Clinical Microbiology and Infection xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

Comparative analysis of surface sanitization protocols on the bacterial community structures in the hospital environment

Tilman E. Klassert [1, *], Cristina Zubiria-Barrera [1], Robert Neubert [1], Magdalena Stock [1], Antony Schneegans [1], Mercedes López [2], Dominik Driesch [3], Gregor Zakonsky [4], Petra Gastmeier [5], Hortense Slevogt [1, 6, †], Rasmus Leistner [5, †]

[1] Jena University Hospital, ZIK Septomics, Host Septomics, Jena, Germany
[2] University Institute of Tropical Diseases and Public Health of the Canary Islands, University of La Laguna, Spain
[3] BioControl Jena GmbH, Jena, Germany
[4] Charité CFM Facility Management GmbH, Berlin, Germany
[5] Institute for Hygiene and Environmental Medicine, Charité – Universitätsmedizin Berlin, Germany
[6] Department of Respiratory Medicine, Medizinische Hochschule Hannover, Germany

ARTICLE INFO

Article history:
Received 20 August 2021
Received in revised form
10 February 2022
Accepted 20 February 2022
Available online xxx

Editor: E.J. Kuipers

Keywords:
Antibiotic resistance
Hygiene
Hospital
Microbiome
Probiotics
Sanitization

ABSTRACT

Objectives: In hospital hygiene, it remains unclear to what extent surface contamination might represent a potential reservoir for nosocomial pathogens. This study investigates the effects of different sanitization strategies on the microbial structures and the ecological balance of the environmental microbiome in the clinical setting.

Methods: Three cleaning regimes (disinfectants, detergents, and probiotics) were applied subsequently in nine independent patient rooms at a neurological ward (Charité, Berlin). Weekly sampling procedures included three different environmental sites: floor, door handle, and sink. Characterization of the environmental microbiota and detection of antibiotic resistance genes (ARGs) were performed by *16S rRNA* sequencing and multiplex Taq-Man qPCR assays, respectively.

Results: Our results showed a displacement of the intrinsic environmental microbiota after probiotic sanitization, which reached statistical significance in the sink samples (median 16S-rRNA copies = 138.3; IQR: 24.38–379.5) when compared to traditional disinfection measures (median *16S rRNA* copies = 1343; IQR: 330.9–9479; $p < 0.05$). This effect was concomitant with a significant increase in the alpha-diversity metrics in both the floor ($p < 0.001$) and the sink samples ($p < 0.01$) during the probiotic strategy. We did not observe a sanitization-dependent change in relative pathogen abundance at any tested site, but there was a significant reduction in the total ARG counts in the sink samples during probiotic cleaning (mean ARGs/sample: 0.095 ± 0.067) when compared to the disinfection strategy (mean ARGs/sample: 0.386 ± 0.116; $p < 0.01$).

Discussion: The data presented in this study suggest that probiotic sanitization is an interesting strategy in hospital hygiene management to be further analyzed and validated in randomized clinical studies.

Tilman E. Klassert, Clin Microbiol Infect 2022;▪:1

Introduction

Hospital-associated infections (HAIs) remain one of the main challenges in healthcare worldwide [1]. In Europe, an annual incidence of 3 million HAIs leads to more deaths in acute care hospitals than all other infectious diseases under surveillance at the European level [1,2]. This problem is further aggravated by the rise of multidrug resistance in nosocomial pathogens, such as

* Corresponding author: Tilman E. Klassert, Jena University Hospital, ZIK Septomics, Host Septomics, Jena, Germany.
E-mail address: Tilman.Klassert@med.uni-jena.com (T.E. Klassert).
† Hortense Slevogt and Rasmus Leistner contributed equally.

https://doi.org/10.1016/j.cmi.2022.02.032

multiresistant *Staphylococcus aureus* (MRSA) [2]. A median annual burden of over 148 000 MRSA-HAIs in the EU/EEA leads to over 7000 deaths every year [2].

Contamination of the hospital environment is a highly debated issue in hospital hygiene; it remains unclear to what extent different surfaces might represent a potential reservoir for clinically relevant pathogens and provide a potential source for the transmission of HAIs [3–8]. Thus, sanitization programs for the clinical environment are monitored as essential steps for the prevention and control of hospital infections [9]. However, traditional disinfectants have shown several major limitations. On one hand, they are not effective against recontamination of the cleaned surface (i.e. disinfection is highly limited in time). Such recontamination phenomena have been reported as early as within the first 30 minutes after disinfection [10]. On the other hand, disinfectants may lead to the selection of resistant bacterial strains, not only against the disinfectant itself [11] but also against diverse antimicrobial agents [11–13]. These limitations translate into persistence of contamination over time with a concomitant increase in resistant microbes in the clinical environment. In addition, disinfectants have a hazardous effect on the environment [14].

In recent years, the health of hospital surfaces has been rethought, driven by the urgent need for an effective and sustainable solution for hospital sanitization. A new concept of surface health is based on the hypothesis that replacement of bacteria with beneficial microbes might be more effective for the control of pathogens than disinfection measures [15,16]. Thus, a sanitization approach based on eco-sustainable detergents with probiotic *Bacillus* spores has been investigated recently for its potential application to healthcare-related surfaces [7]. The first studies have reported a significant improvement in pathogen control on surfaces when compared to traditional disinfection measures [17,18]. Moreover, probiotic sanitization was demonstrated to be safe for patients [19] and did not select for resistant bacteria strains [7]. A multicentre interventional study could further support the clinical effectiveness of this approach, as the use of probiotic sanitization translated into a significant decrease of nosocomial infections [8].

Although the benefits of such probiotic strategies have been demonstrated, it is still unclear to what extent the massive inoculation of particular bacterial species affects the microbial structures and ecological balance of the environment. Moreover, the composition of the environmental microbiome upon different sanitation strategies has not been investigated in the clinical setting. Most microbiological studies addressing infection control strategies have relied on culture-dependent methods, which are not capable of covering the full diversity of the environmental microbiome [20]. In this study, we used *16S rRNA* gene sequencing approaches to characterize the bacterial microbiota on different surfaces of the hospital environment. The longitudinal data were then subjected to comprehensive comparisons between different sanitation strategies (disinfectants, detergents, and probiotics) to measure their potential effect on the microbial community structures and on the incidence of antibiotic resistance determinants in the hospital microbiome.

Methods

Study design and sample collection

This study was designed to investigate the impact of three different sanitization protocols on the environmental microbiome of a neurology ward at the Charité–Universitätsmedizin Berlin. The following cleaning regimes were applied subsequently for 3-month periods each: disinfectants, detergents, and probiotics (details are shown in Fig. S1). The surfaces were cleaned daily using specific products: Incidin Pro 0.5% (ECOLAB) for disinfection; Brial Top 0.5% (ECOLAB) as detergent; and bacilli-containing detergents 0.5% (CHRISAL) as probiotics (see Table S1 for strain details). Microbial sampling was performed once a week in nine independent patient rooms and included three different environmental sites and surface materials: floor (linoleum), door handle (steel), and sink (ceramic). Patient material sampling included nasal and rectal swabs. All patients gave written informed consent in accordance with the Declaration of Helsinki and the local ethics committee (Ethikausschuss Campus Charité–Mitte; approval EA1/387/16).

DNA extraction and 16S rRNA *gene quantification*

The DNA of all samples was extracted with the innuPrep Bacteria DNA + Lysis Booster Kit (Analytik, Jena, Germany) following the manufacturer's instructions. This validated DNA extraction method [21] without mechanical lysis procedures was chosen to minimize the impact of the extrinsic bacilli spores contained in the probiotic products (Fig. S2). This way, the intrinsic environmental microbiome could be characterized in a comparable manner across all three sanitization protocols. The bacterial biomass in the samples was measured by quantification of the *16S rRNA* gene copies using a qPCR approach as described elsewhere [21]. Detailed methods can be found in the supplementary material.

16S rRNA *gene amplicon sequencing*

The library construction and sequencing were performed as described elsewhere [21–23]. Resulting Fastq files were demultiplexed with QIIME v1.9.1 scripts [24] and filtered for the removal of potential contaminants, as described elsewhere [21,25]. Taxonomic assignment was performed using the SILVA REF NR 99 (release 132) database [26]. Co-occurrence network analyses to measure nonrandom interactions between bacteria were performed with SparCC software v.0.1.0 [27]. Pathogenicity status of the bacteria was defined in accordance to their reference in the KEGG Pathogens and ISID databases (see also Table S2). The data sets generated for this study are available at the SRA database under the accession number PRJNA774945 (https://www.ncbi.nlm.nih.gov/sra/PRJNA774945).

Antibiotic resistance gene detection

The presence of 12 antibiotic resistance genes (ARGs) (Table S3) conferring resistance to β-lactams, quinolones, or polymyxins was analyzed throughout the course of the study. The following determinants were addressed: *blaKPC, blaNDM, blaOXA48, blaVIM, blaCMY, blaGES, blaSHV, blaTEM, blaCTX-M1, qnrB1, mcr1,* and *mecA*. For their detection, custom multiplex Taq-Man assays were performed as described previously [21].

Statistics

All comparative analyses (ANOVA, Permanova, and Kruskal-Wallis tests) of the sanitization protocols targeted only the last 7 weeks of each cleaning regime to allow the microbiota to adapt to the different sanitization products during the first 6 weeks after regime change. This time frame was chosen based on previous observations of environmental microbiota dynamics [21]. Statistical analyses and graphic presentations were performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). Statistical tests and settings are detailed in the supplementary material.

Please cite this article as: Klassert TE et al., Comparative analysis of surface sanitization protocols on the bacterial community structures in the hospital environment, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2022.02.032

Results

Impact of surface sanitization protocols on the bacterial community structures

The bacterial load was quantified on the different environmental sites using *16S rRNA* qPCR. In agreement with our previous findings [21], the sink samples showed the highest bacterial load, followed by the door handle. The lowest biomass was detected on the floor (Fig. S3). When the bacterial biomass was compared among different cleaning regimes, a slight decrease in the intrinsic microbiota was observed after probiotic sanitization measures (see Table S4). This effect was most pronounced in the probiotics-cleaned sink samples (median *16S rRNA* copies = 138.3; IQR: 24.38–379.5), reaching statistical significance when compared to the sinks during traditional disinfection (median *16S rRNA* copies = 1343; IQR: 330.9–9479; $p < 0.05$; Fig. 1A).

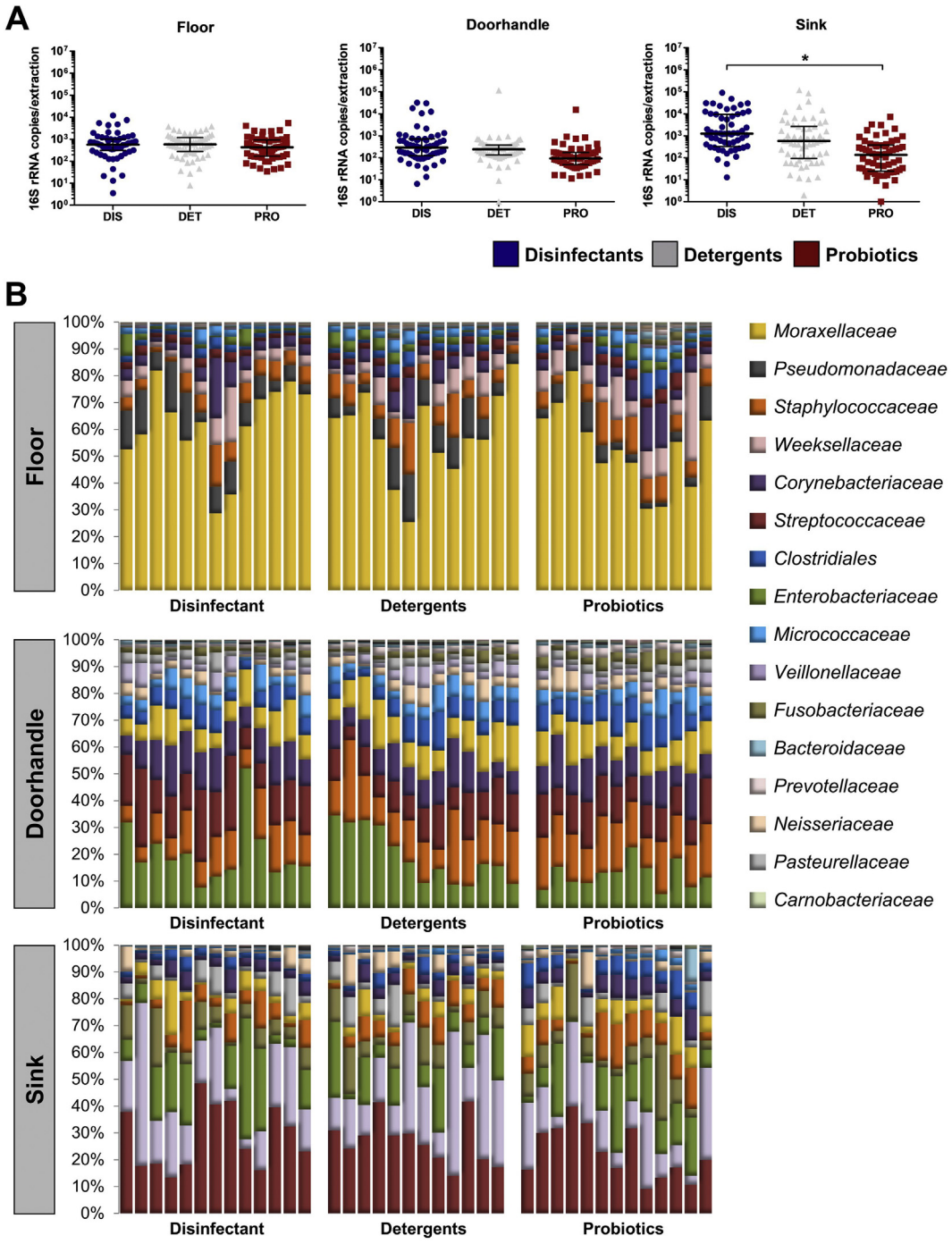

Fig. 1. Quantitative and compositional analysis of the bacterial communities of floor, door handle, and sink during different sanitization strategies. (A) Quantitative analysis of the bacterial biomass across different cleaning regimes (DET, detergents; DIS, disinfectants; PRO, probiotics). Shown are the *16S rRNA* gene copies in each sample (median ± IQR; *$p < 0.05$). (B) Taxonomic summary of the compositional changes of each environmental site after different sanitization protocols. Shown are the relative abundance of the collapsed main taxa (>0.5%) at family level for each week.

Please cite this article as: Klassert TE et al., Comparative analysis of surface sanitization protocols on the bacterial community structures in the hospital environment, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2022.02.032

In a next step, we analyzed whether the sanitization-dependent biomass variability was associated with structural changes in the microbial communities. The analysis at family level revealed only limited variation in the community composition across the weeks and wash regime blocks. In general, the community structures were highly site specific and consistent across time (Fig. 1B). The floor was dominated by Moraxellacea, the door handle samples by Enterobacteriaceae and Staphylococcaceae, and the sink by

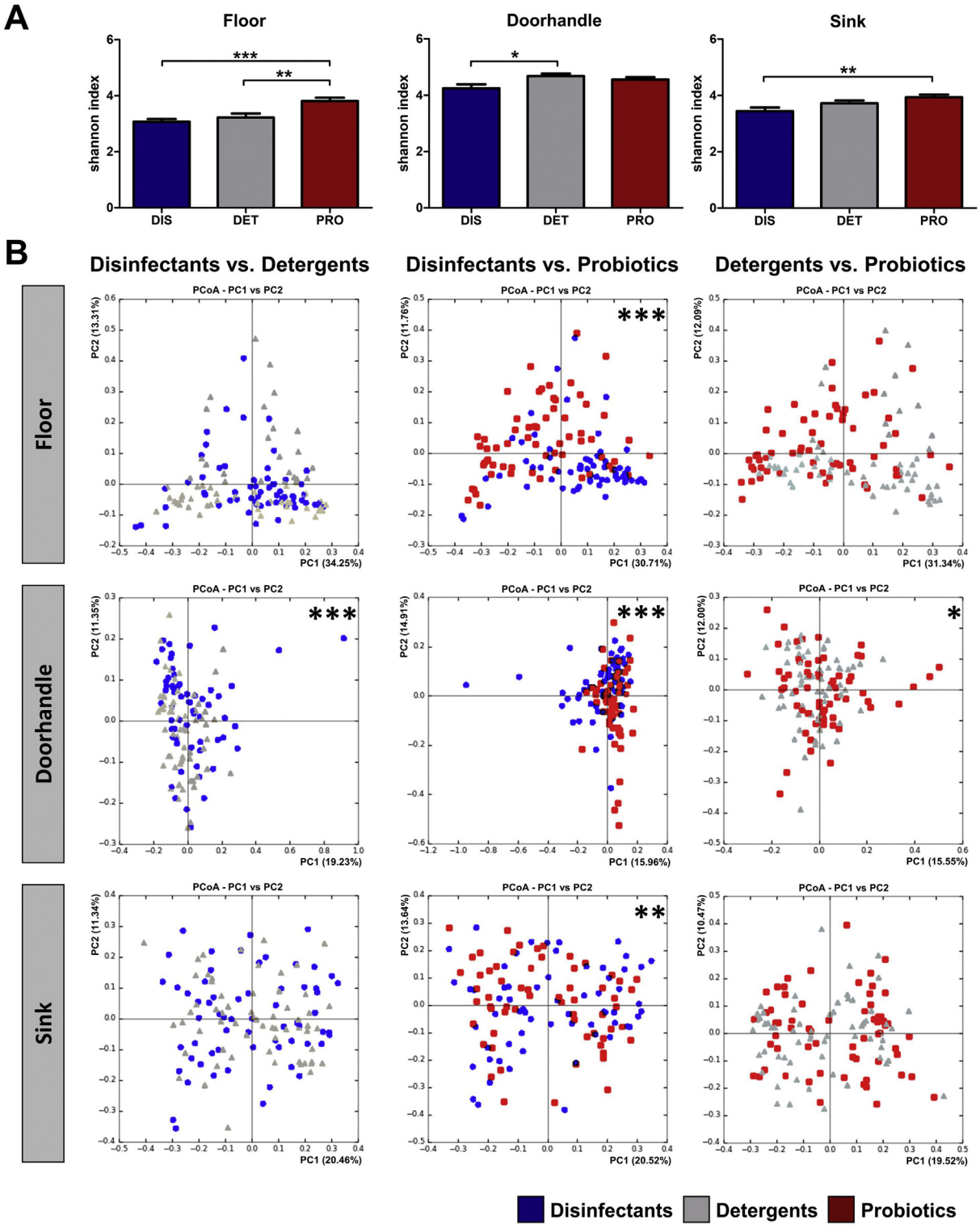

Fig. 2. Diversity metrics of the environmental microbiota upon different sanitization strategies. (A) Alpha diversity of each environmental site after different sanitization strategies. Shown is the Shannon index (mean ± standard error of the mean, *p < 0.05; **p < 0.01; ***p < 0.001, ANOVA). (B) Principal coordinates analysis of the beta diversity using weighted Unifrac distances. Shown are pairwise comparisons between different sanitization strategies. Statistical significance was calculated by Permanova (*p < 0.05; **p < 0.01; ***p < 0.001).

Please cite this article as: Klassert TE et al., Comparative analysis of surface sanitization protocols on the bacterial community structures in the hospital environment, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2022.02.032

Streptococacceae and Veillonellaceae (Fig. 1B). However, analysis of the alpha diversity revealed significant differences in the community-associated Shannon indices between different sanitization strategies. The disinfectant protocol led to the lowest diversity values of the environmental microbiota at all three tested sites. The probiotic strategy showed the highest diversity on the floor and in the sink, which was significantly increased in comparison to the disinfection strategy ($p < 0.001$ and $p < 0.01$, respectively; Fig. 2A). For the door handle, both the probiotic and detergent protocols showed an increased diversity, although only the latter reached statistical significance when compared to conventional disinfection measures ($p < 0.05$; Fig. 2A).

The observed differences were further supported by the results obtained after pairwise comparisons of the beta diversity metrics in a PCoA space (Fig. 2B). Permanova analyses revealed differences in the PCoA distribution among sanitization strategies, especially between disinfectant-treated and probiotic-treated samples, as shown by significant segregation for all three tested surfaces (Permanova; $p < 0.001$ for floor and door handle, $p < 0.01$ for the sink samples). In contrast, the samples from the patients who occupied the tested rooms did not show any significant stratification among the different cleaning regimes (Fig. S4). Thus, the alpha- and beta-diversity metrics suggest a significant impact of the sanitization protocol on the bacterial community structures in the hospital environment. To specifically address these differences, further analyses at lower taxonomic levels were performed.

Selective effect of sanitization strategies on specific bacterial taxa

At the genus level, the most representative taxa on each surface type did not significantly change their relative abundance across different sanitization strategies (Fig. 3A). Kruskal-Wallis testing was then applied on the relative abundance data to detect those taxa with significant differences between cleaning regimes (Table S5). The top significantly changed taxa are shown in Fig. 3B. These included, among others, a reduced abundance of *Pseudomonas* on all tested surfaces during the probiotic cleaning and an increase in Anaerococci observed in door handle and sink samples during the same sanitization strategy (Fig. 3B). Overall, a very limited number of taxa showed any significant changes across cleaning regimes. Moreover, the overall bacterial community structures did not change significantly across sanitization strategies, as measured by

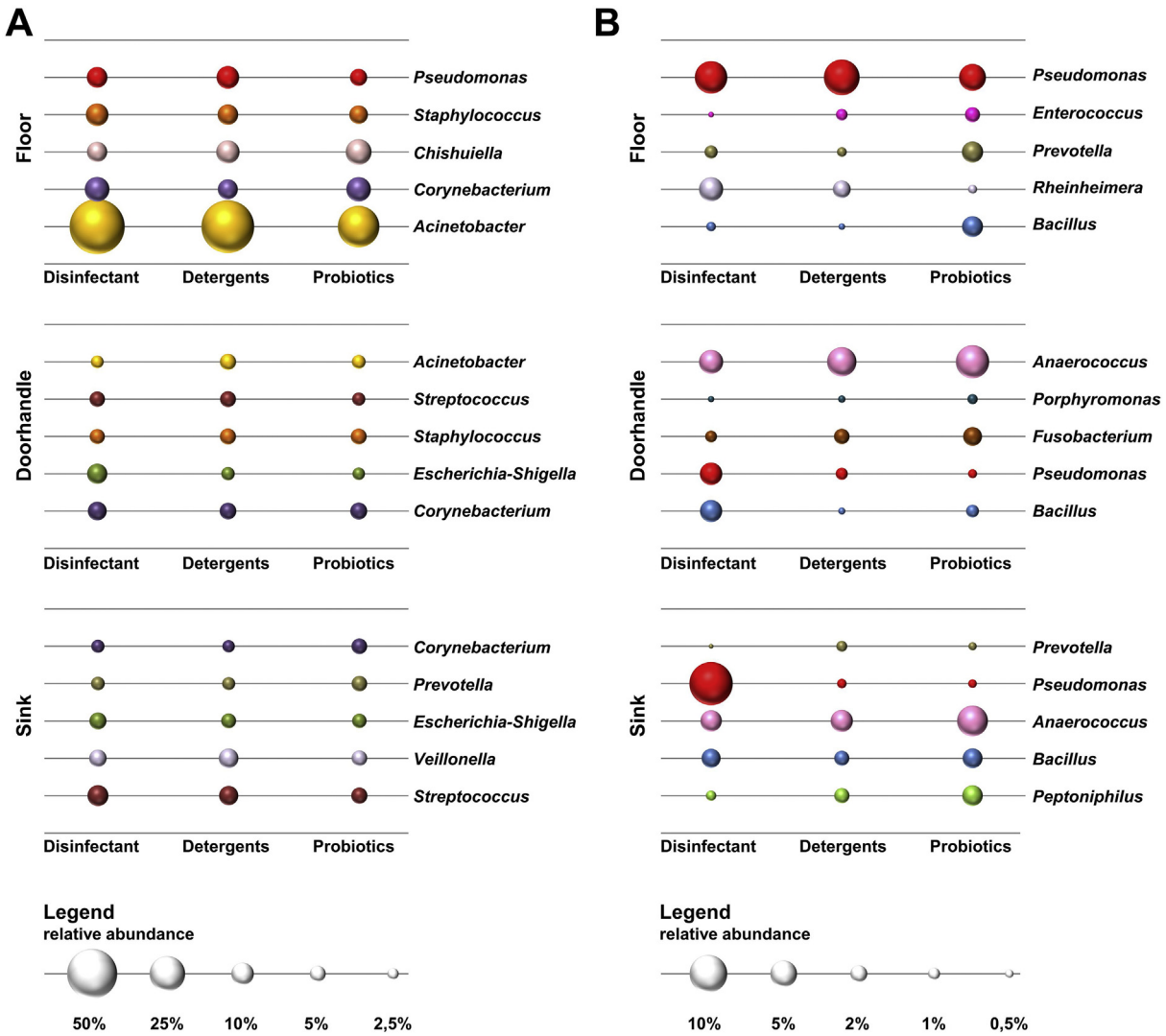

Fig. 3. Relative abundance of specific bacterial taxa after different sanitization strategies. (A) Relative abundance changes over time of the most abundant genera. Bubble size represents the proportional abundance across different sanitization strategies. (B) Relative abundance dynamics of the top significantly changed taxa (genus level, with >0.5% relative abundance) across the different cleaning regimes.

Please cite this article as: Klassert TE et al., Comparative analysis of surface sanitization protocols on the bacterial community structures in the hospital environment, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2022.02.032

network analyses and their associated clustering coefficients and modularity indices (Fig. S5).

To determine whether the relative abundance of specific pathogens changed across different sanitization strategies, sequencing data were analyzed using the subclassifying genus option implemented in the SILVA database. Only three frequent pathogens (present in more than 20% of the samples) could be identified in the sequencing data at the species level: *Staphylococcus aureus*, *Enterococcus faecalis,* and *Escherichia coli*. However, we could not detect any significant change in their relative abundance across the different cleaning regimes (Fig. S6).

Influence of cleaning regimes on ARG dissemination

In line with our previous study [21], the highest amounts of antibiotic resistance determinants were detected in the floor samples. This observation was consistent across all nine tested rooms (Fig. S7). In total, nine of the ARGs were detected in any of the samples tested. Among these ARGs, *mecA* was ubiquitously found on all surfaces. Other ARGs were mainly detected on a specific surface type, such as *blaVIM* and *blaNDM* on the floor and *blaCMY* in the sink samples (Fig. 4A). When the ARG occurrence was compared between different sanitization regimes, a significant reduction in the ARG counts was observed in the sink samples during both probiotic (mean ARGs/sample: 0.095 ± 0.067) and detergent (mean ARGs/sample: 0.127 ± 0.037) sanitization strategies when compared to the disinfection strategy (mean ARGs/sample: 0.386 ± 0.116; $p < 0.01$; Fig. 4B). This was especially striking during the probiotic protocol, as seven of the nine analyzed rooms did not show the presence of any of the tested ARG during this particular sanitization window. When each specific ARG incidence was analyzed independently, significant differences between cleaning regimes were only detected for *mecA* (Fig. S8). When compared to conventional disinfection measures, the probiotic approach led to a reduction in *mecA* at all surfaces, reaching statistical significance for the sink samples (Fig. 4C). As measured by rectal and nasal swabs, the probiotic cleaning-dependent reduction of ARGs in the environment was only partially detected in patient samples and did not reach statistical significance across sanitization protocols (Fig. S9).

Discussion

This study comprehensively characterizes the compositional changes of the microbiome in the clinical environment upon different sanitization protocols. Overall, the microbial community structures of the hospital environment were stable during the whole study; the most representative taxa of each surface type remained unchanged across the different sanitization protocols. Only minor genera changed their relative abundance across the different cleaning regimes, and these did not include any of the three most abundant pathogens detected in this study. However, our results showed that the probiotic strategy led to a displacement of the absolute biomass of the intrinsic microbiota and a

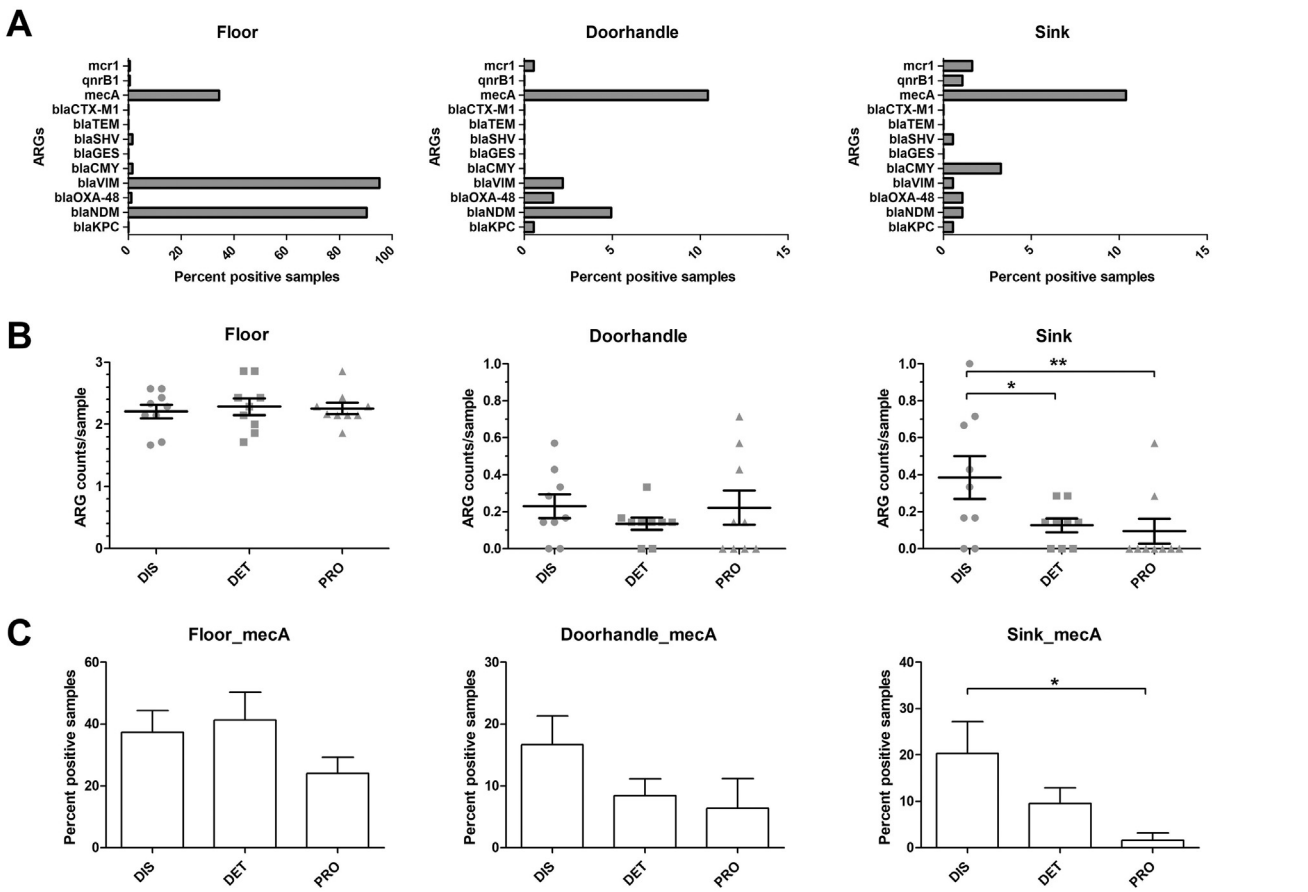

Fig. 4. Antibiotic resistance gene (ARG) detection in the hospital environment after different sanitization protocols. (A) Bar chart depicting the ARG expression across all environmental samples analyzed. Bars represent percentage of samples with positive ARG detection. (B) ARG detection across different sanitization strategies. Shown are the mean ARG counts detected in each of the nine rooms analyzed during the different sanitization windows. (C) Chart depicting the percentage of positive *mecA* samples detected during each cleaning regime at each environmental site.

Please cite this article as: Klassert TE et al., Comparative analysis of surface sanitization protocols on the bacterial community structures in the hospital environment, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2022.02.032

concomitant increase in its alpha diversity metrics. These effects reached statistical significance in the sink samples of the tested rooms. In addition, our data showed a significant reduction of the total ARG counts in the sink samples during probiotic cleaning when compared to traditional disinfection strategies. In particular, the *mecA* prevalence could be significantly reduced upon application of probiotic cleaning protocols.

The concept of using nonpathogenic microorganisms to counteract the growth of other bacterial species was introduced over a decade ago and called *biocontrol* [28]. Although the efficacy of probiotics in critically ill patients is not conclusive [29], its application to water systems [30] or breeding farms [31] has been reported as successful. In recent years, several *in vitro* and interventional studies have suggested its application to hospital environments as a promising alternative to disinfection measures [8,18] and reported it to be safe for patients [19]. However, the impact of such biocontrol measures on the microbial structures and the ecological balance of the hospital environment had not yet been analyzed. Our results on probiotics-mediated displacement of the intrinsic environmental microbiota by *Bacillus* spp. are in line with mechanistic *in vitro* studies showing a selective reduction in specific taxa based on the competitive exclusion principle [18,32]. As a consequence of such displacement, the absolute number of specific bacterial taxa, including potential pathogens, might be reduced and replaced by nonpathogenic bacteria. Although the absolute intrinsic microbial biomass is decreased, our study shows that the community structure and relative abundances of its members remain mainly unaltered. Furthermore, the community fitness did not change significantly across different cleaning strategies, as assessed by network analyses. It might be speculated, however, that the increased biodiversity upon probiotic cleaning might translate into an improved resilience of the environmental ecosystems [33].

Among the taxa that showed significant variation among cleaning regimes, *Pseudomonas* and *Anaerococcus* were the most abundant genera. In general, Anaerococci are mostly commensals of the human microbiome [34], whereas the genus *Pseudomonas* includes several pathogenic and opportunistic species [35]. Interestingly, when comparing different sanitization windows, *Anaerococcus* showed the greatest presence during probiotic cleaning, whereas *Pseudomonas* was most prominent upon disinfection. This might be associated with the reported ability of *Pseudomonas* spp. to adapt and develop resistance to diverse disinfectants [36,37]. However, in this study we did not detect any significant sanitization-dependent increase in relative pathogen abundances when screening the sequencing data at the species level. Interestingly, the search for specific antibiotic resistance determinants allowed the identification of the *mecA* gene as significantly reduced in the clinical environment upon probiotic treatment. This gene is responsible for methicillin resistance and widely disseminated in *S aureus* populations [38]. The reduction in *mecA* might be partially explained by the probiotics-triggered competitive exclusion principle, as the significant reduction of biomass in the sink samples might also translate into a decrease in the total *S aureus* counts and thus correlate with the reduced *mecA* detection in these samples.

Our study has some limitations, which include the inability to discriminate between live and dead bacteria and a limited number of ARGs that were tested. Furthermore, the data were generated from one ward of a single healthcare centre.

The data presented in this study suggest that probiotic sanitization is an interesting strategy in hospital hygiene management and should be further analyzed in larger multicentre studies. In addition, randomized clinical studies with the endpoint of nosocomial infections are required to further validate the impact of our observations.

Transparency declaration

DD and GZ were employed by BioControl Jena GmbH and Charité CFM Facility Management GmbH, respectively. HS reports grants and contracts from/with Gerbion, fzmb GmbH, BioControl GmbH, and Viromed, all unrelated to the submitted work. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

This project was supported by the Federal Ministry of Education and Science (Bundesministerium für Bildung und Forschung) within the framework of InfectControl 2020 (Project KARMIN – 03Z0818C) and a Scholarship by the Jürgen Manchot Stiftung to RN. The data sets generated for this study are available at the SRA database [Bioproject PRJNA774945; https://www.ncbi.nlm.nih.gov/sra/PRJNA774945]

Author contributions

TEK, RL, PG, and HS conceived and designed the study and experiments. TEK and CZB wrote the manuscript. TEK, CZB, RN, AS, and ML performed the experiments. TEK, CZB, MS, PG, and HS analyzed the data. PG, GZ, HS, and RL coordinated the sample collection at the Charité (Berlin). All authors reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgements

We want to thank Moira Stark, Elke Lemke, Jennifer Golembus, Solvy Wolke, and Ignacio R. Zubiria Pineda for their excellent technical assistance. This work will be part of the doctoral thesis of CZB.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2022.02.032.

References

[1] Suetens C, Hopkins S, Kolman J, Diaz Högberg L. Point prevalence survey of healthcare associated infections and antimicrobial use in European acute care hospitals. ECDC Surveillance Report; 2013. https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/healthcare-associated-infections-antimicrobial-use-PPS.pdf. [Accessed 28 November 2021].

[2] Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet 2019;19:56–66.

[3] Allerberger F, Ayliffe G, Bassetti M, Braveny I, Bucher A, Damani N, et al. Routine surface disinfection in health care facilities: should we do it. Am J Infect Control 2002;30:318–9.

[4] Otter JA, Yezli S, French GL. The role played by contaminated surfaces in the transmission of nosocomial pathogens. Infect Control Hosp Epidemiol 2011;32:687–99.

[5] Squeri R, Grillo O, La Fauci V. Surveillance and evidence of contamination in hospital environment from methicillin and vancomycin-resistant microbial agents. J Prev Med Hyg 2012;53:143–5.

[6] Weber DJ, Anderson D, Rutala WA. The role of the surface environment in healthcare-associated infections. Curr Opin Infect Dis 2013;26:338–44.

[7] Caselli E, D'Accolti M, Vandini A, Lanzoni L, Camerada MT, Coccagna M, et al. Impact of a probiotic-based cleaning intervention on the microbiota ecosystem of the hospital surfaces: focus on the resistome remodulation. PLoS One 2016;11:e0148857.

[8] Caselli E, Brusaferro S, Coccagna M, Arnoldo L, Berloco F, Antonioli P, et al. Reducing healthcare-associated infections incidence by a probiotic-based sanitation system: a multicentre, prospective, intervention study. PLoS One 2018;13:e0199616.

[9] Dancer SJ. How do we assess hospital cleaning? A proposal for microbiological standards for surface hygiene in hospitals. J Hosp Infect 2004;56:10–5.

[10] D'Accolti M, Soffritti I, Mazzacane S, Caselli E. Fighting AMR in the healthcare environment: microbiome-based sanitation approaches and monitoring tools. Int J Molec Sci 2019;20:1535.
[11] Bock L, Wand M, Sutton J. Varying activity of chlorhexidine-based disinfectants against Klebsiella pneumoniae clinical isolates and adapted strains. J Hosp Infect 2016;93:42–8.
[12] Wand ME, Bock LJ, Bonney LC, Sutton JM. Mechanisms of increased resistance to chlorhexidine and cross-resistance to colistin following exposure of Klebsiella pneumoniae clinical isolates to chlorhexidine. Antimicrob Agents Chemother 2017;61. e01162-16.
[13] Mahnert A, Moissl-Eichinger C, Zojer M, Bogumil D, Mizrahi I, Rattei T, et al. Man-made microbial resistances in built environments. Nat Commun 2019;10:1–12.
[14] Kümmerer K. Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources–a review. Chemosphere 2001;45:957–69.
[15] Al-Ghalith GA, Knights D. Focus: personalized medicine: bygiene: the new paradigm of bidirectional hygiene. Yale J Biol Med 2015;88:359.
[16] Pettigrew MM, Johnson JK, Harris AD. The human microbiota: novel targets for hospital-acquired infections and antibiotic resistance. Ann Epidemiol 2016;26:342–7.
[17] Vandini A, Temmerman R, Frabetti A, Caselli E, Antonioli P, Balboni PG, et al. Hard surface biocontrol in hospitals using microbial-based cleaning products. PLoS One 2014;9:e108598. https://doi.org/10.1371/journal.pone.0108598.
[18] Fauci Vl, Costa G, Anastasi F, Facciolà A, Grillo O, Squeri R. An innovative approach to hospital sanitization using probiotics: in vitro and field trials. J Microb Biochem Technol 2015;7:160–4.
[19] Caselli E, Antonioli P, Mazzacane S. Safety of probiotics used for hospital environmental sanitation. J Hosp Infect 2016;94:193–4.
[20] Lax S, Gilbert JA. Hospital-associated microbiota and implications for nosocomial infections. Trends Mol Med 2015;21:427–32.
[21] Klassert TE, Leistner R, Zubiria-Barrera C, Stock M, López M, Neubert R, et al. Bacterial colonization dynamics and antibiotic resistance gene dissemination in the hospital environment after first patient occupancy: a longitudinal metagenetic study. Microbiome 2021;9:1–17.
[22] Klassert TE, Zubiria-Barrera C, Kankel S, Stock M, Neubert R, Lorenzo-Diaz F, et al. Early bacterial colonization and antibiotic resistance gene acquisition in newborns. Front Cell Infect Microbiol 2020;10:332.
[23] Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 2012;6:1621–4.
[24] Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7:335–6.
[25] Zubiria-Barrera C, Stock M, Neubert R, Vester A, Kulle A, Schneegans A, et al. A simple sequence-based filtering method for the removal of contaminants in low-biomass 16S rRNA amplicon sequencing approaches. J Microbiol Methods 2020;178:106060.
[26] Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 2012;41:D590–6.
[27] Friedman J, Alm EJ. Inferring correlation networks from genomic survey data. PLoS Comput Biol 2012;8:e1002687.
[28] Falagas ME, Makris GC. Probiotic bacteria and biosurfactants for nosocomial infection control: a hypothesis. J Hosp Infect 2009;71:301–6.
[29] Oudhuis GJ, Bergmans DC, Verbon A. Probiotics for prevention of nosocomial infections: efficacy and adverse effects. Curr Opin Crit Care 2011;17:487–92.
[30] Temmerman R, Vervaeren H, Noseda B, Boon N, Verstraete W. Inhibition of Legionella pneumophila by Bacillus sp. Eng Life Sci 2007;7:497–503.
[31] Dünner B, Birrer S, Nathues C, Hässig M, Stephan R, Sidler X. Influence of probiotics on the establishment of a competitive flora, as well as on antibiotic use and performance parameters in pig breeding farms. Schweizer Archiv Fur Tierheilkunde 2017;159:429–35.
[32] Stone W, Tolmay J, Tucker K, Wolfaardt GM. Disinfectant, soap or probiotic cleaning? Surface microbiome diversity and biofilm competitive exclusion. Microorganisms 2020;8:1726.
[33] Xun W, Liu Y, Li W, Ren Y, Xiong W, Xu Z, et al. Specialized metabolic functions of keystone taxa sustain soil microbiome stability. Microbiome 2021;9:35.
[34] Murphy EC, Frick I-M. Gram-positive anaerobic cocci—commensals and opportunistic pathogens. FEMS Microbiol Rev 2013;37:520–53.
[35] Silby M, Winstanley C, Godfrey SC, Levy SB, Jackson RW. Pseudomonas genomes: diverse and adaptable. FEMS Microbiol Rev 2011;35:652–80.
[36] Tong C, Hu H, Chen G, Li Z, Li A, Zhang J. Chlorine disinfectants promote microbial resistance in Pseudomonas sp. Environ Res 2021;199:111296.
[37] Sagripanti J-L, Bonifacino A. Resistance of Pseudomonas aeruginosa to liquid disinfectants on contaminated surfaces before formation of biofilms. J AOAC Int 2000;83:1415–22.
[38] Wielders C, Fluit A, Brisse S, Verhoef J, Schmitz F. mecA gene is widely disseminated in Staphylococcus aureus population. J Clin Microbiol 2002;40:3970–5.

Please cite this article as: Klassert TE et al., Comparative analysis of surface sanitization protocols on the bacterial community structures in the hospital environment, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2022.02.032