Surface Microbes in the Neonatal Intensive Care Unit: Changes with Routine Cleaning and over Time

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Premature infants in neonatal intensive care units (NICUs) are highly susceptible to infection due to the immaturity of their immune systems, and nosocomial infections are a significant risk factor for death and poor neurodevelopmental outcome in this population. To investigate the impact of cleaning within a NICU, a high-throughput short-amplicon-sequencing approach was used to profile bacterial and fungal surface communities before and after cleaning. Intensive cleaning of surfaces in contact with neonates decreased the total bacterial load and the percentage of Streptococcus species with similar trends for total fungal load and Staphylococcus species; this may have clinical relevance since staphylococci and streptococci are the most common causes of nosocomial NICU infections. Surfaces generally had low levels of other taxa containing species that commonly cause nosocomial infections (e.g., Enterobacteriaceae) that were not significantly altered with cleaning. Several opportunistic yeasts were detected in the NICU environment, demonstrating that these NICU surfaces represent a potential vector for spreading fungal pathogens. These results underline the importance of routine cleaning as a means of managing the microbial ecosystem of NICUs and of future opportunities to minimize exposures of vulnerable neonates to potential pathogens and to use amplicon-sequencing tools for microbial surveillance and hygienic testing in hospital environments.

Materials and Methods

Data availability. The sequencing data sets generated in this study are publicly available in the Quantitative Insights into Microbial Ecology (QIIME) database (www.microbio.me/qiime/) as study identification number 1797 (fungal sequencing run) and study identification number 1798 (bacterial sequencing run).

Cleaning procedures in the NICU. (i) Intensive cleaning. When a piece of equipment (isolate, radiant warmer, etc.) was no longer needed for an infant, it was cleaned by the housekeeping staff with a liquid solution (3M Quat disinfectant 25H [active ingredients, 13% ethylbenzyl ammonium chloride and 13% n-alkyl dimethyl benzyl ammonium chloride]) on a soft cloth.

(ii) Routine cleaning. At the beginning of each 12-h shift, a registered nurse (RN) washed his or her hands, put on nonsterile gloves, and wiped surfaces (computers, computer mice, tables, cribs, monitors, etc.) with an antibacterial wipe (PDI Super Sani-Cloth germicidal disposable wipe [active ingredients, 0.25% n-alkyl dimethyl ethylbenzyl ammonium chloride and 0.25% n-alkyl dimethyl benzyl ammonium chloride]). The only surfaces not cleaned with the antibacterial wipes were the isolettes, which were cleaned with a soft cloth moistened with water. Each stethoscope remained at a given bedside and then was cleaned with an alcohol wipe after the baby in that bed was discharged.
Disposable items (e.g., tubing, nose pieces for continuous positive airway pressure [CPAP] machines, pacifiers, and suction catheters) were not cleaned or reused.

**Sample collection and DNA extraction.** A total of 147 samples were collected from the NICU at UC Davis Children’s Hospital (Sacramento, CA) by a single investigator from August to December 2012. Multiple sites on three isolettes, two radiant warmers, and one ventilator were sampled before and after intensive cleaning. A list of all sites tested is included in Table S1 in the supplemental material, and the sites are illustrated in Fig. 1.

The surfaces were sampled with sterile cotton-tipped swabs (Covidien, Mansfield, MA). The swabs were moistened with sterile phosphate-buffered saline and streaked across a 4-in² area (or the entire surface for surfaces of >4 in²) in two perpendicular series of firm overlapping S strokes; swabs were rotated to ensure full contact of all parts of the swab tip and the surface. Swab tips were snapped off into sterile 1.5-ml polyethylene tubes against the inner edge of the tube without manual contact. The samples were frozen immediately in a −40°C freezer for storage. The cotton tip of each swab was aseptically removed from the shaft and placed directly into the 96-well lysis plate provided in the ZR-96 fecal DNA extraction kit (Zymo Research, Irvine, CA). DNA was extracted using the standard protocol for the ZR-96 kit, with bead beating done with a Geno/Grinder high-throughput tissue homogenizer (SPEX SamplePrep, Metuchen, NJ), and stored at −20°C until further processing.

**Sequencing library construction.** Amplification and sequencing were performed as described previously for bacterial (16) and fungal communities (8). Briefly, the V4 domain of bacterial 16S rRNA genes was amplified using the primers F515 (5′-NNNNNNNNNNGTGTCCAGCMGCCGCGGTAA-3′) and R806 (5′-GGACTACHVGGGTWTCTAAT-3′) (17), with the forward primer modified to contain a unique 8-nucleotide (nt) barcode (italicized poly-N section of the F515 primer) and a 2-nt linker sequence (the bold underlined portion of the F515 primer) at the 5′ terminus. The PCR mixtures contained 5 to 100 ng DNA template, 1× GoTaq green master mix (Promega, Madison, WI), 1 mM MgCl₂, and 2 pmol of each primer. The reaction conditions consisted of an initial 94°C for 3 min, followed by 35 cycles of 94°C for 45 s, 50°C for 60 s, and 72°C for 90 s, and a final extension of 72°C for 10 min. Fungal internal transcribed spacer (ITS)-1 loci were amplified with the primers BITS (5′-NNNNNNNNNNNNCTAATCCTGCGGARGGATCA-3′) and B58S3 (5′-GAGATCCRTTGYTRAAAGTT-3′) (8), with a unique 8-nt barcode and linker sequence incorporated in each forward primer. The PCR mixtures contained 5 to 100 ng DNA template, 1× GoTaq green master mix (Promega), 1 mM MgCl₂, and 2 pmol of each primer. The reaction conditions consisted of an initial 95°C for 2 min, followed by 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 60 s, and a final extension of 72°C for 5 min. Amplicons were combined into two separate pooled samples (keeping the bacterial and fungal amplicons separate) at equal ratios, purified using the QIAquick spin kit (Qiagen), and submitted to the UC Davis Genome Center DNA Technologies Core for Illumina paired-end library preparation, cluster generation, and 250-bp paired-end sequencing on an Illumina MiSeq instrument in two separate runs.

**Data analysis.** The raw FASTQ files were demultiplexed, quality filtered, and analyzed using QIIME 1.6.0 (18). The 250-bp sequences were truncated at any site of more than three sequential bases receiving a quality score of <20, and any sequence containing ambiguous base calls or barcode/primer errors was discarded, as were sequences with <75% (of total read length) consecutive high-quality base calls (19). For the ITS sequences, the primer sequences were trimmed from the ends of each sequence, and operational taxonomic units (OTUs) were clustered de novo using the QIIME implementation of UCLUST (20), with a threshold of 97% pairwise identity. The bacterial 16S rRNA gene sequences were
clustered using the QIIME subsampled reference OTU-picking pipeline with the UCLUST reference 20 against the Greengenes 16S rRNA gene database (February 2011 release) (21), clustered at 97% pairwise identity. The OTUs were classified taxonomically using a QIIME-based wrapper of BLAST (22) against the Greengenes 16S rRNA gene database (for 16S rRNA gene sequences) or the UNITE (23, 24) database (for ITS sequences), modified as described previously (8). Any OTU comprising <0.0001% of the total sequences for each run was removed prior to further analysis, calibrating against the defined mock communities included in both sequencing runs (19). The bacterial 16S rRNA gene sequences were aligned using PyNAST (25) against a reference alignment of the Greengenes core set (21). From this alignment, chimeric sequences were identified and removed using ChimeraSlayer (26), and a phylogenetic tree was generated from the filtered alignment using FastTree (27). Sequences that failed to be aligned or those identified as chimeras were removed prior to the downstream analysis. Any sample yielding less than 800 filtered sequences was removed prior to the diversity and statistical analyses.

Beta diversity estimates (between-sample diversity comparisons) were calculated within QIIME using the weighted UniFrac (28) distance between the samples for the bacterial 16S rRNA gene sequences (evenly sampled at 800 sequences per sample), subsampled 10 times without replacement. From these estimates, the principal coordinates were computed to compact dimensionality into three-dimensional principal coordinate analysis (PCoA) biplots.

In order to test whether routine cleaning resulted in significant differences in OTU diversity between time points and between the host-associated and environmental (room) surfaces, a permutational multivariate analysis of variance (MANOVA) (29) with 999 permutations was used to test (with the QIIME-wrapped R module Adonis) the null hypothesis that the sample groups were not statistically different based on the evenly sampled UniFrac distance. All other statistical tests were performed using R software. A one-way analysis of variance (ANOVA) was used to determine whether the relative abundances of known neonatal pathogens differed significantly between host-associated surfaces before and after cleaning, disposable items before and after use, and environmental surfaces over time. One-tailed paired-sample t tests were used to test whether the relative abundances of known neonatal pathogens differed significantly between host-associated surfaces before and after cleaning and between disposable items before and after use. Repeated-measures ANOVAs were used to test whether bacterial taxa differed significantly between weekly sampling time points for environmental samples from routinely cleaned rooms.

Environmental surveillance heat maps were generated based on taxonomic abundance tables generated in QIIME and visualized using SitePainter 1.1 (30).

**Quantitative PCR.** In order to quantify net microbial biomasses on neonate-associated surfaces (isolation, radiant warmers, ventilators, CPAP machines, stethoscopes, and pacifiers), quantitative PCR (qPCRs) were used to enumerate the fungal and bacterial communities. qPCRs were performed in 20-μl reaction mixtures containing 2 μl of DNA template, 5 pmol of each respective primer, and 10 μl of TaKaRa SYBR 2× perfect real-time master mix (TaKaRa Bio, Inc.). For quantification of the fungal community, the primers YEASTF (5′-GAGTCGAGTTGTTTGGG AATGC-3′) and YEASTR (5′-TCTCTTTCCAAAGTTCTTTTCATCTT-3′) were used (31). The reaction conditions involved an initial step at 95°C for 10 min, followed by 40 cycles of 15 s at 95°C, 1 min at 60°C, and 30 s at 72°C. For amplification of the bacterial community, the primers Uni334F (5′-ACTCTTACGGGAGGCAGCTG-3′) and Uni514R (5′-ATTACCG CGGCTGCTGGC-3′) (32) were used. The reaction consisted of an initial hold at 95°C for 20 s, followed by 40 cycles of 4 s at 95°C and 25 s at 65.5°C. All reactions were performed in triplicate in optical-grade 96-well plates on an ABI Prism 7500 fast real-time PCR system (Applied Biosystems). The instrument automatically calculated cycle threshold \((C_0)\) values, efficiencies \((E)\), confidence intervals, and cell concentrations (fungi) or 16S rRNA gene copy numbers (bacteria) by comparing sample threshold \((C_t)\) values to a standard curve of serially diluted genomic DNA extracted from a known concentration of *Saccharomyces cerevisiae* or *Escherichia coli* cells. One-tailed paired-sample t tests were calculated with R software to test significant differences between host-associated surfaces before and after cleaning and between disposable items before and after use. qPCR microbial biomass heat maps were generated using SitePainter 1.1 (30). Given the multiple comparisons performed, P values of <0.1 were reported, and P values of <0.01 were considered significant.

### RESULTS

To address the question of how routine cleaning impacts the microbial ecosystem of a NICU, amplicon sequencing and qPCRs were used to profile the bacterial and fungal communities inhabiting both neonate-associated (isolation, ventilators, CPAP machines, stethoscopes, nasal catheters, radiant warmers, and pacifiers) and nonassociated environmental (room) surfaces (Fig. 1).

All surfaces in the NICU supported complex bacterial communities, primarily dominated by members of the *Gammaproteobacteria* and *Firmicutes* (Fig. 2; see also Table S2 in the supplemental material). The neonate-associated surfaces were inhabited by sporadically elevated relative abundances of *Streptococcus*, *Staphylococcus*, *Neisseria*, and *Enterobacteriaceae* compared to those in the room environment, which exhibited higher abundances of *Geo- bacillus*, *Halomonas*, *Shewanella*, *Acinetobacter*, and *Gemella*, among other genera (Fig. 2). Most surfaces (especially the neonate-associated surfaces) failed to amplify with the fungal ITS primers, most likely because the fungal populations were below the limit of detection for this assay. The surfaces that did amplify displayed the presence of several yeasts, including *Saccharomyces cerevisiae*, *Cryptococcus albidus*, *Debaryomyces fabryi*, and *Candida albicans* (Fig. 3). *S. cerevisiae* was the most dominant fungus on these surfaces, exhibiting as much as 100% relative abundance on some surfaces (Fig. 3; see also Table S3 in the supplemental material).

Table 1 summarizes the relative abundances of the bacterial and fungal taxa detected on the NICU surfaces known to contain the pathogens most commonly associated with neonatal disease outbreaks (33). Table 2 summarizes the most common organisms grown from blood cultures in this NICU in the years 2011 and 2012. Cleaning resulted in significantly \((P = 0.0022)\) decreased abundances of *Streptococcus* (Table 1). *Staphylococcus* and *Streptococcus* were more abundant on the neonate-associated surfaces than on nonassociated surfaces, which exhibited higher abundances of *Acinetobacter* and *Pseudomonas* (Table 1). Disposable items did not exhibit differences in the common NICU pathogens before or after exposure to the neonate with the exception of *Acinetobacter*, the abundance of which was similar to that of the microbiota of the nonclinical room surfaces before contact with the neonate and decreased afterward. The amplicon-sequencing protocol employed here did not differentiate the bacterial species, so not all of the genera detected necessarily represent pathogenic species. None of these taxa demonstrated significant changes on the environmental surfaces of the room for 4 weekly sampling times, but repeated-measures ANOVAs indicated that abundances of *Acinetobacter* and *Pseudomonas* (Table 1).
dance-weighted UniFrac PCoA of the bacterial communities revealed that the surface samples formed three clusters, neonate-associated surfaces after use (before cleaning) and before use (after cleaning) and nonassociated environmental surfaces in the room, associated with different bacterial taxa (Fig. 4A). A significant separation of these three groups was confirmed with a permutational MANOVA ($P = 0.006$). The precleaning and used disposable samples demonstrated diffuse clustering (Fig. 4A), due to the sporadic dominance of Streptococcus and Staphylococcus on these surfaces (Fig. 2), and this clustering appears associated with the abun-

**FIG 2** Microbial heat maps reveal bacterial distribution in the NICU. Heat maps of select, most abundant bacterial genera detected across each surface type using 16S rRNA gene amplicon sequencing are shown. The keys for each plot indicate the relative abundance scale from blue (absent/low) to red (high). Sites that appear white had bacterial DNA below the limit of detection for this assay.
dance of Firmicutes, primarily Streptococcus, Staphylococcus, and Clostridium (Fig. 4A). After being cleaned, these surfaces clustered closer to environmental samples from the room and unused disposable samples, associated with the dominance of Acinetobacter, Enterobacteriaceae, Shewanella, and Halomonas (Fig. 4A). The separation of bacteria found in the environmental samples into a different cluster appears to be due to the association with the minor bacterial populations present in these samples, including those of Fusobacterium and Actinomyces (Fig. 4A).

To test whether cleaning also changed the microbial loads on the neonate-associated surfaces, qPCRs were used to enumerate the bacterial and fungal biomasses. Cleaning resulted in significant decreases for fungi ($P = 0.04$) (Fig. 4B and C). All the surfaces exhibited low levels of bacterial ($10^4$ to $10^5$ 16S rRNA gene copies/cm$^2$) and fungal ($10^4$ to $10^6$ cells/cm$^2$) biomasses, visualized in quantitative heat maps (Fig. 4D and E).

**DISCUSSION**

NICU surfaces contain delicate microbial ecosystems, heavily influenced by contact with their fragile residents—premature and sick neonates—who are innately vulnerable to opportunistic infections. It is impossible to operate these environments in complete sterility, as the infants themselves, the milk they consume, the adults caring for them, and the multiple pieces of equipment required for their care all represent fertile vectors for microbial transmission. Thus, cleaning regimens are necessary to prevent the retention and spread of virulent microbial pathogens in this sensitive environment.

To assess the impact of cleaning on bacterial and fungal com-

![FIG 3 Microbial heat maps reveal fungal distribution in the NICU. Heat maps of select, most abundant fungal genera detected across each surface type using ITS amplicon sequencing are shown. The keys for each plot indicate the relative abundance scale from blue (absent/low) to red (high). Sites that appear white had fungal DNA below the limit of detection for this assay.](http://jcm.asm.org/)

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Before intensive cleaning (n = 38)</th>
<th>After intensive cleaning (n = 38)</th>
<th>In the environment (routine cleaning, n = 52)</th>
<th>In disposables before contact (n = 6)</th>
<th>In disposables after contact (n = 6)</th>
<th>A &gt; B (paired t test)</th>
<th>D &lt; E (paired t test)</th>
<th>A ≠ B ≠ C ≠ D ≠ E (one-way ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus</em></td>
<td>0.0549 (±0.0804)</td>
<td>0.0353 (±0.0671)</td>
<td>0.0237 (±0.0260)</td>
<td>0.0106 (±0.0051)</td>
<td>0.1444 (±0.2358)</td>
<td>0.07196</td>
<td>0.0891</td>
<td>0.0017</td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td>0.0006 (±0.0019)</td>
<td>0.0007 (±0.0020)</td>
<td>0.0011 (±0.0046)</td>
<td>0.0000 (±0.0001)</td>
<td>0.0000 (±0.0001)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>0.0250 (±0.0445)</td>
<td>0.0119 (±0.0217)</td>
<td>0.0216 (±0.0317)</td>
<td>0.0024 (±0.0004)</td>
<td>0.1344 (±0.2805)</td>
<td>0.0022</td>
<td>NS</td>
<td>0.0006</td>
</tr>
<tr>
<td><em>Escherichia</em></td>
<td>0.0003 (±0.0005)</td>
<td>0.0004 (±0.0006)</td>
<td>0.0003 (±0.0006)</td>
<td>0.0005 (±0.0004)</td>
<td>0.0004 (±0.0005)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td>0.0005 (±0.0003)</td>
<td>0.0005 (±0.0004)</td>
<td>0.0005 (±0.0003)</td>
<td>0.0005 (±0.0004)</td>
<td>0.0003 (±0.0001)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><em>Acinetobacter</em></td>
<td>0.0655 (±0.0240)</td>
<td>0.0696 (±0.0199)</td>
<td>0.0758 (±0.0303)</td>
<td>0.0820 (±0.0107)</td>
<td>0.0520 (±0.0311)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>0.0166 (±0.0054)</td>
<td>0.0199 (±0.0065)</td>
<td>0.0221 (±0.0140)</td>
<td>0.0201 (±0.0038)</td>
<td>0.0159 (±0.0096)</td>
<td>0.0233$^a$</td>
<td>0.0998$^b$</td>
<td>NS</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>0</td>
<td>0</td>
<td>0.0346</td>
<td>0</td>
<td>0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^a$ NS, not significant ($P < 0.1$); the significance of *Candida albicans* was not tested, as fungal amplification was only possible from one sample of the room environment.

$^b$ Only paired before and after the cleaning sites were tested ($n = 70$).

$^c$ A < B; D > E.
Saliva bacteria of the skin and mucosal surfaces and have been breaks (33), these two genera are also common human communities associated with neonatal disease outbreaks (14). In the current study, levels of these organisms were relatively low but were not altered with intensive cleaning. These observations raise questions as to whether the colonization of NICU surfaces with Enterobacteriaceae is related to increased numbers of nosocomial infections and whether improved hand-washing or surface clean-

TABLE 2 Neonatal blood culture results, 2011-2012*

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>No. of infants with positive cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus</em>, coagulase negative</td>
<td>24</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>9</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>6</td>
</tr>
<tr>
<td>Enterococcus species</td>
<td>6</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>4</td>
</tr>
<tr>
<td><em>Streptococcus viridans</em> group</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>2</td>
</tr>
<tr>
<td>Other Gram-negative rod</td>
<td>3</td>
</tr>
</tbody>
</table>

*A One patient had a positive blood culture for each of the following: Leuconostoc sp., Acinetobacter baumannii, Candida albicans, Candida parapsilosis, Listeria monocytogenes, Pseudomonas aeruginosa, Serratia marcescens, *Streptococcus agalactiae* (group B), *Streptococcus anginosus* group, and an unidentified yeast.

Fungal (E) bioloads on each surface, as determined by qPCRs. The keys for each plot indicate the absolute abundance scale from blue (absent/low) to red (high). Other Gram-negative rod

Cleaning drives shifts in microbial structures and abundances. (A) A weighted UniFrac PCoA biplot demonstrates the abundance-weighted clustering of clinical surfaces before cleaning (red) and after cleaning (blue), disposable items before (purple) and after use (yellow), and nonclinical surfaces in the room environment (orange). Each colored orb represents a single sample, and the proximity indicates the degree of similarity between individual samples. In this biplot, bacterial taxa are coplotted as gray orbs to indicate the association with different sample clusters; orb size is a function of the mean abundance. The P value in the top right corner indicates the result of a permutational MANOVA test comparing the bacterial community similarity of pre-/postcleaning and environmental sample groups. (B and C) qPCRs indicate drops in bacterial (B) and fungal (C) surface bioloads in response to cleaning. The error bars represent the standard deviation (SD); P values indicate the results of paired t tests. (D and E) Quantitative heat maps of clinical surfaces illustrate changes in bacterial (D) and fungal (E) bioloads on each surface, as determined by qPCRs. The keys for each plot indicate the absolute abundance scale from blue (absent/low) to red (high). Sites that appear white had DNA below the limit of detection for this assay.
ing techniques might further decrease this class of infection. It may be that NICU surface ecologies are not significantly different than those of other hospital environments, a situation that can only be assessed as hospital ecological surveillance data grow. Rather, the unique challenge in preventing nosocomial infections in the NICU may be the increased susceptibility of premature and otherwise compromised infants to opportunistic infections, even from organisms that are commensals in healthy adults. The sources of most outbreak episodes are difficult to diagnose, but infected patients and colonized parents and personnel appear to be prevalent sources of infection (33). As with previous studies, NICU surfaces appear to be important reservoirs for potentially pathogenic microbes in these environments. Cleaning significantly reduces the total microbial load and reshapes the microbial assemblages on these surfaces to be dominated by nonpathogenic organisms. It is noteworthy, however, that some of the more abundant surface microbiota groups with known neonatal pathogen members are not common causes of infection in this NICU (e.g., Acinetobacter), while some organisms that commonly cause infection in this NICU are present in very low abundance on the NICU surfaces tested in this study (e.g., Enterococcus and Klebsiella).

The observation that streptococci and staphylococci colonize the NICU surfaces that are in contact with the neonate suggests that these microbes are able to outcompete the normal surface microbes. It is tempting to speculate that more potent cleaning techniques or agents will lead to further decreases in nosocomial infections; however, surveys of microbial communities like the present study have suggested that the prevailing paradigm (all bacteria are potentially dangerous, and therefore effective destruction of all bacteria on surfaces is a reasonable goal) may be inadequate. Future improvement may require innovative approaches such as selective antimicrobial cleansers or purposeful colonization of surfaces with as yet undetermined nonpathogenic species that can outcompete known pathogens.

The decreasing cost of high-throughput sequencing technologies will likely make testing future interventions and routine environmental surveillance of hospital surfaces feasible. The similarities in detection of several host-associated groups of Enterobacteriaceae and Firmicutes (e.g., Staphylococcus, Streptococcus, and Enterococcus) between this and the previous amplicon-sequencing survey of NICU surfaces (14) begin to define what constitutes a “typical” NICU surface ecosystem. Defining routine (i.e., nonthreatening) surface microbiota will require additional amplicon-sequencing studies of NICUs, and such an approach may enable future assessments of individual NICU “health” and early warning systems for predicting and preventing infection outbreaks.

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