Are We Aware of Microbial Hotspots in Our Household?

Abstract
Household microorganisms are found in unexpected places. Therefore, the authors conducted a study to investigate the microbial hotspots and reveal the misconceptions regarding the most contaminated objects in the household. In the authors’ study, 26 daily use objects in 22 households were sampled to determine the levels of heterotrophic plate count (HPC), coliforms, E. coli, yeast and mold, and Staphylococcus aureus. High microbial concentration was found in the kitchen area and the dish sponge was the most contaminated item in the household, followed by the toothbrush holder. Coliforms were most prevalent in the kitchen on items such as sponges, sinks, and cutting boards. Yeast and molds were found on leather, fabric, porcelain, and laminate, and S. aureus was found on personal objects and pet’s items. Overall, HPC and the presence of coliforms were significantly related to surface type (p < .05). In the kitchen, cleaning frequency (p < .03) and type of cleaning (p < .0003) had significant effects on HPC. The authors’ study provides information that will help the general population to make an educated decision in developing a proper and routine cleaning regime in their homes. This baseline data might contribute to designing appropriate sanitation guidelines or standards that will help to implement proper sanitation practices in households and to conducting further research in the area of foodborne and household communicable diseases.

Introduction
Microorganisms such as bacteria and fungi are ubiquitous in the environment and we are continuously interacting with them. Human activities can influence the level and diversity of microorganisms associated within a particular environment (Hunter, 2007; Paerl et al., 2002). The household is one such environment where we live, interact, and spend most of our time apart from our workplace. Based on our routine activities and common knowledge we tend to pay more attention to certain areas and neglect others in the household in terms of cleanliness and cleaning activities; therefore, we unknowingly become prone to infections and transmit pathogens or opportunistic pathogens. Understanding our own environment such as the household and its objects in terms of microorganisms is crucial to improving overall health and safety and also in revealing some of our misconceptions about the habitat of these microorganisms within the household.

With the advent of globalization, we use a wide variety of household products including food items from different parts of the world that might expose us to different types of microbial strains with atypical characteristics (Kaferstein, Motarjemi, & Bettecher, 1997). It has been previously reported that fomites contribute to the transmission of pathogens and outbreak of foodborne illnesses at home (Boone & Gerba, 2007; Cogan, Bloomfield, & Humphrey, 2002).

The recent media exposure of outbreaks of microbial infections has led to an increase in the practice of using antimicrobial agents in the form of hand sanitizers, dishwasher solutions, disinfection wipes, and several other kitchen and bathroom items, which might impact the levels and diversity of different microorganisms. Due to the lack of proper guidelines and knowledge regarding home sanitation and hygiene, the overuse of wide varieties of these antimicrobial products available might give rise to more resistant organisms (Levy, 2001).

Given the over 48 million incidences (Centers for Disease Control and Prevention [CDC], 2012a) of food safety illness reported each year, it is important to note where microorganisms are most prevalent (i.e., hotspots) within our homes so that we can take proper steps to regularly sanitize them and safeguard against foodborne illness. We therefore conducted a study to investigate the “hotspots” for microorganisms in household objects and to reveal the general public’s certain misconceptions about the most contaminated areas in their households. The baseline data generated in our study are expected to contribute to further research for the risk assessment of various transmissible and foodborne illnesses.
The specific objectives of our study were a) to determine the levels of heterotrophic plate count (HPC), coliforms, E. coli, yeast and molds, and Staphylococcus aureus in different household objects and personal items and b) to make a comparative analysis of the overall levels of microbial contamination in household environments based on different parameters such as type of surface, frequency of cleaning, type of cleaning agents, and method of cleaning. Our study is expected to aid the general public in understanding better household sanitation issues and in making educated decisions to implement proper household sanitation practices.

**Materials and Methods**

**The Study Design**

Twenty-two households in southeast Michigan were selected for inclusion for a microbiological survey. The volunteer households were selected randomly. Ethnicity and economic status of the volunteers did not factor into their...
selection for the study. Households possessing children and pet(s) were targeted in our study. Four main classifications of objects and surfaces were focused on within the research project: kitchen, bathroom, pet(s) objects, and personal items. Twenty-six locations that were considered "high-touch" areas, given the activity of the typical household population, were subjected to microbiological sampling. The locations were as follows: kitchen (eight surfaces—dish sponge, kitchen sink, coffee reservoir, counter top, stove knobs, cutting board, microwave handle, and refrigerator handle); bathroom (six surfaces—toothbrush holder, faucet handle, toilet seat, door knob, light switch, and toilet handle); pet items (two surfaces—drinking bowl and toy [includes balls and rubber toys]); and personal objects (10 surfaces—pen, keys, cellular phone, iPod, lunch box, video game controller, remote control, bottom of purse, wallet, and keyboard). Each item or surface was sampled for microbiological bioburden and characterization as described below.

Collection of Samples
A laboratory representative trained in aseptic sampling was responsible for procuring microbiological swab samples from each of the 26 locations listed previously. Sampling occurred during the months of December 2010 and January 2011. The swabs utilized in this study were 3M Quick Swabs, rayon-tipped swabs containing lutein neutralizing buffer. For the sponge samples, the entire sponge was transferred to a sterile Whirl-Pak bag. The analyst obtained swabs from the 26 locations per the directions provided by swab manufacturer (3M Microbiology, 2002).

Following surface sampling, the swabs were flooded with lutein neutralizing and placed at 4°C for transport back to the NSF International laboratory for processing. If delivery could not be achieved the same day as sampling, the samples were held overnight at 4°C and then delivered. At the time of sampling, the analyst recorded the surface area sampled in cm². The following additional data per each surface location were recorded: surface type (sponge, stainless steel, plastic, laminate, porcelain, fabric, leather, or metal); cleaning frequency (never, daily, weekly, or monthly); type of cleaning agent used (quaternary ammonium, chlorine, or other); type of cleaning (aggressive scrub, light wipes, or other).

Microbial Analysis of the Samples
A 4-mL aliquot of phosphate-buffered saline with 0.05% Tween 20 was added to each swab container to bring the total volume within the container to 5 mL. Unless otherwise noted, all reagents and chemicals were American Chemical Society reagent grade or higher. The swab samples were vortexed for three durations of 30 seconds each. The swabs were removed and disposed of. The eluent was serially diluted in phosphate-buffered saline. The dilutions were processed for microbial content using the following nonselective and selective media: total aerobic plate count bacteria used R2A agar; total coliforms and E. coli used the 3M Petrifilm E. coli/Coliform Count Plate; total yeast and mold used the 3M Petrifilm Yeast and Mold Count Plate; and S. aureus used the 3M Petrifilm Staph Express Count Plate. For the sponge sample, a 1-g subsample was aseptically removed and placed into phosphate-buffered saline with 0.05% Tween 20. The volume of eluent buffer varied depending on the absorbency of the sponge. The final volume amended was recorded for use in calculating observed bacterial and fungal densities. Serial dilution and organism plating for the sponge samples were carried out as specified for the swab samples. The R2A plates were incubated for 72 ± 4 hours at 30°C ± 1°C. The yeast and mold plates were incubated for five days at 30°C ± 1°C. The coliform/E. coli and S. aureus plates were incubated for 24 hours ± 2 hours at 37°C ± 1°C. Confirmation of presumptive positive S. aureus colonies was performed through the addition of a 3M Petrifilm Staph Express Disk and incubation of the plate for three hours at 37°C ± 1°C. Following incubation, the plates were enumerated and the bacterial and fungal concentrations for each sample were calculated. All plates possessing growth were stored at 4°C for future isolate identification and examination.

To confirm adequate performance of the swabs and growth media, the following bacteria and fungi were utilized as control organisms: S. aureus American Type Culture Collection (ATCC) 8833, E. coli ATCC 11229, Saccharomyces cerevisiae ATCC 18824, and Aspergillus niger ATCC 2075. All cultures were obtained from ATCC. The microorganism strains were grown according to ATCC's instructions. Individually, 0.1-mL aliquots of 24-hour-old bacterial and yeast strain suspensions were inocu-
Median (95% Confidence Interval) Heterotrophic Plate Count Bacteria (HPC) Values Found in Kitchen Area

Data Analysis and Statistical Methods
The median HPC, rather than the mean values, are represented in the figures because the HPC had levels of contamination that varied from <1 up to 10^6 CFU/10 cm², and the data failed to meet the assumptions of normal distribution (Kolmogorov-Smirnov test, p < .05). Univariate ANOVA was conducted with the log-transformed HPC data as the response variable and surface type, cleaning frequency, cleaning agent, and type of cleaning as independent variables to see if these predictors had any effect on HPC. The predictor variables considered in our study were in the form of nominal categorical data. The bacterial count data of coliforms, yeast and molds, and S. aureus were converted into binary response variables (presence-absence). For each of coliform, yeast and mold, and S. aureus, separate logistic regression was used to determine the relationship, if any, between presence of these microbes and the independent variables. All statistical analyses were done using the SPSS 17 software package.

Results
HPC from the surfaces of different household items sampled in our study exhibited large variations; values obtained ranged from <1 to 1.6 X 10^6 CFU/10 cm². The median (99% confidence interval (CI)) HPC values for each of the objects sampled are represented in Figure 1. The figure illustrates that the bacterial counts were highest in the dishwashing sponges in the kitchen followed by toothbrush holder, pet bowls, and kitchen sinks, whereas personal items such as purse, wallet, and cellular phone reported the lowest counts. An overall comparison of HPC showed that the median bacterial counts were highest in the pets' items followed by kitchen and bathroom (Figure 2a). It is interesting to note that even though a majority of the fomites that exhibited high levels of contamination belonged either to the kitchen or bath-
the light switch in the bathroom and microwave handle in the kitchen had comparatively lower levels of HPC in our study sample. When HPC was categorized based on surface type, porcelain and laminate topped the list apart from sponges (Figure 2b).

Results of univariate ANOVA on the log-transformed data revealed that only surface type (F = 19.6, df = 8, p < .05) had a significant effect on HPC (Table 1). Even though HPC was lower on the surfaces that were cleaned with chlorine-based cleaner (3.9 x 10^5 CFU/10 cm^2), compared to quaternary ammonium (9.0 x 10^5 CFU/10 cm^2), the overall differences were not significant. Interestingly, the use of light wipes showed a lower HPC value than an aggressive scrub. An ANOVA was performed separately on the kitchen data to see if the cleaning parameters had any effect on HPC (Figure 3). The kitchen was a focal point given that a majority of the kitchen surface locations ranked in the top 10 in terms of highest bacterial concentration. The results indicated that cleaning frequency (p < .05) and type of cleaning (p < .0003) had significant effects on HPC.

A total of 572 surfaces were sampled, of which yeast and molds were found to be positive for a maximum number of surfaces, followed by coliforms, S. aureus, and E. coli (Figure 4). For each of the objects sampled, the percentage of surfaces positive for coliforms, yeasts, and molds, and S. aureus is presented in Figure 5. We observed that coliforms were more prevalent on the kitchen surfaces (Figure 6a), including dish sponge/ rags (22.7%), kitchen sinks (13.3%), countertops (9.3%), and cutting boards (5.3%). Yeasts and molds were predominant on fabric (85.7%), leather (45.4%), plastic (38.4%), porcelain (61.3%), and stainless steel surfaces (49.7%), whereas S. aureus was found mostly on pet's items (39.5%) and personal objects (23.3%) (Figures 6a, 6b). Only two factors showed highly significant (p < .001) associations with the presence of coliform bacteria: surface type and cleaning agent (Table 2). The coliform colonies were significantly higher in dish sponges (odds ratio [OR] = 122.07, p < .05) and porcelain (OR = 23.65, p < .01). The presence of yeast and molds and S. aureus was not significantly related to any of the factors except for S. aureus, in which cleaning frequency had a significant relation (p < .05) (Table 2).

**Discussion**

Our study was conducted to help identify the microbial "hotspots" in an average person's home, with an intention to help people understand how they can better protect themselves from different household microorganisms, some of which might be pathogens or opportunistic pathogens. Not all of the microorganisms that we interact with are harmful; some are beneficial and vital for our existence. A typical human microbiota has been estimated to have 10 times as many microbial cells as the human body (Selikow, Russell, Antunes, & Finlay, 2010) and many of these bacteria are critical to our health in that they actually help fight off disease and chronic conditions (Ley, Turnbaugh, Klein, & Gordon, 2006).

Before conducting the swab analysis, NSF International asked a member of each volunteer household to rank the items they thought would have the most contamination in their home. The survey revealed misconceptions about which household items have the most microorganisms. Items found in the bathroom were most frequently ranked by survey respondents to be most contaminated in the home. The swab analysis, however, revealed that kitchen items actually had higher HPC compared to the bathroom items. More specifically, the volunteers perceived the rank of contamination in the following order (from highest to lowest): toothbrush holder, dish sponge, money, pet toy, kitchen countertop, bathroom door knob, kitchen sink, pet bowl, toilet handle, and bathroom light switch. On the contrary, the top 10 items in our study that exhibited HPC (highest to lowest) were dish sponge, toothbrush holder, pet bowl, kitchen sink, coffee reservoir, kitchen countertop, stove knob, pet toy, toilet seat, and bathroom faucet handle (Figure 1).

The results indicated that the kitchen area, where sanitation is an important concern, had the highest HPC. In fact, sponges and dishrags, the very items used to clean the kitchen, topped the list. The high HPC could probably
be a function of their use. The porous nature of sponges allows them to pick up and hold bacteria and nutrients through the cleaning process. They are often not properly left to dry and instead are left in damp areas, which provide an optimal environment for microbial growth. Additionally, they are not properly sanitized before their next use. The results obtained in our study are also supported by other works that have demonstrated that despite the use of dishwashing liquid agents, it was difficult to reduce contamination levels in used dish sponges (Kusumaningrum, van Putten, Romhouts, & Beumer, 2002).

The kitchen was also the area of the home where coliform bacteria were most prevalent, probably due to the presence of raw food products such as poultry, fruits, and vegetables. Because of cross contamination, the high coliform count on these surfaces can cause potential foodborne illnesses at home (CDC, 2012b). This generally occurs when hands or items are not properly sanitized after coming in contact with a contaminated surface. Similar results were reported in other studies irrespective of geographic locations and lifestyle (Chaidze & Gerba, 2000; Ojima et al., 2010; Shruti, Pankaj, Shelhar, & Rachika, 2011).

Our study showed that porcelain, laminate, and stainless steel had high numbers of positive surfaces for coliforms along with yeast and molds (Figure 6b). This result is well supported by other works that have highlighted that pathogens can remain viable on dry utensils and stainless steel surfaces in the kitchen environment for considerable periods of time (Kusumaningrum, Riboldi, Hazleger, & Beumer, 2003; Scott, Bloomfield, & Barlow, 1982). While certain household items like the kitchen sink and sponge are “hotspots” for microorganisms because of their function in the home, others allow for...
### TABLE 2

Logistic Regression Model of Coliform, Yeast and Mold, and *Staphylococcus aureus* in Different Household Objects

<table>
<thead>
<tr>
<th>Variables</th>
<th>df</th>
<th>Coliform</th>
<th></th>
<th></th>
<th>Yeast and Mold</th>
<th></th>
<th>S. aureus</th>
<th></th>
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<tr>
<td></td>
<td></td>
<td>B</td>
<td>p-Value</td>
<td>OR</td>
<td>B</td>
<td>p-Value</td>
<td>OR</td>
<td>B</td>
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<td>1.80</td>
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<td>1.80</td>
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<td>.31</td>
<td>3.40</td>
<td>0.59</td>
<td>.12</td>
<td>1.80</td>
<td>1.53</td>
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<td>.998</td>
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<td>2.00</td>
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<td>.45</td>
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<td>0.72</td>
<td>.82</td>
<td>1.24</td>
<td>.098</td>
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<td>.82</td>
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<td>.098</td>
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<tr>
<td>Others†</td>
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*df = variable coefficient; OR = odds ratio.

*The values in bold indicate significant effect.

*For the category "cleaning agent," the group "others" included natural products.

*For the category "type of cleaning," the group "others" included rinsing by hand in water.

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substantial microbial growth because they are easily overlooked. This holds true for the coffee reservoir (where water is held in the coffee maker before brewing), which contained high counts of yeast and mold. Another example of a neglected item that showed high levels of bacteria was the toothbrush holder. In smaller bathrooms, it is generally placed in close proximity to the toilet and flushing often causes aerosols, containing fecal bacteria, to land on items near the toilet (Gerba, Walls, & Melmick, 1975). Microbes quickly multiply because the toothbrush holder sits in a damp area and is often neglected in the cleaning process, since it often just gets a quick rinse after daily use.

Our study highlighted that cleaning frequency, agent, type of cleaning, and their interactions did not have any significant effect on HPC values (Table 1). This was also evident from the fact that objects like the dish sponge, countertop, cutting board, kitchen sink, pet's bowl, toilet seat, toilet handle, and bathroom faucet handle, which were cleaned frequently (either daily or weekly), exhibited high HPC values. Possible explanations could be that a) cleaning procedures were not effective against microorganisms in the households sampled in this study; b) these items had greater probability of cross contamination compared to personal items like remote controls, cellular phones, and purses (the items never/rarely cleaned); c) kitchen and bathroom items provide a moist and damp environment, conducive for microbial growth compared to personal items; or d) the more aggressive cleaning method may result in liberating higher concentrations of bacteria to the surface.

Earlier studies conducted by Rustin and co-authors (1998) demonstrated that frequent cleaning following a strict regimen with a combination of different hypochlorite products was successful in reducing the levels of microbial contamination in different kitchen and bathroom objects. Our study group was randomly selected, however, with an aim to evaluate the real-time scenario of microbial contamination in an average household. Also, we tested the effects of the different factors on the pooled HPC data obtained from all the objects sampled in our study. Further studies with increased sample size are required to specifically investigate if cleaning frequency, cleaning agent along with contact time, and proper cleaning procedure could influence the overall levels of microbial contamination in household environments. Additional
studies also could be performed to examine if microbial concentrations are reduced over time if a more routine, aggressive cleaning regimen is implemented for these hotspots.

**Conclusion**

Our study was successful in indicating the microbial hotspots in a general household and also clarified some of the misconceptions regarding the most contaminated areas in a household. The findings of our study will help environmental health professionals to a) educate the general population in understanding the importance of the overall household hygiene and sanitation practices, b) perform risk assessment and help to identify areas and objects contributing to household communicable and foodborne illnesses, c) implement appropriate measures to reduce transmission of diseases through fomites, d) establish a standard or guidelines to implement an effective household environmental monitoring program, and e) identify the main reservoirs or carriers of microorganisms in the household. This will help to investigate further and identify some of the dominant microorganisms, including their source, role, and interactions in household environments such as biofilm-forming capabilities and potential resistance to disinfectant and sanitization agents. Additionally, the risk assessment strategy detailed in this article can be directly applied to other environments and workplace settings. Overall, the baseline data will help to increase awareness and protect public health and safety.

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