii Isolated from Domestic Kitchens in Middle Tennessee, United States

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ABSTRACT

Cronobacter sakazakii is an emerging opportunistic pathogen that has been implicated in cases of severe meningitis, sepsis, and necrotizing enterocolitis in premature and full-term infants. In this study, the prevalence of C. sakazakii was estimated in selected domestic kitchens in middle Tennessee. Antimicrobial resistance patterns of these C. sakazakii isolates were examined for points of public health significance. A total of 234 contact sites in 78 domestic kitchens were tested for C. sakazakii. Consumers’ used dishcloths and cleaning sponges were also tested. Antimicrobial susceptibility of the identified C. sakazakii isolates was determined for 10 antimicrobial drugs by means of the disk diffusion method. C. sakazakii was recovered from 26.9% of domestic kitchens visited. Multidrug resistance was observed; the highest resistance was to penicillin (76.1% of isolates) followed by tetracycline (66.6%), ciprofloxacin (57.1%), and nalidixic acid (47.6%). None of the C. sakazakii isolates were resistant to gentamicin. These results suggest that antibiotic-resistant C. sakazakii could be present at various sites in domestic kitchens.

Cronobacter sakazakii, initially referred to as “yellow-pigmented Enterobacter cloacae,” is a member of the Enterobacteriaceae family and is widely distributed in nature (45). It is an invasive emerging foodborne pathogen that causes life-threatening meningitis (7, 17), septicemia, and enterocolitis in infants (38). The results of a study conducted at 25 neonatal care units in 15 hospitals indicated that the growth of C. sakazakii in bottles depends on initial water temperature, room temperature, cold storage temperature, and reheating temperature and time (51). Infant infections with this pathogen have been associated with contaminated reconstituted infant formula (5), but other environmental sources of contamination are possible (59). During previous investigations into outbreaks of C. sakazakii infection in premature babies and neonates, this pathogen was isolated from blenders and bottle-cleaning brushes (46). C. sakazakii also has been linked in at least nine cases of infection in adults, all of whom were immunocompromised with underlying medical conditions (11, 25, 38). C. sakazakii has been isolated from various foods, including dried vegetables, eggs, and cheese products, and from water (19, 37, 39). This pathogen also has been detected in commercial food processing and domestic environments (6, 33, 34), thereby posing some level of safety risk to infants and individuals with impaired immunity. Although restaurants are most often identified as sources of outbreaks of foodborne disease, foodborne illnesses occur three times more frequently in private homes (52). Domestic kitchens are the front line in the battle against foodborne diseases, and consumers must use safe procedures during routine handling, preparation, and storage of foods and liquids to minimize the potential risk of contamination and infection (50). Improper food handling and unhygienic practices can result in the spread of foodborne pathogens to ready-to-eat foods in various parts of the domestic kitchen. C. sakazakii destruction is not always complete in powdered infant formula (PIF); therefore, good hygienic practices are essential during preparation to prevent secondary contamination and spread of this pathogen. Some researchers have suggested that oral and intestinal colonization with C. sakazakii can be linked to ingestion of contaminated food (20, 48).

Acquired antimicrobial resistance by foodborne pathogens increases infectious disease morbidity and mortality with resulting huge socioeconomic costs (27, 30). Many researchers have verified that Cronobacter spp. can be inactivated by antibiotics, but concentrations of antibiotics currently considered sufficient for treatment may be insufficient in the future (12, 26). C. sakazakii infections, in particular those resulting in meningitis, require effective treatment, and the antibiotic susceptibility of this pathogen has raised concerns (11, 15). Because most Cronobacter infections are treated with antibiotics, drugs must be chosen
with caution to lower the risk of intrinsic resistance to β-lactams and cephalosporins (22). Unwise use of antibiotics in agriculture has been suggested to contribute to antibiotic resistance in foodborne pathogens (42, 55). Carabapenems and antipseudomonal penicillins are recommended drugs of choice for treating Cronobacter infections, with ciprofloxacin as an alternative (22).

Despite the attention paid to cross-contamination by pathogenic microorganisms in the kitchen environment, little attention has been paid to C. sakazakii. Therefore, a better understanding of the prevalence of C. sakazakii in the domestic kitchen and of hygiene guidelines for PIF preparation could play a significant role in control of this emerging foodborne pathogen. The aim of this study was to determine whether C. sakazakii could be found in domestic kitchens and to ascertain antimicrobial resistant patterns of any such C. sakazakii isolates.

MATERIALS AND METHODS

Sample collection. A total of 78 households participated in this study. The participants were recruited through brochures posted at meetings of neighborhood organizations, churches, and other social gatherings. In domestic kitchens, surface swabs (400 cm²) were taken from various locations, including sinks, countertops, refrigerator handles, and meat drawers, with sterile moistened Hydra Sponges (Biotrace International, Muncie, IN). Dishcloths and sponges also were collected for microbial analysis. After sampling, the Hydra Sponges soaked in neutralizing buffer (10 ml) were placed in sterile bags with the participant’s identification number. Immediately after collection, samples were transported to the laboratory in refrigerated coolers and analyzed within 2 h. In this study, participants received a $25 grocery store gift card as remuneration for allowing collection of samples from their kitchens.

Isolation of C. sakazakii. Upon arrival at the laboratory, swabs and other collected samples were placed in 100 ml of buffered peptone water (BPW) and incubated at 37°C for 24 h. Ten milliliters of the BPW preenrichment medium was added to 90 ml of Enterobacteriaceae enrichment (EE) broth and further incubated at 35°C for 20 h. After enrichment, 10 ml of the EE broth culture was streaked onto violet red bile glucose agar (VRBG; BD, Franklin Lakes, NJ), chromogenic Cronobacter Druggan-Forsythe-Iversen agar (DFI; bioMérieux, Hazelwood, MO), and Trypticase soy agar (TSA; Difco, BD, Sparks, MD). VRBG plates were incubated at 37°C for 24 h, DFI plates were incubated at 42°C for 24 h, and TSA plates were incubated at 35°C for 48 to 72 h. Colonies formed on DFI were examined for blue-green pigmenta-

TABLE 1. Primers used for PCR amplification of parts of the genome specific to C. sakazakii

<table>
<thead>
<tr>
<th>Primer</th>
<th>GenBank no.</th>
<th>Primer sequence</th>
<th>Amplicon size (bp)</th>
<th>Annealing temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS-L</td>
<td>AV702097.1</td>
<td>3'-GGTTGTCTCGGAAGGCCGA-5'</td>
<td>282</td>
<td>55</td>
</tr>
<tr>
<td>RS-R</td>
<td>AV702097.1</td>
<td>5'-GTCCTCGGTCTGGAGTTTG-3'</td>
<td>469</td>
<td>55</td>
</tr>
<tr>
<td>PA-L</td>
<td>DQ000206</td>
<td>3'-GGTTTAACCTGAACCTTTCCC-5'</td>
<td>501</td>
<td>55</td>
</tr>
<tr>
<td>PA-R</td>
<td>DQ000206</td>
<td>5'-CGCCAGCGATTTAGAAGA-3'</td>
<td>501</td>
<td>55</td>
</tr>
</tbody>
</table>

GenBank accession numbers for C. sakazakii 16S–23S rDNA intergenic spacer and ompA gene sequences.

Antibiotic susceptibility testing. C. sakazakii isolates were examined for their susceptibility to a panel of 10 antimicrobials. The agar disk diffusion method was used as recommended by the Clinical and Laboratory Standards Institute (10). These antimicrobials were tetracycline (30 μg), gentamicin (10 μg), streptomycin (10 μg), ampicillin (10 μg), kanamycin (30 μg), penicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), and cefoxitin (30 μg) (Fisher Scientific) and nalidixic acid (30 μg; Sigma-Aldrich). Antimicrobial susceptibility testing was performed with
C. sakazakii gene isolates obtained from various locations in domestic (2).

Antimicrobial resistance. According to the disk diffusion test, C. sakazakii isolates were most often resistant to penicillin (76.1% of isolates), tetracycline (66.6%), and ciprofloxacin (57.1%). Lower percentages of isolates were resistant to nalidixic acid (47.6%), ampicillin (33.3%), streptomycin (28.6%), chloramphenicol (19%), and cefoxitin (9.5%). The least resistance was found to kanamycin (4.8% of isolates). In the present study, none of the C. sakazakii isolates were resistant to gentamicin. The most common antibiotic resistant patterns were tetracycline–penicillin–streptomycin–kanamycin–nalidixic acid and ciprofloxacin-ampicillin-chloramphenicol-kanamycin-streptomycin-tetracycline-penicillin. Overall, C. sakazakii isolates were resistant to two or more of the antibiotics tested.

DISCUSSION

Amplification of the rDNA ITS and ompA gene by PCR revealed four false-positive isolates, which were previously identified as C. sakazakii with the API 20E biochemical method. This finding indicates that the API 20E test is not a reliable identification method for C. sakazakii. Therefore, the ID 32 E (bioMérieux) and other appropriate tests such as the PCR assay are recommended (16, 21). Previous studies have indicated that although the ITS and the ompA gene are quite conserved, they have enough variability to distinguish among closely related microorganisms (28, 49). In the present study, food preparation areas including sinks were contaminated with C. sakazakii. Sinks are the most touched surface during food handling, as reported by Henroid et al. (29), and could consequently pose a cross-contamination risk.

Our findings are supported by results of previous studies, which suggested that bacteria can inhabit domestic dishcloths and thus be spread around the kitchen (13, 53, 54, 56). Other researchers have found that dishcloths and kitchen sponges are sources of pathogen cross-contamination to other surfaces in kitchens (32, 41). Consumers should be educated on the need for frequent replacement or decontamination of kitchen cleaning tools as one aspect of kitchen hygiene that decreases the risk of foodborne illness in the home. In our study, the presence of C. sakazakii in meat drawers indicates the possibility of cross-contamination from raw meats. C. sakazakii may be present on raw retail meats; this pathogen has been previously reported in sausages, pork, camel, and poultry meat (19). Bacteria from contaminated raw foods, leaking packages, and hands may be introduced to domestic refrigerators, countertops, and other food preparation areas, posing a risk of indirect contamination during subsequent food preparation activities (43). In previous studies, homes have been identified as an important point of cross-contamination through improper handling of raw food products (4, 40).

C. sakazakii is an emerging opportunistic foodborne pathogen that has been associated with the domestic environment and food production factories (2). This pathogen can survive and grow on the surface of several types of fresh-cut produce and in fruits, vegetables juices, meats, herbs, spices, grains, cheese, and many other foods (31, 36). Because C. sakazakii can be transported into the home via fresh produce, meats, fruits, and ready-to-eat

FIGURE 1. Gel patterns generated from PCR amplification of ITS (a) and ompA gene (b and c) sequences. Lanes 1 through 16, C. sakazakii isolates obtained from various locations in domestic kitchens; lane 17, positive control (Cronobacter muytjensii ATCC 51329); lane 18, negative control (master mix without DNA template); lane 19, blank; lane M, 100-bp ladder molecular size marker.
foods (3), consumers should use good hygienic practices when handling raw foods in domestic kitchens, especially during reconstitution of PIF. C. sakazakii can exist in food and on surfaces that have contact with food (14, 45). It also can survive in dry conditions and therefore has a sanitizing advantage in dry environments (6). Therefore, consumers must be educated in effective procedures for sanitizing kitchens surfaces, utensils, and hands after handling raw meats (24, 41) that might be contaminated with foodborne pathogens. Kitchen hygiene is an important intervention that decreases the risk of foodborne illness in the home.

The emergence of resistance to antimicrobials is a major public health problem and justifies monitoring the antibiotic susceptibility of foodborne pathogens. Although all C. sakazakii isolates tested in the present study were susceptible to gentamicin, in previous studies C. sakazakii from poultry was resistant to gentamicin (35). C. sakazakii infections have been traditionally treated with ampicillin in combination with either gentamicin or chloramphenicol (38). Resistance to ampicillin has emerged owing to the acquisition of transposable genetic elements and the production of β-lactamases (23). Some Enterobacter isolates can inactivate broad spectrum penicillins and cephalosporins owing to β-lactamase production, which is increasing among strains of C. sakazakii (9, 38).

In this study, antibiotic-resistant C. sakazakii isolates found in domestic kitchens may have originated from processed meat products or other raw foods. Multiple antibiotic-resistant foodborne pathogens from raw and processed meat products harbor antibiotic resistance genes similar to those isolated from human pathogens (1, 8). Farm animals exposed to antibiotics for extended periods of time can develop microbial flora resistant to these agents (57). Resistant infections may be costly to control and can compel the use of a less desirable antibiotics (58). Most reported C. sakazakii infections have occurred in hospital nurseries and neonatal units (31); however, bacteria infection also can occur in the home (50). C. sakazakii could be introduced to the kitchen environment through contaminated food products. The current study indicates that C. sakazakii may find its way into domestic kitchens and might be resistant to antibiotics used in human medicine. C. sakazakii might be transferred from contaminated kitchen sites to infant formula and other ready-to-eat foods via unsafe food handling practices.

The growth and survival of C. sakazakii can be controlled with correct PIF preparation practices and regular cleaning and disinfection of food contact sites. These practices are critical in hospitals, where infant formula is prepared for neonates. Mothers and caregivers are advised to reduce the holding and feeding times for reconstituted PIF because the reconstituted product provides ideal conditions for the growth of harmful bacteria. All equipment and food preparation areas associated with feeding infants and preparing PIF should be thoroughly cleaned before use. Hygiene practices must be improved to reduce the degree of cross-contamination in domestic settings (54), especially when the kitchen is used to reconstitute PIF. C. sakazakii has relatively short lag and generation times; therefore, improper storage of reconstituted PIF at ambient temperature, including on a bedside table for night feeding, may permit the growth of C. sakazakii (18).

In conclusion, this study was conducted to identify and characterize antibiotic-resistant C. sakazakii isolates from various sites in domestic kitchens. New information was gathered about resistance profiles of environmental (household) C. sakazakii isolates. These findings indicate that antibiotic-resistant foodborne pathogens in domestic kitchens may present some risk to consumers and highlight the need for high levels of hygiene, particularly when preparing and storing PIF and food for elderly and immunocompromised individuals. PIF is not a sterile product and can be an ideal medium for bacterial growth. Only knowledgeable consumers can become active advocates for food safety. Because governmental food safety objectives are aimed at consumer protection and apply to food when it is consumed, more studies of foods at the point of consumption are needed.

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