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Research Article

Hospital Microbiome Variations As Analyzed by High-Throughput Sequencing

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Abstract

Hospital-acquired infections remain a serious threat to human life and are becoming a top public health issue. As the latest advances in sequencing technologies have allowed the unbiased identification of bacterial communities, we aimed to implement emerging omics technologies to characterize a hospital's microbiome at the center of Cairo, Egypt. To this end, we screened surfaces and inanimate objects in the hospital, focusing on bed sheets and door knobs, with additional screening for resistant microbes and resistance genes. While bacterial load and community composition were not dramatically different between door knobs of hospital units with different hygiene levels, the bacterial communities on door knob samples were richer and more diverse than those detected on bed sheets. Bacteria detected on door knobs were a mix of those associated with dust/particulate matter/debris (e.g., *Bacillus*, *Geobacillus*, *Aeribacillus*) and skin-associated bacteria (e.g., *Staphylococcus*, *Corynebacterium*). The latter were among the core genera shared by all analyzed samples. Conversely, bacteria that were more abundant in bed sheets were not associated with a particular source (e.g., *Pseudomonas* and *Nitrobacter*). Resistance screening indicated an expansion of a mobile beta-lactamase-encoding gene (bla_{TEM}), reflecting its current global spread. This study is a first step toward more comprehensive screening of hospital surfaces and correlating their microbiome with hospital outbreaks or chronic infections. We conclude that, as hospitals are unique built environments, these findings can inform future infection control strategies in hospitals and health care-related built environments, and attest to the importance of the emerging hospital microbiome research field.

Keywords: microbiome, built environment, bioinformatics, public health, 16S rRNA, infection control, antimicrobial resistance

Introduction

Despite of the tremendous advances in medical care in the past century, hospital-acquired infections (HAIs) remain a threat to human life and have actually become among the most serious public health problems of the 21st century. Even in a developed country such as the United States, one in every 31 patients suffers from at least one HAI every day (Centers for Disease Control and Prevention [CDC], 2017).

This public health problem is further complicated by the unprecedented rise in bacterial infections that are resistant to one or more antibiotics, while the number of novel antibiotics approved for the market has dropped to all-time low since the year 2000 (Ventola, 2015).

Several reasons are behind the rapid spread of antibiotic-resistant microbes, the most important of which are (i) the misuse or abuse of antibiotics (e.g., using them prophylactically, in animal feed, or not completing the prescribed therapeutic regimen); (ii) the failure to adhere to good policies in prescribing and administering antibiotics (e.g., starting with the strongest broad-spectrum antibiotics for trivial infections, prescribing novel antibiotics without appropriate culturing or sensitivity testing, and administering antibiotics without prescriptions); and (iii) the alarming spread of multidrug-resistant microbes in hospitals.

The latter problem could be regarded as the most dangerous among all reasons behind spread of antibiotic resistance genes, not only because it leads to the spread of resistant pathogens amidst the most vulnerable members of the population

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(hospitalized patients, including immune-compromised and/or elderly patients), but also because hospitals are fertile grounds for the exchange of resistance genes between species leading to the emergence of novel strains (often described by the media as ''superbugs'') that are even more resistant than their ancestors. Even more alarming is the fact that those resistant strains can reside in the hospitals for long time (on surfaces and inanimate objects), and can be asymptomatically carried by physicians, nurses, and other health care workers, who are going to interact with more and more patients, posing a formidable public health threat.

The problems of HAIs and hospital-emerged multiresistant pathogens are not new and have been dealt with in the past nonspecifically by the adoption of high hygienic standards and by the use of sterilization, disinfection, and antisepsis measures. However, despite the advance in science and health care, the emergence of HAIs continues and needs more clever diagnostic measures and unconventional solutions. Although there is continuous and extensive screening for bacteria isolated from infected patients or to a lesser extent from health care workers, there are fewer efforts directed to monitoring the environment, including inanimate objects and surfaces, for detecting the spread of resistant bacteria, which are potential sources for novel epidemics.

The latest advances in molecular biology and genomics have provided new promises to better track epidemics of infectious diseases caused by pathogenic or opportunistic bacteria and fungi, or by deadly viruses. Genome sequencing and comparative genomics allow the tracking and containment of epidemics.

In parallel with these techniques, the metagenomics technology has emerged in the early years of the 21st century and popularized the concept of random sequencing of different environments followed by computationally identifying the microbes therein as well as the genes of interest, whether they are metabolic, virulence, or antibiotic resistance genes (Breitbart et al., 2002; Riesenfeld et al., 2004; Schmieder and Edwards, 2012). The concept of the microbiome has emerged to describe the combined genomes of a microbial community within a particular environment (Turnbaugh et al., 2007) and has since been applied to study various common complex human diseases (Laudadio et al., 2018; Malan-Muller et al., 2018).

Recently, a ''Hospital Microbiome Project'' emerged in the United States to define the microbiome dynamics over time in a new hospital. This project, which we abbreviate as HoMP, is important and promising, as it aims to detect microbial communities in hospitals and track the changes of the composition of these microbial communities to preemptively fight any potential emergence of novel pathogenesis or resistance mechanisms (Lax and Gilbert, 2015).

Such project is of particular importance to Egypt, where the economic situation and population growth compromise the application of hygienic standards in several hospitals. Surveillance performed in collaboration with the United States CDC concluded that HAIs and multidrug-resistant pathogens constitute a real threat in Egyptian hospitals, with a special prevalence of *Klebsiella* spp. (28.7%) and *Acinetobacter* spp. causing hospital-acquired pneumonia, primary bloodstream infections, and urinary tract infections (Talaat et al., 2016).

Although starting such a HoMP with the current limited resources in Egypt might be challenging, launching preliminary studies and pilot projects promises to lead the way to a similar nationwide project in the near future.

Here, we present a pilot study, in which we used molecular tools and high-throughput sequencing (also known as next-generation sequencing), to estimate the distribution of antibiotic resistance genes and microbial communities in an Egyptian hospital and to evaluate the contribution of the hospital environment in the type and distribution of pathogenic and non-pathogenic bacteria. This work is a first step on the way toward establishing an Egyptian HoMP and will present a promising tool for monitoring hospital environments and infection control.

Materials and Methods

Ethics statement

All experiments were conducted according to national and international guidelines for research integrity and ethics. In this study, no animal or human samples were used; however, the umbrella project, of which this study is a part, has been approved by the Ethics Committee of Faculty of Pharmacy, Cairo University (Approval No. MI 1026; year 2014).

Sample collection

Samples were collected from different units in an Egyptian hospital in Cairo. Various inanimate surfaces (door knobs, bed sheets, basin, cupboard, medical devices, and changing carts) were sampled in triplicates, and the final number of swabbed surfaces was 31. The hospital units could be classified into three categories according to their level of disinfection (Table 1). The chosen surfaces were sampled with sterile cotton swabs premoistened with sterile phosphate-buffered saline. An area of 10 cm × 10 cm was swabbed once in one direction for each sample. The swabs were then transferred directly to the laboratory under chilled conditions. One of each triplicate swabs was used for culturing, the other was used for DNA extraction, and the third was stored at −20°C as a backup (or if further processing and experiments are needed).

Determination of antibiotic resistance by the Kirby-Bauer disk diffusion test

One of each collected triplicate swabs was tested for its resistance against five of the most commonly used antibiotics in the hospital by the Kirby-Bauer disk diffusion method (Bauer et al., 1966). In brief, each swab was incubated overnight under agitation in brain heart broth at 37°C. After incubation, the optical density of each culture was adjusted to 0.125 at 560 nm using a spectrophotometer (Jenway 6800 UV/Vis, United Kingdom). Aseptically, plates of Mueller-Hinton agar were surface-inoculated with a sterile swab dipped into each of the adjusted suspension. Plates were left to dry, and then the five antibiotic disks (Bioanalyse, Ankara, Turkey) were placed on the agar surface 24 mm apart from each other and from the edges of the plate. The disks used were for Sulbactam/Ampicillin (10 μg/10 μg), Cefixime (5 μg), Kanamycin (30 μg), Levofloxacin (5 μg), and Vancomycin (30 μg).

Finally, the plates were incubated at 37°C for 24 h. The diameter of the zone of inhibition for each antibiotic was measured to the nearest millimeter. The sensitivity and resistance patterns were recorded according to the zone size interpretative chart following the guidelines of Clinical and Laboratory Standards Institute (2014).

Table 1. Classification of the Samples Collected from Each Unit Based on Surface Type

Inanimate surface tested (sample type)	*Hospital unit*	*Number of samples collected*
Door knob	Clinics Endoscopy ICU Intestinal catarrhal Main entrance Main pharmacy Peritoneal dialysis	12
Bed sheet	Endoscopy Intestinal catarrhal care Intestinal catarrhal clinic Peritoneal dialysis Surgical care	5
Basin	Endoscopy Intestinal catarrhal clinic Peritoneal dialysis	3
Device	Dialysis ICU	1
Cupboard	Endoscopy Intestinal catarrhal care	2
Changing cart	Intestinal catarrhal care Surgical care	2
Drug preparation desk	Intestinal catarrhal clinic	1
Floor	ICU surgical	1
Counter	Main pharmacy	1
Refrigerator	Main pharmacy	1
Ventilator	Surgical care	1
Balance	Intestinal catarrhal clinic	1

The surfaces were sampled in triplicates, and the total number of swabbed surfaces was 31.
ICU, intensive care unit.

DNA extraction from the collected samples

DNA was extracted from the collected swabs by the use of the PowerSoil® DNA Isolation Kit (MO-BIO Laboratories, Carlsbad, CA, USA), and the MO-BIO vortex adapter in accordance to the manufacturer's instructions. The extracted DNA was purified and concentrated by ethanol precipitation. In brief, 5 μL 5 M sodium chloride was added to each sample, mixed well, and then 200 μL of 100% cold ethanol was added to the mixture, which was subsequently centrifuged at maximum speed for 5 min. After the supernatant was decanted, the tube was left to air dry. Finally, DNA was resuspended in 25 μL of nuclease-free water.

Detection of the presence of resistance genes by the polymerase chain reaction

Polymerase chain reaction (PCR) was used to screen for the presence of genes encoding antibiotic resistance factors using specific primers (Supplementary Table S1) adopted from different studies (Aziz et al., 2017; Card et al., 2013, 2014; Ramadan et al., 2019; Saladin et al., 2002). Each PCR mix consisted of 10 μL MyTaq HS Mix (Bioline, Swedesboro, NJ, USA), 1 μL of 10 μM of each of the primer pair (Thermo Fisher Scientific, Waltham, MA, USA), 3 μL of the extracted DNA, and 5 μL of nuclease-free water.

PCR amplification was carried out in a temperature gradient thermocycler (Techne Gradient, Essex, United Kingdom) in 20 μL reaction volume as per the following steps: an initial denaturation step at 95°C for 3 min, followed by 32 cycles of denaturation at 94°C for 30 sec. The annealing step was performed at different temperatures according to the melting temperature (Tm) of each primer pair for 30 sec followed by an extension step at 72°C for 40 sec and a final extension step at 72°C for 10 min. The PCR products were detected by gel electrophoresis on 1% agarose gels prestained with ethidium bromide (Sigma-Aldrich, Taufkirchen, Germany) and visualized by UV transillumination.

16S rRNA gene sequencing

The concentrated DNA was sequenced at Centros FISABIO, Valencia, Spain (courtesy of Dr. Alex Mira) using Illumina MiSeq Sequencer as per the manufacturer's instructions. The 2×300 bp paired-end protocol was followed, and the sequencing library was generated by the Illumina amplicon library protocol. Gene-specific primer sequences used in this protocol were selected from Klindworth et al. (2013) to target the V3 and V4 regions of the 16S rRNA gene.

Bioinformatics analysis of 16S rRNA sequence data

The resulting sequence reads were checked and filtered for quality using FastQC (Andrews, 2010) and PRINSEQ (Schmieder and Edwards, 2011). All subsequent analyses, including clustering and phylogenetic analysis, were performed with QIIME version 1.9.1 (Caporaso et al., 2010). The closed reference method was implemented for picking operational taxonomic units (OTUs) based on 97% identity with the Greengenes database version 13.8 (DeSantis et al., 2006).

Statistical analyses

Several statistical tests are built in QIIME and were automatically performed as a part of the pipeline. In addition, the R Project for Statistical Computing (https://r-project.org) was used for data visualization, and statistical analyses were performed for testing the significance of differences between sample types or other parameters.

Sequence deposition

All raw sequence reads have been deposited in the Sequence Read Archive under BioProject PRJNA544954 and assigned Biosample numbers SAMN11867245 through SAMN11867256. All deposited raw sequence reads have been made publicly available before submission.

Results

A hospital is a unique built environment: on the one hand, it is enriched with a heterogeneous and unusually large number of patients; on the other hand, it is routinely cleaned and monitored for pathogenic- and antibiotic-resistant organisms. The hospital personnel consist of multidisciplinary teams of well-trained health care professionals, practicing

Table 2. Classification of the Screened Hospital Units Based on Their Level of Disinfection and the Number of Samples Sequenced from Each Unit

Level of disinfection	*Hospital unit*	*Sample type*	*Number of samples*	*Sample ID*
Highest	ICU	Door knob	1	Kn8-ICU
	Peritoneal dialysis	Door knob	1	Kn2-PD
		Bed sheet	1	Sh3-PD
	Surgical care	Bed sheet	1	Sh1-SCU
Moderate	Endoscopy	Door knob	3	Kn1-Endo2 Kn5-Endo1 Kn6-Endo3
	Intestinal catarrhal	Door knob	1	Kn9-IntCU
		Bed sheet	1	Sh2-IntCCU
Lowest	Pharmacy	Door Knob	1	Kn7-Ph
	Main entrance	Door knob	1	Kn4-Ent
	Clinics	Door knob	1	Kn10-Cl2

Table 3. Susceptibility Pattern of the Tested Samples to the Five Most Commonly Prescribed Antibiotics in the Hospital and the Target Resistance Genes Detected in Each Sample

		Antibiotic							
Level of disinfection	*Hospital unit*	*Sulbactam/ ampicillin*	*Cefixime*	*Kanamycin*	*Vanco-mycin*	*Levo-floxacin*	R *score*	*Resistance gene detected*	*Sample ID*
Lowest	Clinics	R	R	R	R	I	4.5	–	Kn3-Cl1
		R	R	R	R	S	4	bla_{TEM}	Kn1-Cl2
		S	R	S	R	S	2	bla_{TEM}	B1-IntCl
		S	R	S	S	S	1	bla_{TEM}	Desk-IntCl
		R	R	S	S	S	2	bla_{TEM}	Sh4-IntCCl
		R	R	S	R	S	3	bla_{TEM}/*kan*	BL-IntCl
	Entrance	I	R	I	R	S	3	bla_{TEM}	Kn4-Ent
	Pharmacy	I	R	R	S	R	3.5	bla_{TEM}	Kn7-Ph
		S	R	S	R	S	2	NT	Ref-Ph
		S	R	I	S	S	1.5	NT	Kn13-IntCPh
		S	R	S	R	S	2	bla_{TEM}	Counter-Ph
Moderate	Endoscopy	S	R	R	S	S	2	bla_{TEM}	Kn5-Endo1
		R	R	S	R	S	3	bla_{TEM}	Kn1-Endo2
		I	R	R	R	S	3.5	bla_{TEM}	Kn6-Endo3
		S	R	S	S	S	1	bla_{TEM}	B2-Endo
		S	R	S	R	S	2	NT	Sh5-Endo
		I	R	S	S	S	1.5	bla_{TEM}	C3-Endo
	Intestinal Catarrhal	S	R	R	R	S	3	bla_{TEM}	Kn9-IntCU
Highest	ICU	R	R	R	S	S	3	–	Kn88-ICU
		S	R	R	R	S	3	bla_{TEM}	Kn8-ICU
	Intestinal Catarrhal care	S	R	S	S	S	1	bla_{TEM}	Sh2-IntCCU
		I	R	S	S	S	1.5	bla_{TEM}	C4-IntCCU
		S	R	S	S	S	1	bla_{TEM}	Cart1-IntCCU
	Peritoneal dialysis	S	R	S	S	S	1	bla_{TEM}	Kn2-PD
		S	R	S	S	S	1	NT	B3-PD
		S	R	S	S	S	1	bla_{TEM}	Sh3-PD
		S	R	S	R	S	2	bla_{TEM}	D1-PDCU
	Surgical care	I	I	S	R	S	2	–	Sh1-SCU
		R	R	R	R	R	5	–	Cart3-SCU
		R	R	S	R	S	3	*Kan*	Vent-SCU
		S	R	S	S	S	1	bla_{TEM}	Floor-SCU
% Resistant		25.81	96.77	29.03	51.61	6.45			
% Intermediate		19.35	3.23	6.45	0.00	3.23			
% Sensitive		54.84	0.00	64.52	48.39	90.32			
Chi-square *p*-value (vs. overall average)		1.01×10^{-7}	7.41×10^{-28}	2.74×10^{-2}	1.18×10^{-2}	6.69×10^{-14}			

R-score: a value reflecting the number of antibiotics to which the collective microbes within a sample are resistant (The score calculates 1 point per antibiotic to which the community is resistant and 0.5 point per antibiotic to which it is intermediate).

NT, not tested; –, negative PCR; R, resistant; S, susceptible; I, intermediate.

standard infection control measures. The outcome of the aforementioned interacting factors widely varies from hospital to hospital, and the risk of health care-associated infections remains a major public health threat.

In this pilot study, various inanimate surfaces inside an Egyptian hospital were sampled and screened for the composition and antibiotic resistance of their microbial communities. Overall, 31 samples were successfully swabbed and screened for the distribution of antibiotic resistance genes in the hospital (Table 1). Twelve out of those 31 samples were sequenced for microbiome structure estimation (Table 2).

Culture-based analysis: resistance patterns of different microbial communities

Samples were screened for their susceptibility/resistance to five antibiotics that are most frequently used in the hospital. The respective antibiogram was determined by the Kirby-Bauer disk diffusion method and results were interpreted based on the guidelines provided by the Clinical and Laboratory Standards Institute (2014).

All tested samples ($n=31$) were resistant to at least one antibiotic, while 19 out of 31 (61%) samples were resistant to two or more antibiotics. One sample from the intensive care unit's (ICU's) changing cart was resistant to all tested antibiotics (Table 3).

Out of the 31 tested samples, 30 (97%) were resistant to cefixime, whereas only two samples were resistant to levofloxacin. The proportions of resistance to other antibiotics were 26% to sulbactam/ampicillin, 29% to kanamycin, and 52% to vancomycin (Fig. 1; Table 3). Cefixime resistance was significantly overrepresented among samples (Chi-square p-value $=7.41\times10^{-28}$), while levofloxacin resistance was significantly underrepresented (Chi-square p-value $=6.69\times10^{-14}$). Resistance to kanamycin and vancomycin was closest to average, yet, the proportion of resistant samples remained below the overall average (Table 3).

Culture-independent analysis: PCR for detection of resistance genes in representative samples

Twenty-seven samples were selected according to their *in vitro* resistance patterns to be screened for the presence of target resistance genes by PCR.

The bla_{TEM} gene was detected in 22 out of 27 (81%) of the tested samples, while only 2 (7%) samples harbored the tested kanamycin resistance gene. *vanA*, *vanB*, and *vanC* resistance genes for vancomycin and bla_{SHV}, $bla_{\text{NDM-1}}$, $bla_{\text{CTX-M1}}$, and $bla_{\text{CTX-M15}}$, resistance genes for beta-lactams were absent from all the tested samples.

Samples that were resistant to cefixime (based on the disk diffusion method criteria) also carried the bla_{TEM} gene. However, samples that tested positive for the presence of the kanamycin resistance gene (from the surgical care unit and intestinal catarrhal clinic) were sensitive to the antibiotic when tested *in vitro* (Table 3).

Culture-independent microbiome analysis: overview

From the collected samples, we selected a representative set of door knobs and bed sheet samples to perform this pilot exploration of their microbiome. DNA sequences of 12 samples were preprocessed, filtered, and then paired ends were joined to yield 1.2 million joined reads (range: 38,724–150,979) totaling 548,442,336 bp, with an average size of 458 bp per read. Samples were analyzed by the closed reference methods against the Greengenes database.

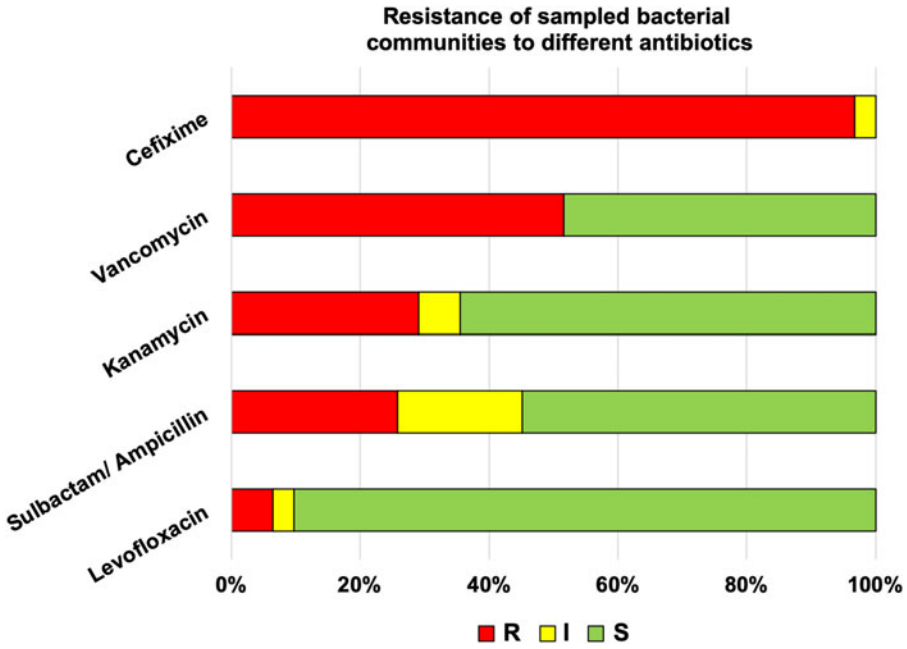

FIG. 1. A *stacked bar* plot summarizing the disk diffusion resistance patterns of the 31 samples to five representative antibiotics/antibiotic mixtures (*Y* axis). Percentages of resistant, intermediate, and susceptible samples are shown in *red*, *yellow*, and *green*, respectively.

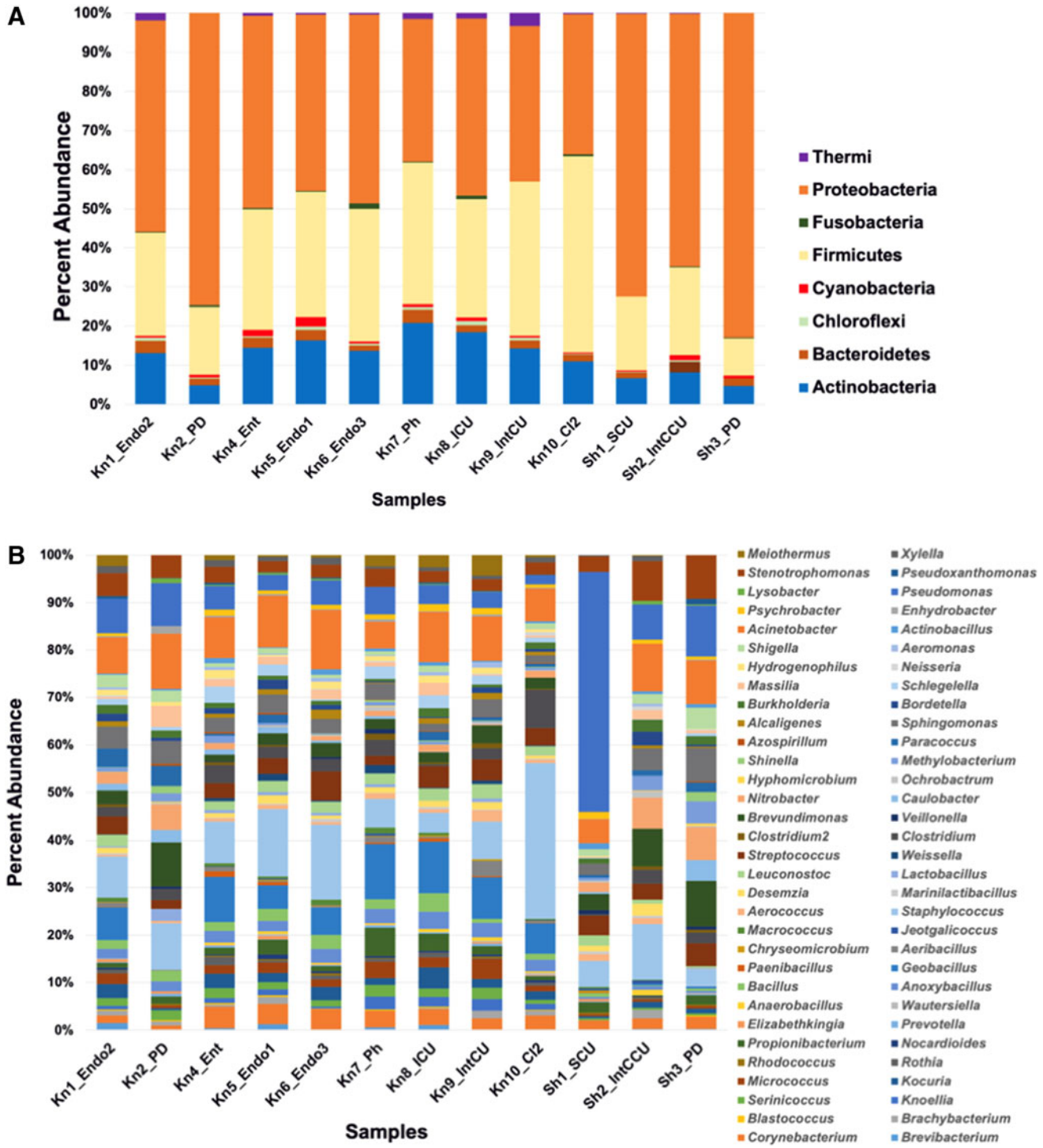

FIG. 2. **(A)** Phylum-level composition of the microbial communities detected in the samples collected from different units of the hospital. *Bar* charts represent the relative proportions of the major phyla identified in the door knobs and bed sheets. **(B)** Genus-level composition of the microbial communities detected in the samples collected from different units of the hospital. *Bar* charts represent the relative proportions of the genera identified in the door knobs and bed sheets.

On the phylum level, 22 groups were detected, the most abundant of which was Proteobacteria, with abundance ranging from 35.5% to 81.8% (mean = 53.5%), followed by Firmicutes (abundance range: 9.4–49.8%, mean = 28.6%), Actinobacteria (abundance range = 4.6–20.3, mean = 12.1%), and Bacteroidetes (abundance range 1.4–3.3%, mean = 2.2%) (Fig. 2A).

The samples could be classified into 63 OTUs to the genus level (with a number of unresolved OTUs). Out of these, 22 genera were shared by all 12 samples and could as such be

considered a core microbiome; 27 (22 core +5) were shared by all door knob samples; and 29 (22 core +7) genera were shared by all bed sheet samples. Additional genera that were in all door knob samples are *Aeribacillus*, *Bordetella*, *Enhydrobacter*, *Hydrogenophilus*, and *Prevotella*. Among these five genera, *Aeribacillus* was absent in all three bed sheet samples (Figs. 2 and 5A).

Genera that were shared by all bed sheets but not all samples are *Jeotgalicoccus*, *Leuconostoc*, *Ochrobactrum*, *Paracoccus*, *Psychrobacter*, *Rhodococcus*, and *Shigella*. Of note, none of these genera was absent from all bed sheet samples.

The top 10 genera in all samples combined were *Staphylococcus*, *Pseudomonas*, *Acinetobacter*, *Geobacillus*, *Stenotrophomonas*, *Brevundimonas*, *Streptococcus*, *Sphingomonas*, *Corynebacterium*, and *Clostridium*, in descending order of overall abundance. However, the relative abundance of these top genera obviously varied from sample to sample (Fig. 2B). Interestingly, 9 out of the 10 most abundant genera were among the core taxa (shared by all 12), while *Geobacillus* was only detected in 10 samples (as it was missing in samples from one door knob and one bed sheet).

Per sample alpha diversity

The alpha diversity, or diversity within each sample, is typically expressed in terms of richness (number of types or OTUs) and diversity index (e.g., Shannon diversity index), which takes into account richness as well as evenness of distribution.

Species richness within the collected samples was calculated as the total number of observed OTUs present in each sample. By plotting the rarefaction curves, we compared the level of species diversity in each sample (Supplementary Fig. S1). The highest species diversity was observed in a door knob sample collected from the main entrance, while the bed sheet sample collected from the intestinal catarrhal care unit had the lowest number of observed OTUs (Supplementary Fig. S1). This unit maintains the highest level of disinfection and is expected to have the lowest bacterial load among the hospital units.

The curves of all tested samples approached the sampling saturation point (asymptote or plateau), with slopes near zero at a sequencing depth of around 5000. This means that further sequencing would not add any significant additional OTUs to the already discovered OTUs in each sample, and that the communities identified in each sample were not complex, which is expected from inanimate surfaces regularly disinfected.

Shannon diversity index, computed on genus-level taxonomic assignments, ranged from 2.28 to 3.58 and was more or less similar to richness in its pattern.

Beta diversity

Beta diversity is typically assessed through principal component analysis of distance between the compositions of different taxa. Principal component analysis of Bray–Curtis distances showed no strong pattern of clustering, but there was partial clustering of bed sheet samples, although two door knob samples were clustered close to the three bed sheet samples (Supplementary Fig. S2). As the analysis with Bray–Curtis distance did not explain most of the variance (C1: 15%, C2: 11%, and C3: 10%), resorting to the weighted UNIFRAC method (Lozupone and Knight, 2005) improved the visualization of beta diversity and accentuated the clustering of all bed sheets in distinction of all but one door knob samples (Fig. 3). In either method, no particular clustering pattern was observed with different levels of hygiene.

These results directed us to concentrate the subsequent analyses on the comparison between door knob and bed sheet samples in terms of their microbial composition and diversity.

Phylum-level comparison

At the phylum level, a statistically significant difference (Wilcoxon rank sum test p-value <0.05) was observed

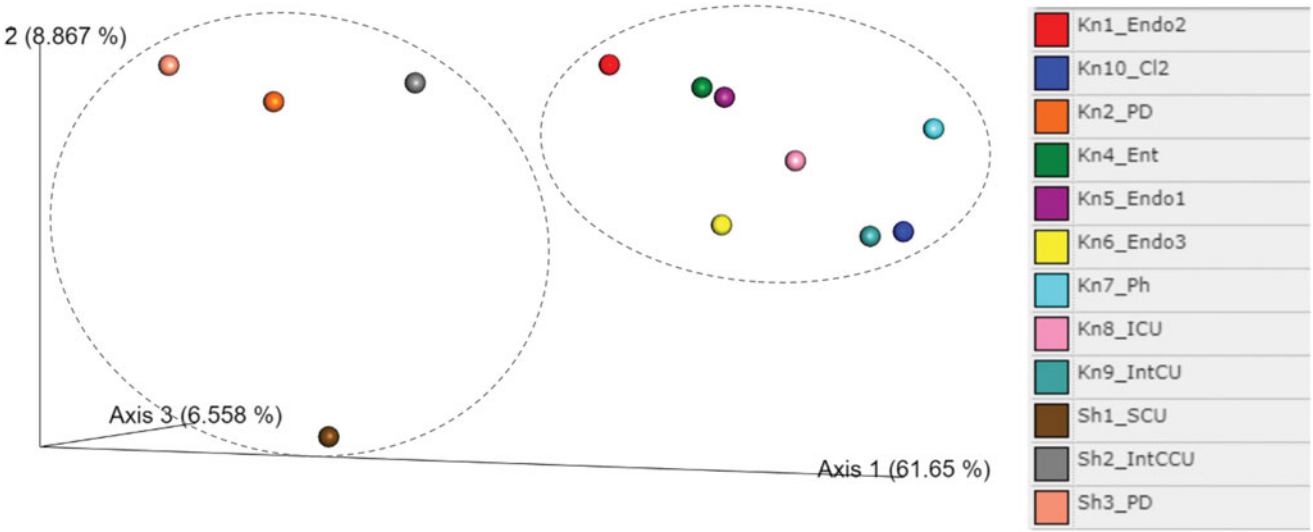

FIG. 3. Beta diversity analysis represented as a three-dimensional plot of the C1, C2, and C3 of a principal coordinate analysis of weighted UNIFRAC distance metric. The distance between any two spheres represents their divergence. Two bed sheet samples (Sh2_IntCCU, Sh3_PD) clustered together with Kn2_PD, and to a lesser extent with Sh1 SCU, while the eight remaining door knob samples clustered together. Clusters are circled by *gray dashed lines*.

between the relative abundance of three phyla in door knobs versus bed sheets. While phylum Proteobacteria was significantly more abundant in bed sheets, phyla Firmicutes and Actinobacteria were more abundant in door knob samples (Fig. 4). Based on these differences, we computed a Proteobacteria-to-Firmicutes ratio and a Proteobacteria-to-Actinobacteria ratio, which were both significantly different between the two sample types. On the contrary, the Firmicutes-to-Actinobacteria ratio was not significantly different between the two sample types, suggesting that the two phyla exhibited similar differential abundance in comparison with phylum Proteobacteria (Fig. 4).

Genus-level comparison

Similarly, the abundance of each of the 63 resolved genera was compared between the two sample types. Fourteen genera had statistically significant differences in abundance (Wilcoxon p-value <0.05) (Supplementary Fig. S3), the most prominent of which are *Aeribacillus*, *Bacillus*, and *Anoxybacillus* (more abundant on door knobs) versus *Pseudomonas*, *Stenotrophomonas*, and *Nitrobacter* (more abundant on bed sheets) (Fig. 5).

Other than the per-sample alpha diversity values shown above, we compared alpha diversity metrics between the two sample types on the genus level. In general, the median taxon abundance in door knob samples was higher than in bed sheets (Fig. 6A). Both richness (estimated as number of resolved genera) and Shannon diversity index (computed on genera distribution) were higher in door knob samples than bed sheet ones (Fig. 6B, C).

Of note, within the door knob samples, those diversity metrics were not statistically different between samples from units with different hygiene levels (Fig. 7).

Discussion

For decades, infection control efforts in hospitals have focused on quality control (via testing the microbial burden in different inanimate surfaces after disinfection) and quality assurance (via different standard operating procedures [SOPs], for disinfection and sanitation). However, these efforts have classically relied on culture-based microbiological techniques, such as total plate count, sterility testing, and a set of tests for evaluating antimicrobial agents.

The latest advances in sequencing technologies are driving a revolution in microbiological research, accelerating the discovery of novel microbes, and allowing the unbiased identification of bacterial communities whether they can be cultured or not.

This sequencing-driven revolution has not yet picked up steam in the areas of quality control and infection control. As we have previously proposed implementing sequence-based technologies in quality assurance and quality control of clean

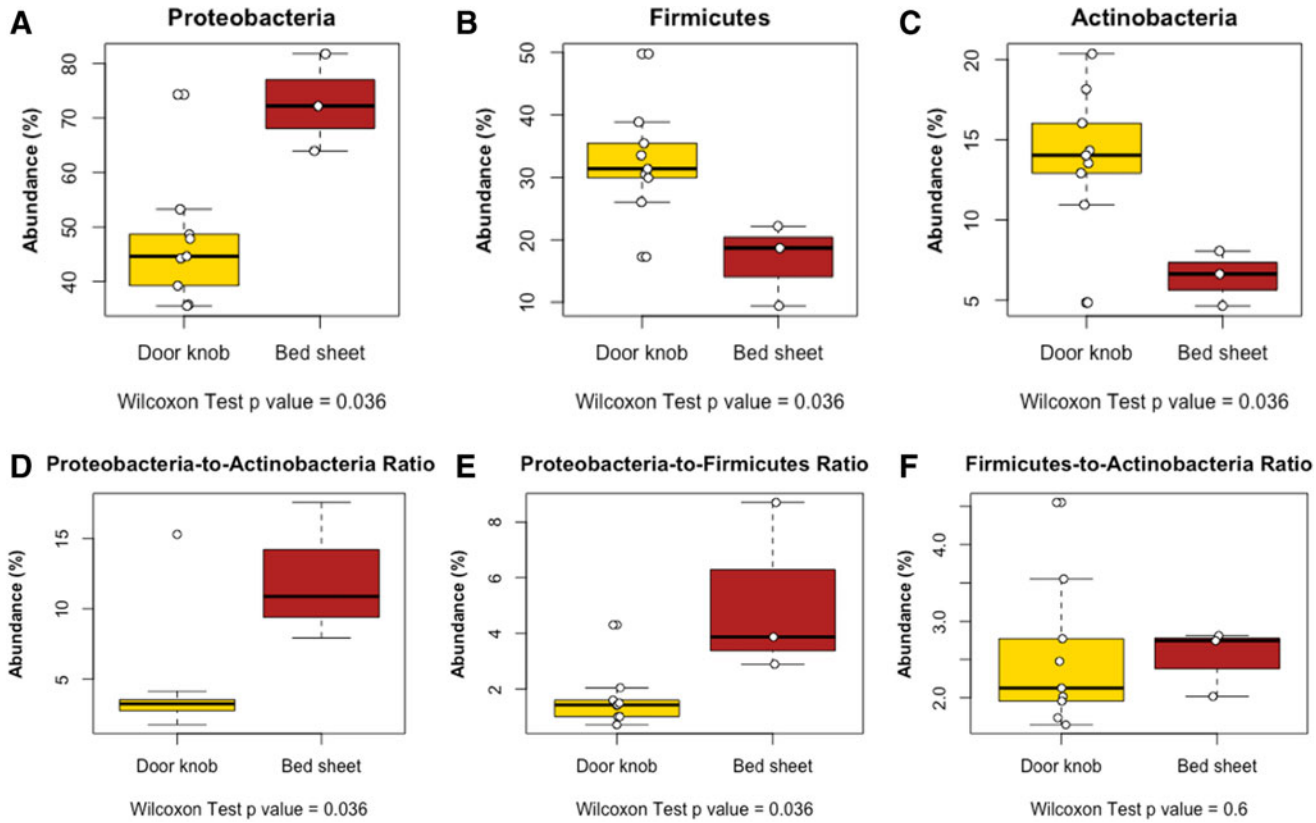

FIG. 4. Dot-overlaid boxplots comparing the abundance of different phyla **(A–C)** between door knob (*yellow*) and bed sheet samples (*firebrick color*). Differences between the two sets have been tested with Wilcoxon rank sum nonparametric test for significance, and p-values are shown below each plot. The ratios between pairs of the three most abundant phyla are also compared **(D–F)** between door knob and bed sheet samples (*same color* code).

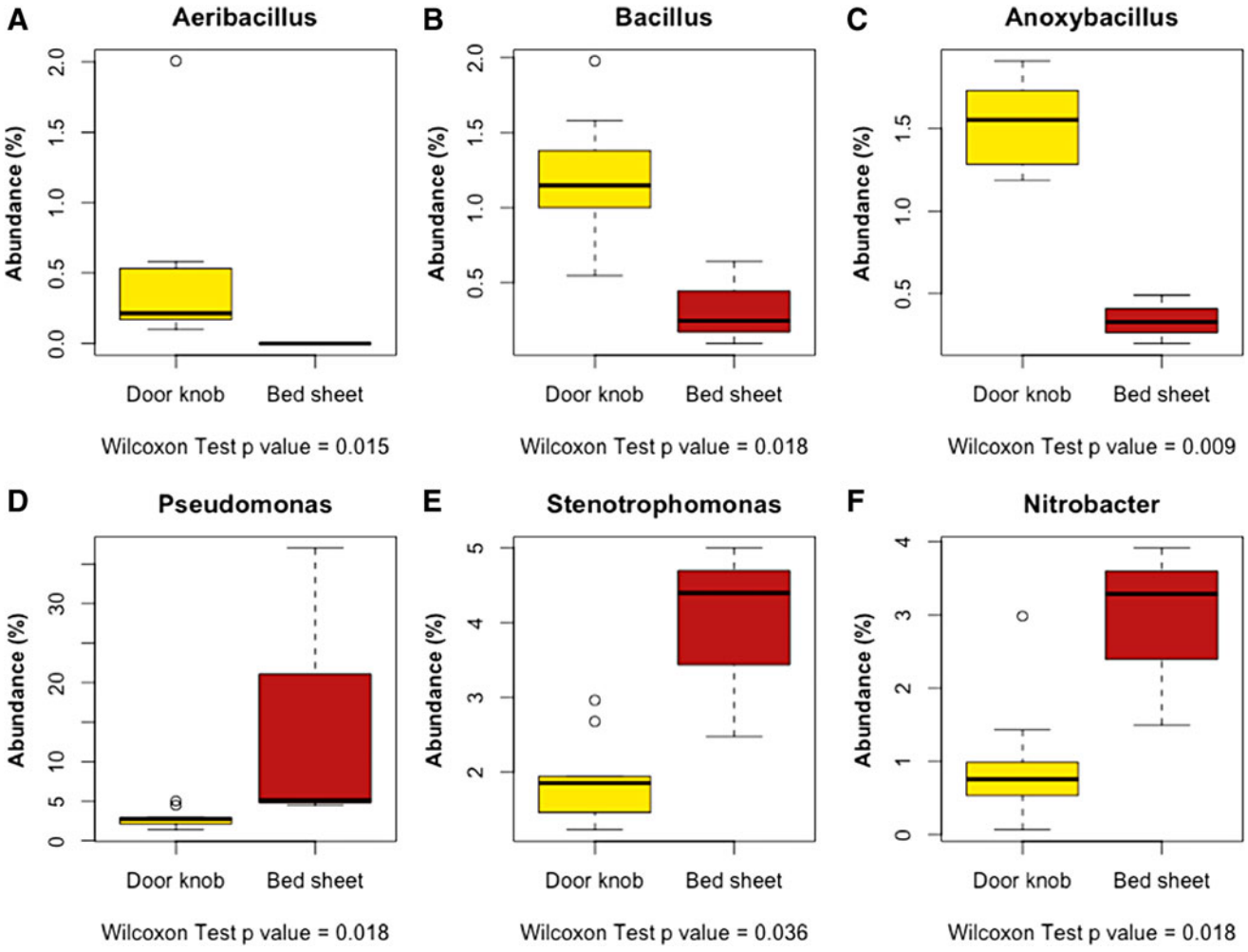

FIG. 5. Boxplots comparing the abundance of selected genera between door knob (*yellow*) and bed sheet samples (*firebrick color*). Differences between the two sets have been tested with Wilcoxon rank sum nonparametric test for significance, and *p*-values are shown below each plot. Genera **(A–C)** are more abundant in door knob samples while genera **(D–F)** are more abundant in bed sheet samples.

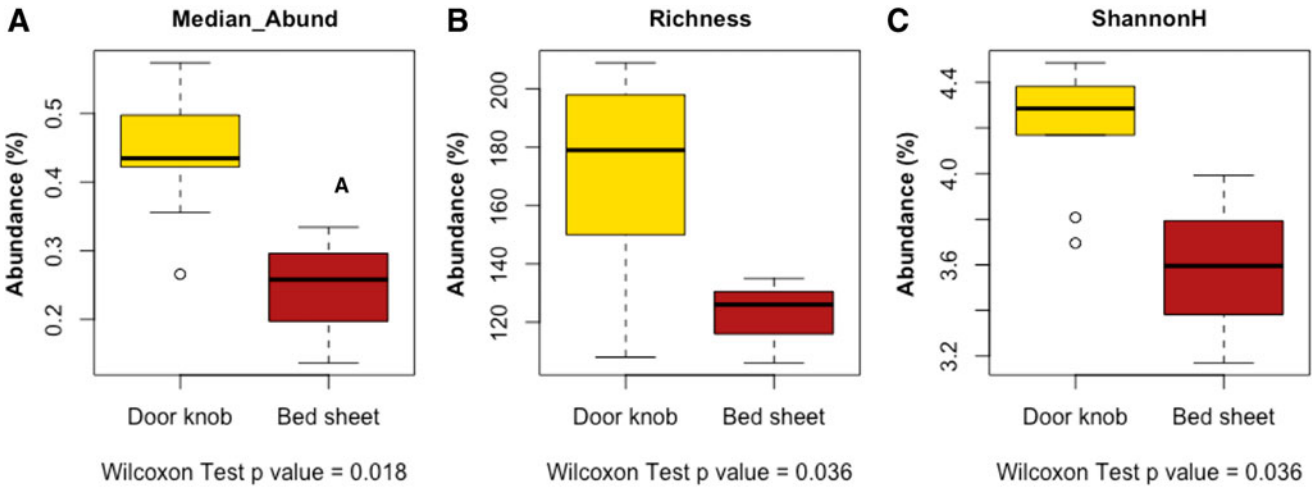

FIG. 6. Difference in median abundance **(A)**, richness **(B)**, and Shannon diversity **(C)** between door knob and bed sheet samples, visualized by boxplots. Wilcoxon rank sum test *p*-values are shown below each plot.

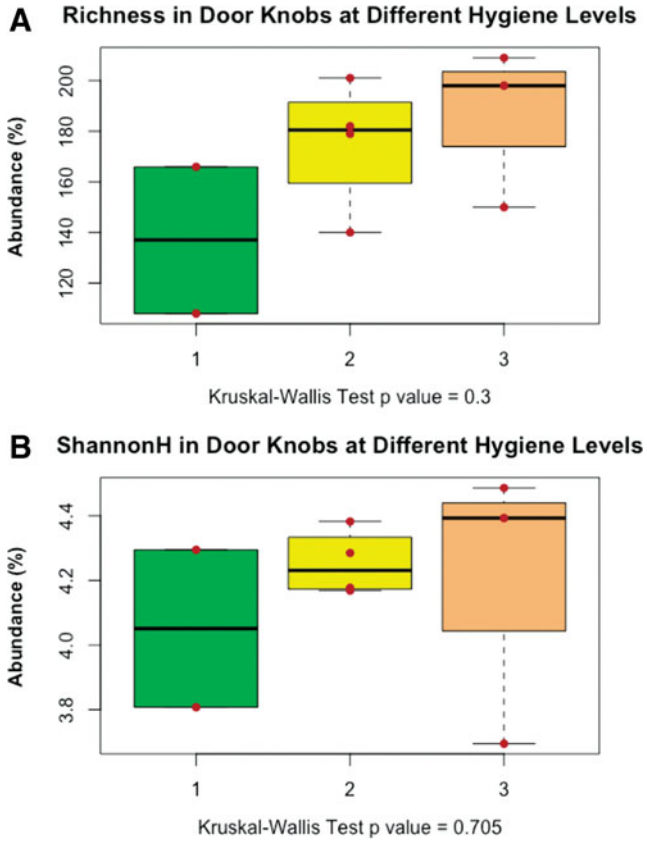

FIG. 7. Dot-overlaid boxplots comparing the richness **(A)** and Shannon diversity **(B)** of door knob samples at different hygiene levels. Level 1 (*green*) = lowest disinfection; Level 2 (*yellow*) moderate disinfection; and Level 3 (*orange*) = highest disinfection. Differences between the three sets have been tested with the nonparametric Kruskal–Wallis test for significance, and *p*-values are shown below each plot. Differences were not statistically significant in either case (at $p \leq 0.05$).

rooms/drug factories (Hamdy et al., 2018), in this work, we assess the feasibility of the same concept in hospitals.

In a hospital context, it is equally important to learn about nonpathogenic microbes as it is to learn about pathogens. These nonpathogenic organisms may not only serve as reservoirs for antimicrobial resistance genes but may also cause diseases in immunocompromised patients who are the most vulnerable in a hospital environment.

In this study, different surfaces and inanimate objects were screened within a busy, public hospital at the center of Cairo, Egypt (one of the world's most populated cities). The study primarily aimed at exploring the microbiomes of two of these surfaces: bed sheets and door knobs. While the main goal was to explore the microbial diversity on these surfaces and how it reflects the cleanliness/usage of different units in the hospital, additional goals included screening for resistant microbes and resistance genes, as well as demonstrating how microbiome analysis would dramatically change the way quality assurance/quality control in a hospital are practiced and integrated in infection control.

A massive HoMP has been launched in the United States (Lax et al., 2017), but its goal and scope were rather to explore the microbial colonization and succession in a newly established hospital, built to the highest standards. This pilot study is on a much smaller scale than the United States HoMP, but it is also with a different scope as it deals with a well-established—yet resource-limited—hospital in a developing country. Thus, the scope is not to explore microbial colonization and impact of humans as they populate this hospital, but rather the daily exposure to microbes and their nature.

This study scope is novel, and not so many published studies had a similar perspective. However, in a few studies, reviewed below, hospital objects/surfaces were screened by culture-based or culture-independent techniques, or—alternatively—similar objects or surfaces were screened in other environments (e.g., a university campus).

For example, in a neonatal ICU, the dominant source of microorganisms identified on surfaces was tracked to human skin as the genera *Streptococcus*, *Staphylococcus*, *Pseudomonas*, *Enterobacter*, and *Neisseria* were the most abundant (Hewitt et al., 2013). The impact of this microbial community was reflected upon studying the gut microbiome of premature infants in a neonatal ICU, as the colonizing gut bacteria were similar to those identified on hospital surfaces in the nursing room (Brooks et al., 2014).

In another study, conducted in a Spanish hospital, high-throughput sequencing was used to study microbial communities on inanimate surfaces. The sample from the main entrance hall was more diverse than that taken from the ICU; however, 1145 taxa were detected in the ICU, emphasizing the microbial diversity of uncultured microbes existing on a surface that is regularly disinfected (Poza et al., 2012).

Door handles were also previously studied in different contexts. For example, door handles were shown to be contaminated by variable bacterial loads, which largely depended on the handles design, location and frequency of use (Wojgani et al., 2012). In a university campus, the dominant phyla identified on all door handles were among those associated with human skin (Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes). Moreover, their relative abundances were consistent with their typical distribution on human skin (Ross and Neufeld, 2015).

In a newly opened hospital, bacteria identified on bedrails of patient rooms significantly mirrored the skin microbiota of the patient in the room. Over time, patients and room surfaces dynamically exchanged and shared similar bacterial communities. At patient admittance, the patient acquired preexisting room-associated taxa; later on, room surface communities shifted toward the patient's skin microbiome (Lax et al., 2017). Alarmingly, antibiotic resistance genes were almost always more abundant on room surfaces than on the skin of the patients residing in these rooms (Lax et al., 2017).

A repeated theme in studies on the microbiome of built environments is the impact of humans and the changes associated with changes in human activity in a built environment. Several built environment studies came to the conclusion that anthropogenic activities have the major impact on a building and not the other way around (Lax et al., 2014, 2015).

In this study, it is not possible to answer such a question since the hospital has not been sampled before its establishment, and rooms have not been sampled before patient

admission; however, based on literature and other microbiome studies, it is possible to trace many identified microbes back to human activities or environmental sources. For example, some microbial taxa can be attributed to dust and other debris, which are not uncommon in the metropolitan Cairo area. Among these taxa are *Bacillus*, *Geobacillus*, and to some extent *Acinetobacter*. Meanwhile, bacteria such as *Staphylococcus* and *Corynebacterium* are known to be human associated.

The nature and usage of analyzed surfaces are a major factor on the bacterial communities detected on them. Unlike bed sheets, which are washed, dried, and mostly limited to contact with patients and nurses, door handles are among the most touched surfaces in the entire hospital, as they are touched by patients, physicians, nurses, janitors, and visitors. While these door handles are probably frequently disinfected, especially in areas with higher hygiene level, and as such, are more frequently cleaned than bed sheets, they are not naturally considered major sources of infection compared to other hospital surfaces.

A remarkable finding in this study is that bacterial contamination (both in terms of bioburden and community composition) was not dramatically different between door knobs of hospital units with different hygiene levels (Fig. 7). However, the bacterial communities on door knob samples had more abundant taxa (Fig. 6A), and were richer and more diverse (higher Shannon diversity) than those detected on bed sheets (Fig. 6C).

The types of bacteria detected on door knobs were a mix of bacteria associated with dust/particulate matter/debris (e.g., *Bacillus*, *Geobacillus*, *Aeribacillus*) in addition to the usual skin-associated bacterial genera (e.g., *Staphylococcus*, *Corynebacterium*). The latter were among the core genera shared by all analyzed samples, but were not significantly different between door knobs and bed sheets.

On the contrary, bacteria that were more abundant in bed sheet samples are not particularly associated with human skin (e.g., *Pseudomonas*, *Nitrobacter*). *Pseudomonas* is associated with several environments, but could be associated with human secretions. On the contrary, bacteria such as *Nitrobacter* are neither human associated nor common hospital contaminants. Thus, the enrichment of *Nitrobacter* in bed sheet samples may be due to misclassification (as there could be a closely related undistinguishable genus). However, since the observation is statistically sound, it may relate to the role of *Nitrobacter* in the nitrogen cycle by generating nitrates from nitrites, always in association with other ammonia-oxidizing bacteria, which could be a sign of urinary contamination of the bed sheets. Of note, both *Nitrobacter* and Pseudomonads, abundant in bed sheet samples, are obligate aerobes, an observation that has yet to be investigated in more depth.

A major feature of 16S-based microbiome studies is the inability to accurately identify bacterial species and strains. This is important because many genera have pathogenic and nonpathogenic members, and some species have pathogenic and nonpathogenic strains. Consequently, it is not possible to make claims/assumptions on pathogenicity based on genera assignments. For example, *Staphylococcus* is host associated and a typical marker of skin or nasal microbiomes; however, since 16S-based microbiome surveys cannot determine the organism detected up to the species level, it will not be possible to conclude whether *Staphylococcus* is pathogenic (e.g., *aureus*), opportunistic (e.g., *epidermidis*), or nonpathogenic (e.g., *equorum*).

Pseudomonas and *Acinetobacter* are versatile genera that include environmental and human-associated bacteria, most of which are nonpathogenic. However, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are among the most frequent and most problematic hospital-associated bacteria, owing to their common development of resistance and their high adaptation ability (rapid mutation and rearrangement rate).

Finally, it is understandable that some hospital surfaces are expected to be sterile or to remain sterile all the time, and many other surfaces are supposed to be pathogen-free at any time. However, identifying a set of ''resident'' microbes that are common to all hospitals and another that is associated with a certain hospital (local or ''endemic'') could be of high value in tracing epidemics caused by external pathogens or by the disproportionate expansion of one of the resident members. As in human microbiome studies, dysbiosis is a sign (and sometimes a direct cause) of an upcoming disease. In a hospital context, one might argue that the expansion of a certain ''harmless'' microbe may be a sign of failure in infection control measures, or of an unexpected source of contamination.

In this study, for example, one of the bed sheet samples had an unusual proportion of *Pseudomonas*. While this may not be a pathogenic strain of *Pseudomonas*, its expansion could be a sign to be followed upon, whether its source is the patient, the health care professionals, or an inadvertent failure in disinfection SOPs.

On another front, resistance screening indicated a manifest expansion of a mobile beta-lactamase—encoding gene (bla_{TEM}), which reflects the current ubiquity of this gene in different bacterial communities, due to the gene's association with mobile genetic elements. On the contrary, a common kanamycin resistance gene was only detected in two samples, reflecting the lower mobility of the *kan* gene. Obviously the presence of a resistance gene (e.g., *kan*) does not have to imply the expression of resistance phenotype, because the gene may not be expressed under the sensitivity testing conditions (or in the site of isolation itself). In some cases, even if the gene is expressed, its product may fail to cause resistance to the tested antibiotic because of systems-level factors such as global gene regulators or other interfering/antagonistic proteins/metabolites.

Limitations and future perspectives

As a pilot study, this work provides preliminary data which are, however, quite important as they shed light on how to establish large-scale hospital microbiome studies in already populated hospitals working at full capacity in urban areas. Pilot studies are exploratory in nature and usually provide proof-of-concept evidence to permit the design of more comprehensive and systematic investigations. They are most suitable for previously uninvestigated topics or, as in this study, novel approaches to otherwise established practices (hospital infection control). Our recommendation in such studies is to focus on a few sample types but collect large number of samples, and preferably at different time intervals so that comparisons can be effectively and efficiently made.

The major limitation of this work is the small number of samples that were sequenced. Future studies should focus on multiple sampling of inanimate surfaces, and should expand from 16S rRNA microbiome surveys to full metagenomic sequencing and possible recovery of complete microbial genomes to allow the analysis of clonal expansion of some bacterial strains or tracing how potential multiresistant strains emerge and spread. Full resistome analysis (Elbehery et al., 2016) is another potential area of expansion that is highly needed (and still not fully implemented) in hospital microbiome studies.

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Author Contributions

M.T.E., M.A.A., and R.K.A. conceived the study and designed experiments; S.G.-E. collected samples and performed laboratory experiments; M.T.E., S.G.-E., and R.K.A. analyzed experimental data; M.T.E. and R.K.A. conducted bioinformatic and statistical analysis; M.T.E. and S.G.-E. analyzed microbiome data; M.T.E., S.G.-E., and R.K.A. drafted the article; M.T.E. and R.K.A. wrote the article in its final format; all authors critically read and approved the final revised article.

Author Disclosure Statement

The authors declare they have no conflicting financial interests.

Supplementary Material

Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3
Supplementary Table S1

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Abbreviations Used

CDC = Centers for Disease Control and Prevention
HAI = hospital-acquired infections
HoMP = Hospital microbiome project
ICU = intensive care unit
NT = not tested
OUT = operational taxonomic unit
PCR = polymerase chain reaction
SOP = standard operating procedure