American Journal of Infection Control 42 (2014) 1049-55

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major article

Microbiological analysis of environmental samples collected from child care facilities in North and South Carolina

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Key Words:
Childcare facilities
Day care
Environmental sampling
Microbiological indicators
Enteric pathogens

Background: Children cared for outside the home are at an increased risk of enteric disease. Microbiological analyses were performed on environmental samples collected from child care facilities in North and South Carolina.

Methods: There were 326 samples collected from 40 facilities corresponding to common surfaces (77% of samples) and the hands of care providers (23% of samples). Samples were analyzed for total aerobic plate counts (APCs), total coliforms, biotype I *Escherichia coli*, and pathogens *Shigella* spp, *Salmonella enterica*, *E coli* O157, *Campylobacter jejuni*, and human norovirus.

Results: Median APCs and coliform counts for hands were 4.6 and 1.0 $\log_{10}$ colony-forming units (CFU) per hand, respectively. Median APCs for surfaces were 2.0 and 2.6 $\log_{10}$ CFU for flat and irregular surfaces, respectively. Coliforms were detected in 16% of samples, with counts ranging from 1.0 $\log_{10}$ to >4.3 $\log_{10}$ CFU, with higher counts most often observed for hand rinse samples. Biotype I *E coli* counts were below assay detection limits (<1 $\log_{10}$ CFU) for all but 1 sample. No samples were positive for any of the 4 bacterial pathogens, whereas 4 samples showed evidence of human norovirus RNA.

Conclusion: The relative absence of pathogens and biotype I *E coli* in environmental samples suggests the child care facilities sampled in this study managed fecal contamination well.

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Organized child care is an integral part of US society. By 2010, 75% of US children (from birth to 4 years of age), whose mothers were employed, spent time in nonparental child care.[1] There are many opportunities for the transmission of enteric and respiratory diseases among young children cared for in these settings. It is estimated that US children cared for outside the home are between 2.3 and 3.5 times more likely to experience a diarrheal disease episode than those cared for in their own homes.[2] The total annual cost of diarrheal disease in children <10 years of age in the US may be as high as $2.3 billion.[3]

The 4 bacterial pathogens causing the highest numbers of laboratory-confirmed acute gastrointestinal disease cases in children aged <5 years are *Salmonella enterica*, *Campylobacter jejuni*, *Shigella* spp, and shiga-toxin producing *Escherichia coli*.[4] In their comprehensive review of 75 enteric disease outbreaks associated with child care settings, Lee and Greig[5] found that bacterial and viral agents together were responsible for 93.4% of these outbreaks, in approximately equal proportion. Other investigators have reported similar findings.[6,7] Viral outbreaks were most often caused by rotavirus, human norovirus (NoV), and astrovirus[8,9]; bacterial outbreaks were most often caused by pathogenic *E coli* strains and *Shigella* spp.[5] Published data also suggest that child care attendance is a risk factor for salmonellosis.[10]

Few previous studies, many of which were completed decades ago, have sought to characterize microbiological contamination in the child care environment. Most have used general microbial tests, such as aerobic plate count or coliform count, as indicators of overall

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This project was supported by the USDA Cooperative State Research, Education, and Extension Service National Research Initiative (project no. 2008-51110-04335).

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service or criticism of similar ones not mentioned.

Conflict of interest: None to report.

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0196-6553/$36.00 - © 2014 Published by Elsevier Inc. on behalf of the Association for Professionals in Infection Control and Epidemiology, Inc.
http://dx.doi.org/10.1016/j.ajic.2014.06.030

microbiological load on hands and surfaces and fecal coliforms or *E coli* to indicate recent fecal contamination.[11-14] To our knowledge, no previous studies were specifically designed to detect common enteric bacterial agents, and only a few have sought to identify environmental surfaces as reservoirs for viral pathogens.[15,16] These latter studies were done in conjunction with identified disease outbreaks.

As part of a broader project, the purpose of which was to identify risk factors associated with enteric disease in the child care environment, the objective of this study was to analyze environmental samples taken from child care facilities located in North and South Carolina for the presence of microbiological indicators and select enteric pathogens. This article presents the data on microbial indicator load and pathogen prevalence.

MATERIALS AND METHODS

The Institutional Review Boards at Clemson University, North Carolina State University, and Research Triangle Institute International approved the study protocol, and informed consent was obtained from all study participants.

Sampling sites and surfaces

A convenience sample of 18 North Carolina and 22 South Carolina child care facilities were visited from September 2010 through February 2011. Of these, 31 (77.5%) were classified as centers and 9 (22.5%) as homes. Centers were defined as a facility in which compensated child care was in a location that was not the permanent residence of the provider or administrator, whereas homes were facilities that provided care in the home of the provider or administrator.

Environmental samples were collected (in duplicate) from common surfaces (faucets, toys, refrigerators, diaper-changing areas, etc) and the hands of care providers and food workers. Samples were collected at the end of each site visit, which was approximately 11 a.m., and always before lunches were served. The average number of sites sampled per facility was 8 (range, 5-10). To maintain confidentiality, all sampling data were coded, and the analyst conducting the microbiological testing was blinded to sample identity.

Environmental sampling protocol

For environmental sampling, surfaces were subdivided into 2 categories, that is, flat surfaces (eg, diaper-changing surfaces, food-serving areas) and surfaces of irregular shape (eg, utensils, toys, sink faucets). Two environmental swab samples were collected per surface, 1 was suspended in a 10 mL volume of buffer (for bacterial analysis) and 1 was suspended in 1 mL of buffer (for human NoV detection). In both cases, the 3M Swab-Sampler Letheen Broth (3M, St Paul, MN) was used for sampling, adjusted for the 2 volumes. For flat surfaces, a 10×10 cm^2 area was demarcated using a disposable cardboard template (Weber Scientific, Hamilton, NJ). If the surface was of irregular shape, swabbing was performed on the entire sample surface without the use of a template. The swabbing procedure consisted of pressing the swab head against the surface of its container to release excess moisture, rubbing it slowly and thoroughly back and forth over the target area one time, and then placing the swab back into its container. This procedure was repeated 2 additional times using different swabbing directions for each replicate. The swab was then deposited back in its broth tube, which was sealed and placed on ice.

For hand sampling, the method described by Kampf et al[17] was used. Briefly, the contents of 2 glass tubes containing 10 mL of Tryptic Soy Broth (Thermo Fisher Scientific, Lenexa, KS) were each poured into 2 plastic Petri dishes (9 cm diameter). The distal phalanges (ie, fingertips) of each hand were immersed in the liquid (1 dish for the left hand, 1 for the right hand) and rubbed together (including thumbs) for 1 minute. The fingertips were removed, and the sampling fluid for each hand was then aseptically transferred in its entirety to the original sample containers, which were immediately placed on ice. After all samples had been collected, they were express shipped the same day or hand delivered to the laboratory at North Carolina State University. Microbiological analyses were initiated within 24 hours (usually 12-18 hours) of sample collection.

Enumerative assays for microbiological indicators

A flow diagram of sample processing for microbiological testing is shown in Figure 1. For indicator bacteria, 1 mL from each 10 mL surface swab solution, or from 1 of the hand rinsates, was plated to 3M Petrifilm (3M, St Paul, MN) aerobic plate count (APC) plates followed by incubation at 35°C for 48 hours. An additional 1 mL aliquot was plated to 3M Petrifilm *E coli* and coliform count plates and incubated at 35°C for 24-48 hours. In accordance with the manufacturer's interpretation guide, coliform colonies were red or blue in color, whereas *E coli* colonies were blue; both were surrounded by gas bubbles. Results for all counts were reported as colony-forming units (CFU) per hand or surface sampled.

Presence or absence assays for bacterial pathogens

Samples from all hands and surfaces surveyed were screened for 4 different bacterial pathogens: *S enterica*, *Shigella* spp, *E coli* O157, and *C jejuni*. Cultural enrichment for the first 3 pathogens was done by placing 2.5 mL of the 10 mL swab or hand rinsate sample into 22.5 mL of Universal Pre-Enrichment Broth (UPB; Difco Laboratories, Sparks, MD), followed by incubation at 37°C for 24 hours. Screening for *E coli* O157 was done using the Tecra E. coli O157 Visual Immunoassay kit (3M, St Paul, MN) with the input of 1 mL of UPB per manufacturer instructions. For detection of *S enterica*, 1 mL of UPB was transferred into the Rappaport-Vassiliadis Medium (Thermo Fisher Scientific, Lenexa, KS), which was incubated at 37°C for 24 hours, after which 1 mL was used in the Tecra Salmonella Visual Immunoassay kit (3M, St Paul, MN). Both kits contained their own positive controls. Negative results indicated the absence of *E coli* O157 or *S enterica*. Any presumptive positive results were confirmed in accordance with methods in the Food and Drug Administration Bacteriological Analytical Manual.[19]

For detection of *Shigella* spp, the UPB was streaked onto XLD Agar (Difco Laboratories, Sparks, MD) and incubated at $35° \pm 2°$C for 18-24 hours; presumptively positive colonies were identified in red per media manufacturer instructions. A 250 μL aliquot of UPB was also extracted for DNA isolation using a NucliSENS easyMAG instrument (bioMérieux, Durham, NC) according to manufacturer instructions, with the extracted DNA reconstituted in proprietary elution buffer to a total volume of 50 μL. The DNA was subjected to quantitative polymerase chain reaction (qPCR) targeting the *ipaH* gene, which encodes the invasion plasmid antigen H for *Shigella* spp.[18] All primers and probes used were obtained from Integrated DNA Technologies (Coralville, IA). The 25 μL reaction mixture consisted of a 2 μL sample of DNA, 0.4 μM primers, 0.2 μM probe, 0.2 mM of each dNTP (Applied Biosystems, Foster City, CA), 0.5 μL of reference dye ROX (Invitrogen, Carlsbad, CA), and 15.5 μL Diethylpyrocarbonate (DEPC)-treated nuclease-free sterile water added to a PCR reaction tube containing the concentrations of a PCR buffer, magnesium chloride, and enzyme mixture recommended by the manufacturer of Platinum Taq Polymerase (Invitrogen,

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