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Microbial Hotspots and Diversity on Common Household Surfaces

Understanding the microbial ecology and distribution in the household environment is crucial to improving overall health and safety. By performing a controlled environmental survey of numerous households, determining the levels of microbial growth, identifying the microorganisms on different objects and personal items, and evaluating cleaning regimens, the data gathered can reveal critical trends in the microbial ecology, as well as some of our misconceptions about the habitats of these microorganisms within the household. This can help to better understand potential sources and transmission of pathogenic organisms, as well as assist in the development of a proper cleaning regimen in the household to improve the health and safety of the family.

Summary

NSF International, in collaboration with the Charles River Accugenix group, conducted a study to investigate microbial hotspots, uncover the types of culturable microorganisms, and reveal the misconceptions regarding the most contaminated objects in the household. 30 daily-use objects in "high-touch" locations around the home from 17 households were obtained and sampled to determine the levels of heterotrophic plate count (HPC), coliforms, *Escherichia coli*, yeast, molds and *Staphylococcus aureus*. From these 30 objects, 340 bacterial isolates on HPC plates were identified using three different platforms: MALDI-TOF mass spectrometry, 16S rDNA sequencing, and Biolog.

Accugenix® AccuPRO-ID®, a polyphasic service consisting of MALDI-TOF backed up by AccuGENX-ID® 16S rDNA sequencing, was accurate to the species level for 86% of the isolates, 10% accurate to the genus level, and resulted in 2% incorrect answers. In contrast, Biolog provided no identification on 66% of the isolates, was 8% correct to the species level, 14% correct to the genus level, and incorrect for 9%.

High microbial concentrations were found in the kitchen, with the dish sponge being the most contaminated item, followed by the toothbrush holder. Coliforms were most prevalent in the kitchen; yeast and molds on leather, fabric, porcelain and laminate; and *S. aureus* on personal and pet objects.

Overall, HPC and the presence of coliforms were significantly related to surface type ($p < 0.05$). In the kitchen, cleaning frequency ($p < 0.03$) and type of cleaning ($p < 0.0003$) had significant effects on HPC. Irrespective of the source, bacteria belonging to the phylum *Actinobacteria* were more predominant. Among the Gram-positive bacteria, species belonging to the genus *Micrococcus* were most prevalent, followed by *Kocuria* and *Microbacterium*. However, the top contaminated object, the dish sponge, contained more Gram-negative organisms (*Stenotrophomonas spp., Pseudomonas spp*. and *Klebsiella spp*.). Several opportunistic pathogens (*S. haemolyticus, K. oxytoca, K. pneumoniae* and *S. maltophilia*) were also recovered. Additionally, it was noted that objects cleaned with quaternary ammonium-based products had less diversity compared to chlorine-based products.

Methods

Individual bacterial isolates were cultured on TSA. R2A medium was used for HPC count while 3M petrifilms containing selective and differential media were used for coliforms, *E. coli, S. aureus*, yeast and molds. Classical techniques were performed to determine Gram reaction. Statistical analyses were performed using the SPSS 17 software package. Biolog was carried out according to the manufacturer's instructions using Microlog 3 software with the GP and GN databases. Molecular identity of the bacterial isolates was determined by AccuGENX-ID® 16S rDNA sequencing and analysis, and the Accugenix® identification reference library. MALDI-TOF microbial identification was performed on either the microflex™ LT or autoflex™, according to the manufacturer's recommendations (Bruker Daltonics, Inc., Fremont, CA).

Conclusions

Not only can the locations and types of surfaces influence the quantity and diversity of microorganisms associated with a particular niche, but human practices and activities can also have a significant impact. Evaluation of household microbial ecology can help elucidate sources of contamination and the effectiveness of cleaning procedures.

Of critical importance in this type of evaluation is the accurate species-level identification of recovered isolates. Accurate classification of these isolates facilitates a better understanding of the impact microorganisms have on an environment, whether it be a manufacturing environment or a home. Reliable microbial identification systems are needed for consistent and accurate results to permit sound data comparisons, interpretations and tracking of organisms to their source. When identifications are based on phenotypic characteristics, such as with Biolog, the methods are highly error-prone and performance is more variable. With the MALDI-TOF-based method of identification that is supported by AccuGENX-ID® 16S rDNA sequencing – the AccuPRO-ID® solution – there is a highly accurate option for routine identifications.

When conducted with reliable microbial identification systems like AccuPRO-ID®, studies such as this can help us to better understand potential sources and transmission of pathogenic organisms and contribute to designing appropriate sanitation guidelines/standards to help the general public make educated decisions in developing a proper, routine cleaning regimen for their homes.

*The content of this Technical Note is an abridged version of a poster originally presented at the 112th Annual Conference of the **American Society for Microbiology** on June 16-19, 2012, in San Francisco, California. Poster No: Q-621. Authors: Ratul Saha, Susan Wheeler, Lorelle Bestervelt, and Robert Donofrio from NSF International of Ann Arbor, MI; Nabaneeta Saha from University of Calcutta, Kolkata, India; Christine Farrance, Bindhu Verghese, and Sunhee Hong from Accugenix, Inc. of Newark, DE.*

Figure 1. Microbial distribution by household object

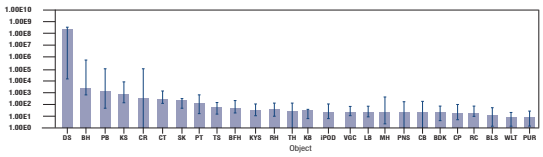

Figure 1. Median HPC of different household objects sampled in the study (95% CI). HPC values are presented in colony-forming units (CFU) per 10 cm^2. The top five locations for microbial counts were the dish sponge (DS), toothbrush holder (TBH), pet bowl (PB), kitchen sink (KS) and the coffee reservoir (CR). The high HPC on these items is mostly likely due to the function of their use and locations. The identification of the flora is presented in Figures 3-7.

Figure 2. Microbial distribution by location and surface

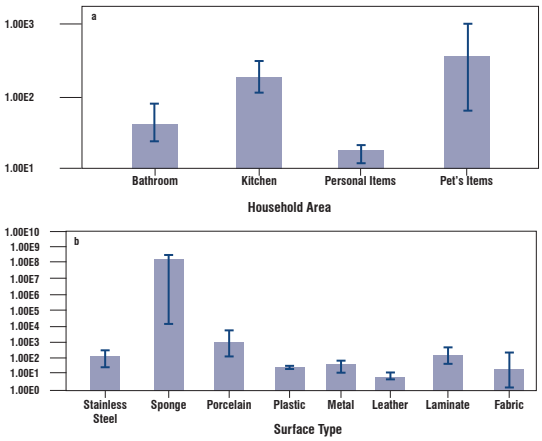

Figure 2. Median HPC values based on (a) household area and (b) type of surface (95% CI). The median bacterial counts were highest on the pet's items, followed by kitchen and bathroom. Sponges, porcelain and laminate topped the list when HPCs were categorized based on surface type.

Table 1. AccuGENX-ID® performance

Table 1. Classification performance of 16S rDNA sequencing of environmental isolates		
Taxonomic Level	Frequency	Number
Species	93.2%	317
Genus	4.7%	16
Family	0.3%	1
No Sequencing Signal	1.8%	6

Table 1. In this study, we compared the accuracy of AccuPRO-ID® and the Biolog GEN II Microplates to the results from AccuGENX-ID®. Of the 340 isolates characterized, 16S sequencing was able to provide a genus- or species-level identification for 97.9% of the samples (Fig. 3). One sample was identified to the family level. Six of the samples resulted in no signal from the sequencing reaction. The biodiversity represented 131 different species in 46 different genera, with *Micrococcus, Kocuria* and *Microbacterium* species comprising approximately 44% of the isolates (Fig. 3). *Micrococcus luteus* was, by far, the most frequently occurring organism, with 17% of the samples, followed by two different *Kocuria* species at 5.9% and 5% (Table 3).

Table 2. Percentage of surfaces positive for *E. coli*, coliforms, *S. aureus*, yeast & mold

Table 2. Organisms of concern selectively isolated from household items	
Organism/Group	Percentage of Positive Surfaces
Yeast/Mold	31.7%
Coliforms	11.7%
S. aureus	6.4%
E. coli	0.3%

Table 2. Organisms were isolated on selective and differential media. Detection of these potential pathogens reinforces the importance of maintaining a routine cleaning regimen to protect the overall health of the family.

Figure 3. Frequency of culturable bacteria recovered from different objects

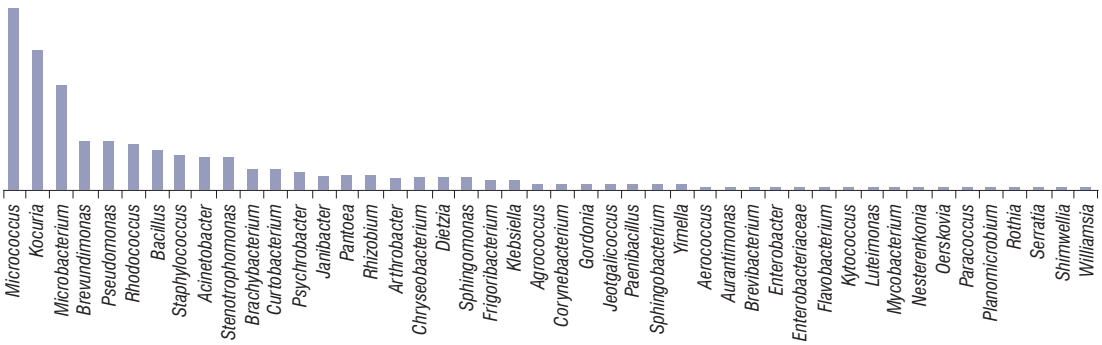

Figure 3. Frequency of occurrence (n=334, isolates). Irrespective of source, the levels of Gram-positive bacteria were higher than Gram-negative bacteria. Bacteria belonging to the phylum Actinobacteria were more predominant. The 3 most frequently present bacteria (*Micrococcus, Kocuria* and *Microbacterium*) are ubiquitous and also found as normal flora of the human skin, so it is not surprising to encounter them as the dominant microorganisms in high-touch household objects. However, it is important to note that they are also considered opportunistic pathogens, in addition to numerous other bacteria identified during this study, and are reported to cause infections in immunocompromised patients. Their survivability on the surfaces of objects even after cleaning could be an issue of concern in terms of transmission to individuals coming in contact with those objects.

Figure 4. Diversity of culturable bacteria on the five most contaminated objects

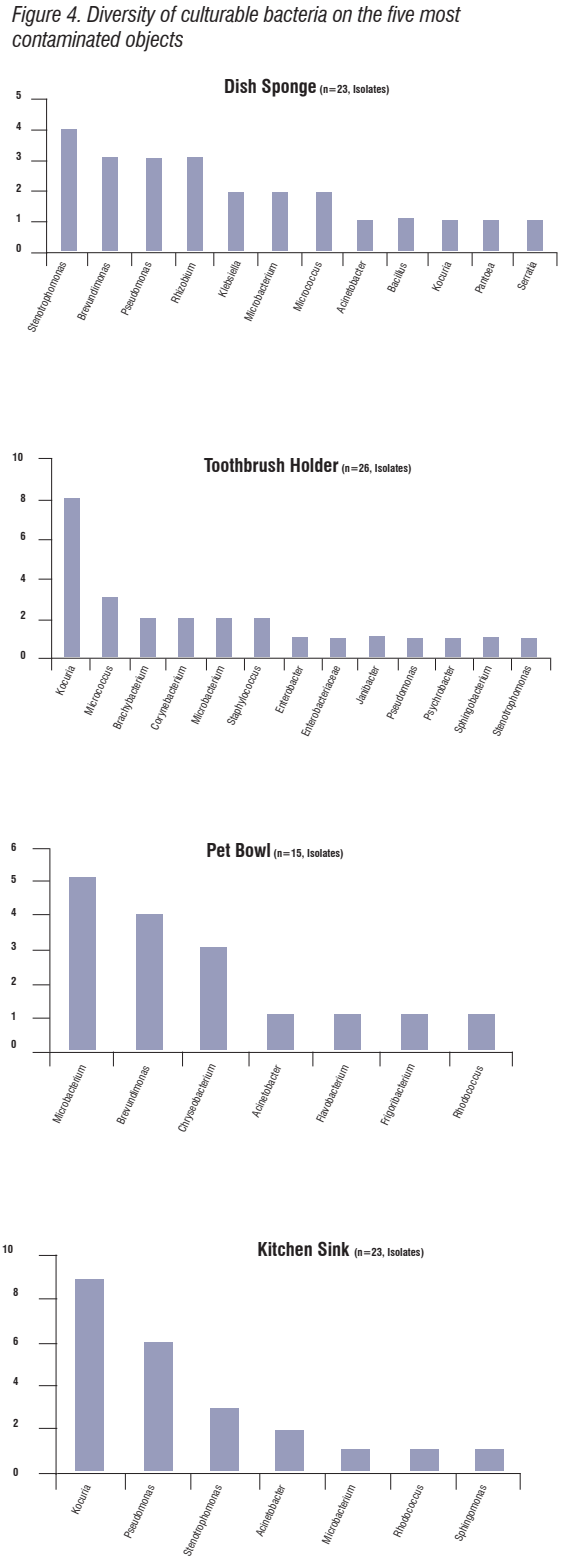

Figure 4. Frequency of occurrence. Gram-negative organisms were more prevalent in the dish sponge compared to other objects. Less diversity was observed in the coffee reservoir. However, organisms such as *Arthrobacter* and *Bacillus*, which are known to be found in extreme environments, were also isolated. Additionally, these organisms are less susceptible to different daily-use disinfectants. The data indicate that some objects might require a more stringent cleaning regimen than others.

Figure 5. Performance of different identification technologies

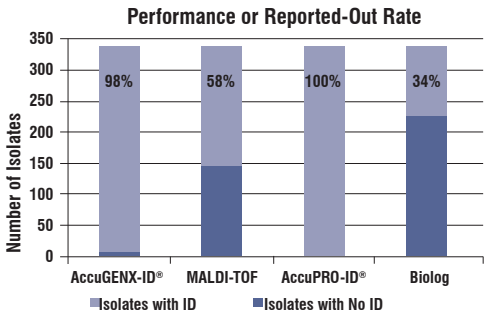

Figure 5. While sequencing identified approximately 98% of the isolates, MALDI-TOF alone identified 58% of isolates and the Biolog GEN II Microplates identified just 34%. AccuPRO-ID®, the combination of AccuGENX-ID® and MALDI-TOF, identified 100% of the isolates. When using a MALDI-TOF identification system, there are different reasons for the failure to produce an identification. A "No ID" result can occur due to the spectra not meeting a quality threshold, the lack of reference spectra in the library, or because the sample was too old and failed to grow upon subculture. It is in these instances that an isolate is subjected to AccuGENX-ID® to provide identification. Lack of an identification when using the Biolog system can occur when the results of the biochemical tests are ambiguous and lead to conflicting or low probability options or, frequently, because the organism in question is not in the library.

Figure 6. Frequency of different bacteria based on surface types

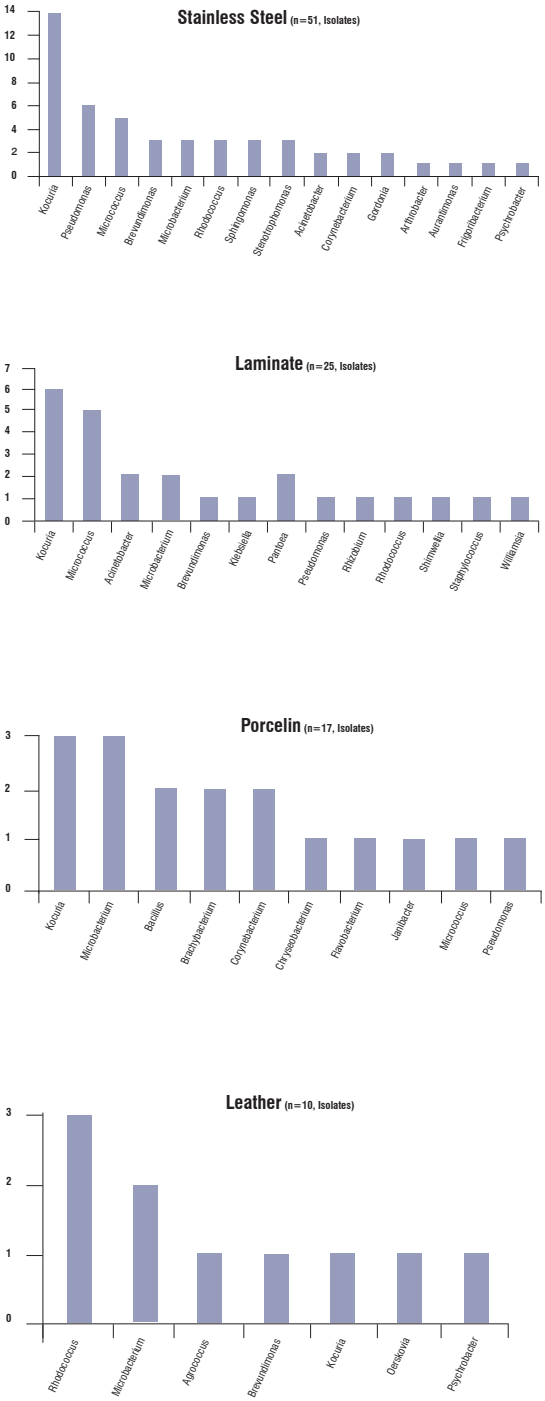

Figure 6. Frequency of occurrence. Stainless steel and laminate-based surfaces showed more diversity compared to other surfaces. Difference in diversity was also observed between cleaning frequency, type of cleaning and cleaning agent (e.g., chlorine- and quaternary ammonium-based agents, data not shown). Even though the dominant taxa were *Micrococcus*, the bacterial diversity was higher for surfaces that were never cleaned compared to those that are cleaned, irrespective of the cleaning frequency.

Figure 7. Accuracy and performance of the identifications made by both AccuPRO-ID® and Biolog as compared to AccuGENX-ID®

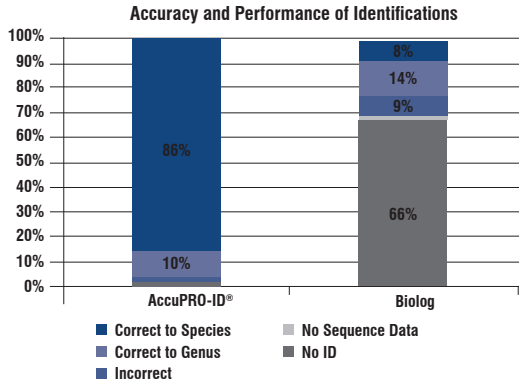

Figure 7. While the ability to assign a name to an environmental isolate is important, it is more critical to have an accurate identification. Comparing the data from AccuPRO-ID® and Biolog to the results obtained with AccuGENX-ID®, the accuracy can be evaluated. AccuPRO-ID® was correct to the species level for 86% of the isolates, to the genus level for 10%, and resulted in 2% incorrect answers. In contrast, Biolog was correct to the species level for 8% of the isolates, to the genus level for 14%, and incorrect for 9%. Overall, the Biolog phenotypic system was 10-fold less accurate to the species level than AccuPRO-ID® and left roughly two-thirds of the samples with no identification.

Table 3. Frequency of "No ID" by Biolog

Table 3. Evaluation of the identification of the most frequently occurring organisms by Biolog

Species Name	Occurrence in Study	Number			
		No ID	Incorrect	Correct to Genus	Correct to Species
Micrococcus luteus	57	48	3	2	4
Kocuria palustris	20	19	1	-	-
Kocuria rhizophila	17	13	4	-	-

Table 3. For phenotypic systems like Biolog, inconclusive results often occur, as organisms isolated from their environment tend to be physiologically stressed. This can lead to a higher rate of erroneous identifications, or, in this study, a high frequency of "No ID." For example, 57 of the environmental samples were identified by AccuGENX-ID® as *M. luteus*. However, Biolog provided "No ID" for 48 of the isolates (even though the organism is present in the library) and an incorrect identification for 3, and was correct to the genus level for 2 and the species level for only 4. The second most frequently occurring organism, *K. palustris*, is not present in the Biolog database and resulted in "No ID" for 19 isolates and a misidentification for one. *K. rhizophila* isolates resulted in "No ID" for 13 out of 17 and classification with outdated nomenclature for 4 isolates, even though the organism is present in the reference database.