Journal of Hospital Infection 148 (2024) 167–177

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Trends in viable microbial bioburden on surfaces within a paediatric bone marrow transplant unit

S. Watkin [a,*], E. Cloutman-Green [a,b], M. Kiernan [c], L. Ciric [a]

[a] Department of Civil, Environmental and Geomatic Engineering, University College London, London, UK
[b] Microbiology, Virology and Infection Prevention and Control, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
[c] Richard Wells Research Centre, University of West London, London, UK

ARTICLE INFO

Article history:
Received 20 February 2024
Accepted 23 March 2024
Available online 15 April 2024

Keywords:
Paediatrics
Healthcare surfaces
Surface cleaning
Infection prevention and control
Haematology and oncology
Healthcare-associated infection

SUMMARY

Background: Despite their role being historically overlooked, environmental surfaces have been shown to play a key role in the transmission of pathogens causative of healthcare-associated infection. To guide infection prevention and control (IPC) interventions and inform clinical risk assessments, more needs to be known about microbial surface bioburdens.

Aim: To identify the trends in culturable bacterial contamination across communal touch sites over time in a hospital setting.

Methods: Swab samples were collected over nine weeks from 22 communal touch sites in a paediatric bone marrow transplant unit. Samples were cultured on Columbia blood agar and aerobic colony counts (ACC) per 100 cm^2 were established for each site. Individual colony morphologies were grouped and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry or 16s rDNA sequencing.

Findings: Highest mean counts were observed for sites associated with ward management activity and computer devices (3.29 and 2.97 ACC/100 cm^2 respectively). A nurses' station keyboard had high mean ACC/100 cm^2 counts (10.67) and diversity, while laundry controls had high mean ACC/100 cm^2 counts (4.70) and low diversity. *Micrococcus luteus* was identified in all sampling groups. Clinical staff usage sites were contaminated with similar proportions of skin and environmental flora (52.19–46.59% respectively), but sites associated with parental activities were predominantly contaminated by environmental microflora (86.53%).

Conclusion: The trends observed suggest patterns in microbial loading based on site activities, surface types and user groups. Improved understanding of environmental surface contamination could help support results interpretation and IPC interventions, improving patient safety.

* Corresponding author. Address: University College London, Civil, Environmental and Geomatic Engineering, Chadwick Building, University College London, Gower Street, London WC1E 6BT, UK.
E-mail address: uceswat@ucl.ac.uk (S. Watkin).

https://doi.org/10.1016/j.jhin.2024.03.015

Introduction

Healthcare-associated infections (HAIs) pose a significant challenge to global healthcare systems, being associated with significant morbidity, mortality, increased length of stay, and costs [1]. Antimicrobial resistance among HAI-associated pathogens compounds the issue by limiting treatment options [2]. This, in conjunction with an increasingly susceptible patient population, highlights the importance of infection prevention and control (IPC) in the hospital setting [3,4].

Historically, the physical hospital environment has not been thought to play a significant part in the transmission of pathogens associated with HAI, and has often been overlooked in IPC interventions [5,6]. Evidence now illustrates the key role environmental surfaces play in the persistence and transmission of HAI-causative pathogens. Environmental touch surfaces can become both transiently and persistently contaminated with micro-organisms, which can disseminate through the clinical space [7]. Multiple studies have shown the propensity for environmental surfaces to harbour HAI-associated pathogens outside of outbreak conditions, and environmental reservoirs of HAI-associated pathogens are frequently implicated in outbreaks [7—10]. Furthermore, there is an increased risk (odds ratio: 2.45; 95% confidence interval: 1.53—3.93) of pathogen acquisition if a patient is admitted to a room which was previously occupied by a patient positive for the pathogen [11].

Studies using whole-genome sequencing have provided further evidence of the involvement of environmental surfaces in HAI-associated pathogen transmission [12,13]. The microbiome of environmental surfaces in hospitals has been investigated through 16S rDNA sequencing of whole bacterial communities by multiple studies [8,14,15]. While this method gives high-quality information into the microbiome present, it has high technical requirements and cost implications, and can be challenging to clinically interpret due to results often reporting to genus level.

Despite the evidence illustrating the role environmental surfaces play in HAI-associated pathogen transmission and persistence, microbial monitoring of environmental surfaces is rarely performed outside of outbreaks. No official guidance or standards exist on acceptable microbial contamination of surfaces in healthcare settings in the UK. Standards relating to aerobic colony counts per cm^2 (ACC/cm^2) values and the presence of indicator organisms have been suggested, although there is no clear agreed method to conduct microbial monitoring [16—18]. When culture-based microbiology techniques are used, they are often investigating a single species, or are done to determine the ACC on a surface, which limits the information that can be gathered from environmental sampling.

Here, a nine-week sampling campaign of 22 communal touch surfaces in a paediatric bone marrow transplant unit was conducted. Culturable bacterial isolates were identified and this information was used to determine trends in the micro-organisms observed. This method was selected to balance ease of use in the clinical setting with the quality of data obtained.

Methods

Study setting

Investigations were conducted at Great Ormond Street Hospital for Children in London, UK. Sampling was undertaken within the bone marrow transplant (BMT) and haematology/oncology unit, comprising of two connected wards. Each ward contained a nurses' station, intravenous medication preparation room, oral medication preparation room, linen cupboard and sluice. One housed a kitchen for parent use, while the other contained a milk preparation room and a kitchen for BMT patient food preparation. The connecting corridor housed the ward sample chute and laundry facilities.

Study design

Sampling was initiated following deep cleaning of both wards in response to an adenovirus outbreak. This involved the removal of all equipment and consumables and cleaning of all surfaces (including walls, floors, and ceilings) with a 1000 ppm chlorine-based agent. Sampling was conducted weekly on 22 surfaces in communal spaces across both wards and the connecting corridor which were associated with a wide range of user groups and activities. Sample sites are listed in Table I, with their locations across the unit illustrated in Figure 1. Surface areas for all sites were calculated to allow for comparison between ACC on sites. Sample sites were categorized based on: predominant contact groups (e.g. clinical staff), sample site type (e.g. computer devices), associated activities (e.g. ward management), potential transmission link to patients, and perceived risk to patients. Potential transmission link to patients and risk perceptions were determined through consultation with the IPC team at Great Ormond Street Hospital for Children. Enhanced environmental cleaning was undertaken (twice daily cleans with a chlorine-based agent), with surfaces regularly cleaned with sodium hypochlorite wipes (5200 ppm) (GAMA Healthcare, Hemel Hempstead, UK).

Sample collection and culture

Samples were collected using rayon flocked swabs, with Amies charcoal transport medium used for sample transport (Copan Diagnostics, Murrieta, CA, USA), with a standardized swabbing technique. Swabs were pre-moistened in nuclease-free water prior to sample collection. Swabs were used to inoculate Columbia blood agar plates (Oxoid, Basingstoke, UK), which were cultured at 37 °C for 48 h. Aerobic colony counts were identified for 100 cm^2 of each surface sampled (ACC/100 cm^2). Glycerol stocks of each colony were prepared and stored at −80 °C.

Species identification

Colonies were grouped by morphologies on each plate and up to ten representative colonies from each grouping per plate were selected for species identification. If all representative colonies returned the same species identification, this identity

Table I
Sample site groupings by categorizations

Surface name		Contact groups	Sample site type	Activity	Link to patient	Risk perception
S1	Sample chute keypad	Clinical staff	Computer devices	Sample processing	Contact	High
S2	Laundry washing machine controls	Parent	White good control	Parental	Contact	Low
S3	Laundry drier machine controls	Parent	White good control	Parental	Contact	Low
S4	Parent kitchen fridge handle	Parent	Handle	Parental	Contact	Low
S5	Parent kitchen microwave handle	Parent	Handle	Parental	Contact	Low
S6	Parent kitchen toaster	Parent	White good control	Parental	Contact	Low
S7	Ward 1 linen cupboard handle	Clinical staff	Handle	Ward management	Intermediate	Medium
S8	Ward 1 nurses' station keyboard	Clinical staff	Computer devices	Ward management	Contact	Medium
S9	Ward 1 IV room keyboard	Clinical staff	Computer devices	IV medication preparation	Intermediate	High
S10	Ward 1 IV room scanner	Clinical staff	Computer devices	IV medication preparation	Intermediate	High
S11	Ward 1 sluice entrance door handle	Clinical staff	Handle	Sample processing	Contact	High
S12	Ward 1 oral room keyboard	Clinical staff	Computer devices	Patient oral ingestion	Ingestion	High
S13	Milk kitchen entrance keypad	Clinical staff	Computer devices	Patient oral ingestion	Ingestion	High
S14	Ward 2 sluice entrance door handle	Clinical staff	Handle	Sample processing	Contact	High
S15	BMT kitchen toaster	Non-clinical staff	White good control	Patient oral ingestion	Ingestion	Medium
S16	BMT kitchen fridge handle	Non-clinical staff	Handle	Patient oral ingestion	Ingestion	Medium
S17	BMT kitchen microwave handle	Non-clinical staff	Handle	Patient oral ingestion	Ingestion	Medium
S18	Ward 2 linen door handle	Clinical staff	Handle	Ward management	Intermediate	Medium
S19	Ward 2 nurses' station keyboard	Clinical staff	Computer devices	Ward management	Contact	Medium
S20	Ward 2 IV room keyboard	Clinical staff	Computer devices	IV medication preparation	Intermediate	High
S21	Ward 2 IV room scanner	Clinical staff	Computer devices	IV medication preparation	Intermediate	High
S22	Ward 2 oral drug room keyboard	Clinical staff	Computer devices	Patient oral ingestion	Ingestion	High

IV, intravenous.
Sample sites were categorized according to predominant contact group, sample site type, activity, transmission link to patient, and risk perception.

was assigned to all isolates in the grouping. If representative colonies did not return the same species identification, all colonies in the grouping were selected for identification.

Species identification was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS), using the MALDI Biotyper® Sirius (Bruker Daltonics GmbH & Co. KG, Bremen, Germany). Samples of the leading edge of freshly cultured isolates were applied to a stainless-steel target plate and fixed with α-cyano-4-hydroxycinnamic acid (HCCA) ('direct transfer').

Species identification was determined by the quality score associated with each identity — assigned via the MBT Compass reference library (Bruker Daltonics). Identification scores of ≥2.0 indicated successful species-level identification, while scores between 1.99 and 1.60 indicated genus-level identification. For isolates that did not retrieve species-level identifications when prepared using the above method, extended direct transfer was used. This followed the same method as direct transfer, with the addition of 70% formic acid prior to fixing with HCCA. Isolates that were still not identified to species level were processed by ethanol extraction prior to MALDI-ToF as previously described [19].

Sequencing of the 16S rDNA was performed for isolates that could not be identified to species level via MALDI-ToF MS. Isolates were cultured on tryptone soy agar (37 °C, 24 h). Crude DNA extracts were produced via boiling samples at 95 °C for 10 min. Polymerase chain reaction (PCR) of 16S rDNA was performed using the 27F (AGAGTTTGATCMTGGCTCA) and 1492R (GGTTACCTTGTTACGACTT) primer set and MyTaq™ master mix (Meridian, Cincinnati, OH, USA). PCR conditions were as follows: 95 °C for 3 min denaturation followed by 30 cycles at 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s with a final stage of 72 °C for 7 min. Amplification of 16S rDNA was confirmed via agarose gel electrophoresis. Cleanup of PCR products was performed via a QIAquick PCR Cleanup Kit as per the manufacturer's instructions (Qiagen, Hilden, Germany). Post-cleanup PCR products were sequenced by Sanger sequencing (performed by Source BioScience, Nottingham, UK). Sequences were quality-checked and matched using the Basic Local Alignment Search Tool (National Center for

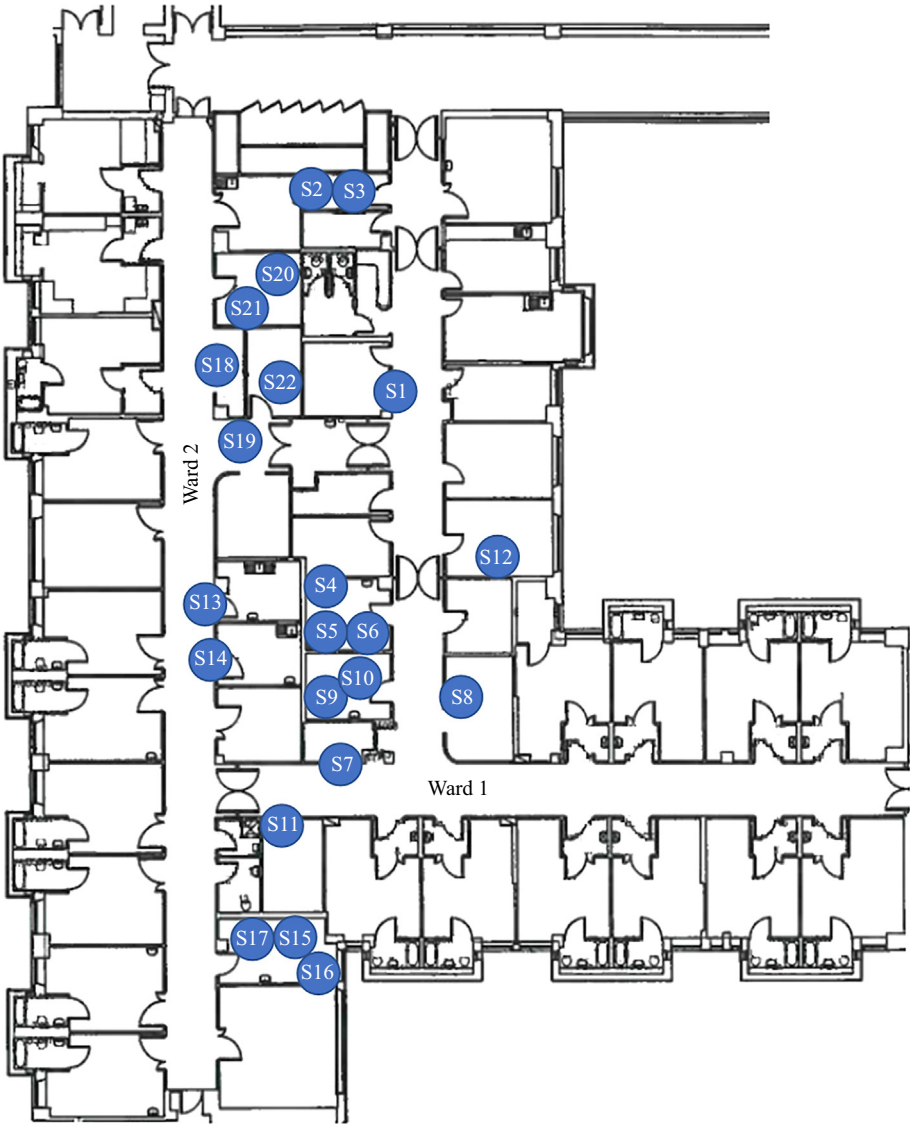

Figure 1. Distribution of sample sites across the two wards composing the bone marrow transplant unit. Sample sites and their categorisations are listed in Table I.

Biotechnology Information, Bethesda, MD, USA). Species-level identifications were confirmed by identity percentages >99% between sequenced isolates and reference genome sequences.

Species categorization

Following species identification, isolates were clustered based on the ecological niche they inhabit, determined through a search of available literature. For species with multiple niches, a single classification was determined based on the likely source of the organism within the context of the study setting.

Data analysis

Data handling and analysis were performed in Microsoft Excel (Office 2021; Microsoft, Redmond, CA, USA) and SPSS (version 27; IBM, Armonk, NY, USA).

Microbial loading was analysed through the determination of the geometric mean ACC/100 cm^2 for sample sites and site groupings. The formula for geometric mean calculation is:

$$\left(\prod_{i=1}^{n} x_i\right)^{\frac{1}{n}} = \sqrt[n]{x_1 x_2 \ldots x_n}$$

where $\prod$ = geometric mean, n = number of values, and x_i = the values to average. Briefly, geometric mean calculations refer to finding the n^{th} root of the product of a dataset with n numbers. For example, calculating the geometric mean of one sample site over the study duration would entail calculating the sum of all nine ACC/100 cm^2 counts obtained for the site over the study duration, then calculating the 9^{th} root of this value. This allows for more representative comparison between the ACC/100 cm^2 counts observed over time here due to the tendency for these data to have a high positive skew.

Shannon–Weiner Diversity Index (H) scores were calculated for sample sites and sample site groupings using the vegan

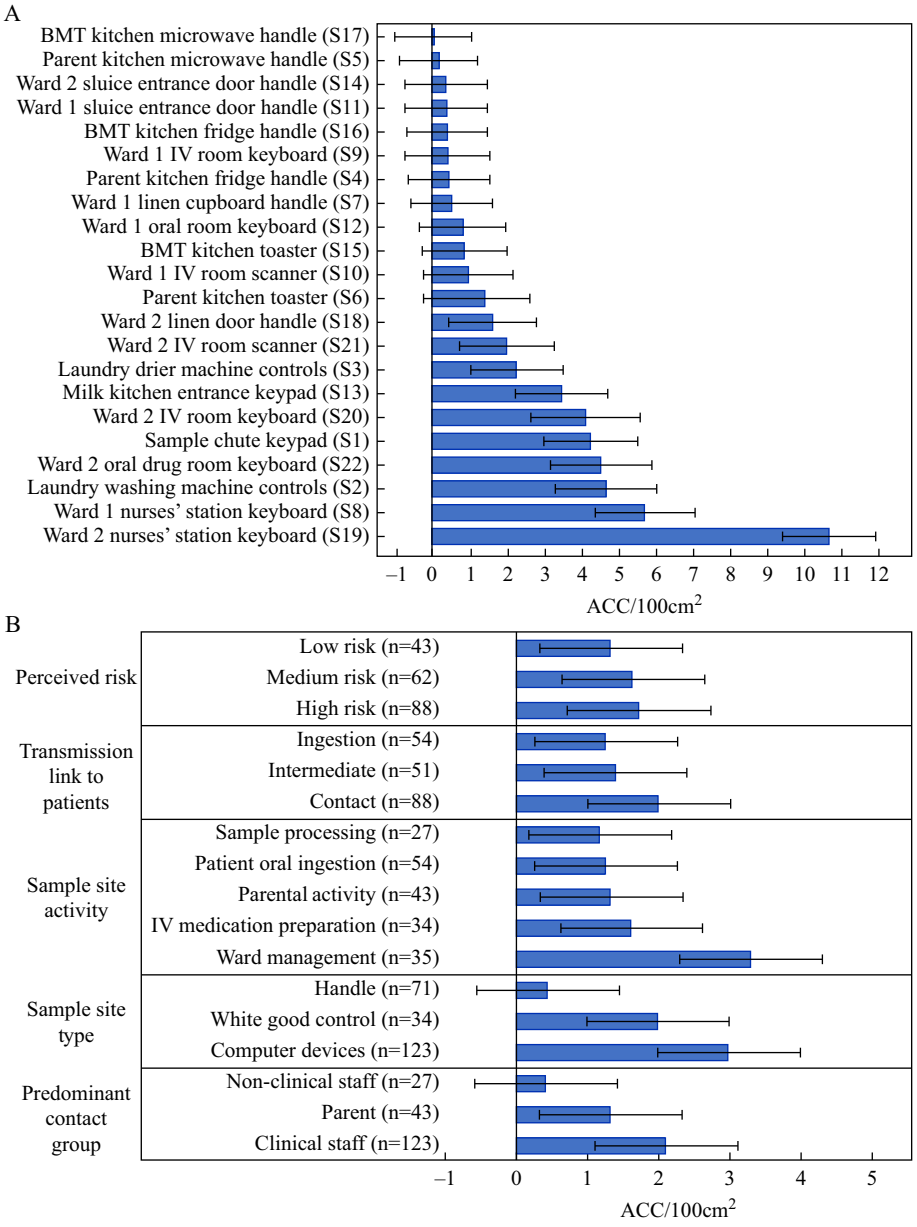

Figure 2. Geometric mean aerobic colony counts (ACC) per 100 cm^2 during the study period. (A) Geometric mean ACC/100 cm^2 were determined for each sample site included in the study. All samples were $N = 9$, except S2 ($N = 8$), S10 ($N = 7$), and S18 ($N = 8$). (B) Sample sites were grouped by predominant contact group, sample site type, sample site activity, transmission link to patients, and perceived risk. Each sample group classifies all 193 samples collected through the duration of the study. Error bars represent ± geometric standard error of the mean (GSE).

package in R (version 4.2.2). *H* scores reflect the diversity in a population, accounting for both the quantity and relative abundance of species in a sample, with higher *H* scores reflecting greater diversity.

Results

Aerobic colony count per 100 cm² analysis

A total of 193 samples were collected from environmental surfaces within the units. Ward 2 nurses' station keyboard had the highest geometric mean ACC/100 cm^2 (10.67 ± 1.25 geometric standard error (GSE)) over the study duration. Keyboards accounted for five of the six sites with highest geometric mean average ACC/100 cm^2 counts. The sample site with lowest ACC/100 cm^2 was the BMT kitchen microwave handle (0.057 ± 1.02 GSE) (Figure 2A). When sample sites were clustered by predominant contact group, sites associated with clinical staff had the highest geometric ACC/100 cm^2 mean (2.10 ± 1.06), while non-clinical staff sites had the lowest (0.45 ± 1.07 ACC/100 cm^2) (Figure 2B). Sample site type grouping showed computer devices as having a

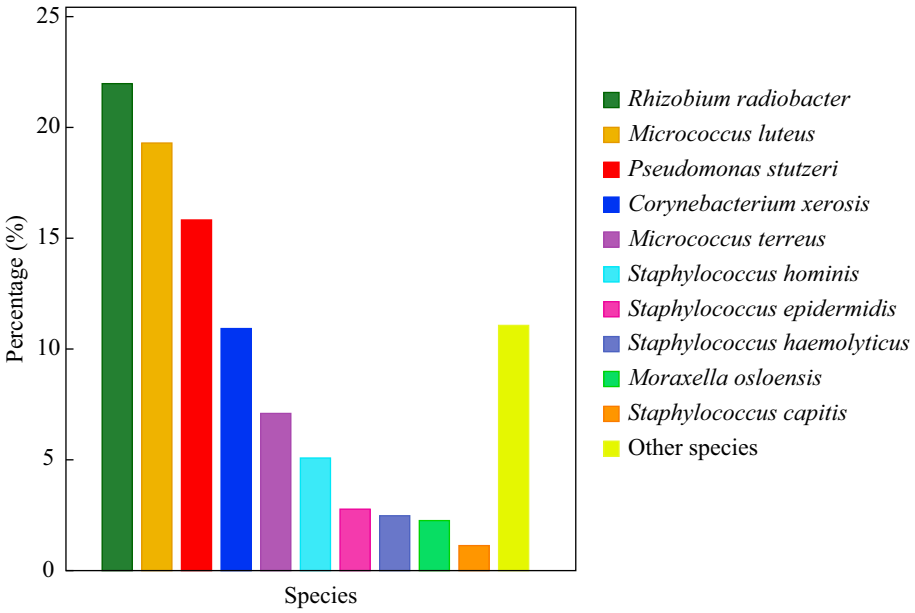

Figure 3. Distribution of species during the period of environmental monitoring. Species constituting ≥1.00% of the total count of identified species are shown. Species accounting for <1.00% are grouped as 'Other species'.

geometric mean of 2.79 ± 1.08 ACC/100 cm^2, while handles had a geometric mean of 0.45 ± 1.04 ACC/100 cm^2. Sample site activity categorization demonstrated that ward management sites had the highest ACC/100 cm^2 (3.29 ± 1.12), in contrast to sample processing sites (1.17 ± 1.13 ACC/100 cm^2). Categorization by transmission link to patients showed that sites with a contact transmission link had the highest ACC/100 cm^2 (2.00 ± 1.08), with ingestion sites being the lowest (1.25 ± 1.08 ACC/100 cm^2). Groups within the perceived risk categorization exhibited a small range in geometric ACC/100 cm^2 mean (1.72–1.33). Overlap in GSE values was seen for most grouping in all categories, except between ward management sites and patient oral ingestion/sample processing sites (site activity), and computer devices and handles (site type).

Organism identification analysis

Overall, 1853 colonies were observed in this study. Of these, 995 (58.50%) were identified via MALDI-ToF (895 (52.62%) by direct transfer, 83 (4.88%) by extended direct transfer, and 17 (1.76%) by full extraction), with 676 (39.74%) identified by morphology grouping. A total of 30 (1.76%) were identified by 16S rDNA sequencing. In total, 1701 isolates were identified to species level (91.80%); 58.90% were Gram positive, 41.00% were Gram negative, and 0.10% were Gram variable.

A total of 57 distinct species were identified in this investigation. Ten species comprised >1.00% of the overall identified species count, with four of these comprising ≥10.00% (*Rhizobium radiobacter*, *Micrococcus luteus*, *Pseudomonas stutzeri*, and *Corynebacterium xerosis*) (Figure 3). *R. radiobacter* was the most frequently identified species overall (21.99% of identified species, although it was predominantly only identified on one sampling day). A further 47 species comprised <1.00% of the overall number of identified species, which, when clustered, account for 11.05% of isolates identified.

Shannon–Weiner Diversity Index (*H*) scores were calculated for sample sites, with the most diverse sample site being the nurses' station keyboard on ward 1 (Figure 4A). The microwave handle in the BMT kitchen was observed to have an *H* score of 0, reflecting a lack of diversity of culturable microbes. Keyboards accounted for three of the five most diverse sample sites over the study duration. The nurses' station keyboards in ward 1 (S8) and ward 2 (S19) had the highest counts of distinct species identified, with 21 and 28 different species isolated respectively. The intravenous drug preparation room scanner in ward 2 (S21) had nine distinct species isolated despite the high *H* score, compared to the sample chute keypad (S1) which had 10 distinct species, with an *H* score of only 0.32.

Figure 4B shows that all sampling weeks yielded *H* scores of >1.00. Week 7 was the most diverse sampling week ($H = 2.26$). Week 3 had the least diversity ($H = 1.04$). This contrasts with the geometric mean ACC/100 cm^2 counts for each sampling week, where week 3 had the highest count (5.86 ± 1.22 GSE) and week 7 had the second highest count (4.79 ± 1.19 GSE). Surface samples on week 9 had the lowest geometric mean average at 0.39 ACC/100 cm^2 ± 1.06 GSE.

Figure 5 shows the dominant species on each sample site grouping category. *R. radiobacter* was dominant on sites associated with parents as the user group. *P. stutzeri* was dominant in the sample processing category, with sites grouped by site activity. Coagulase-negative *Staphylococci* (CoNS) and *M. luteus* were identified across all classifications. Parent user group sites had low diversity scores, while sites associated with clinical activity had higher diversities. Large differences in species counts between some groups were not consistently reflected in diversity scores. For example, computer devices ($N = 1378$) had $H = 1.37$, while handles ($N = 134$) had

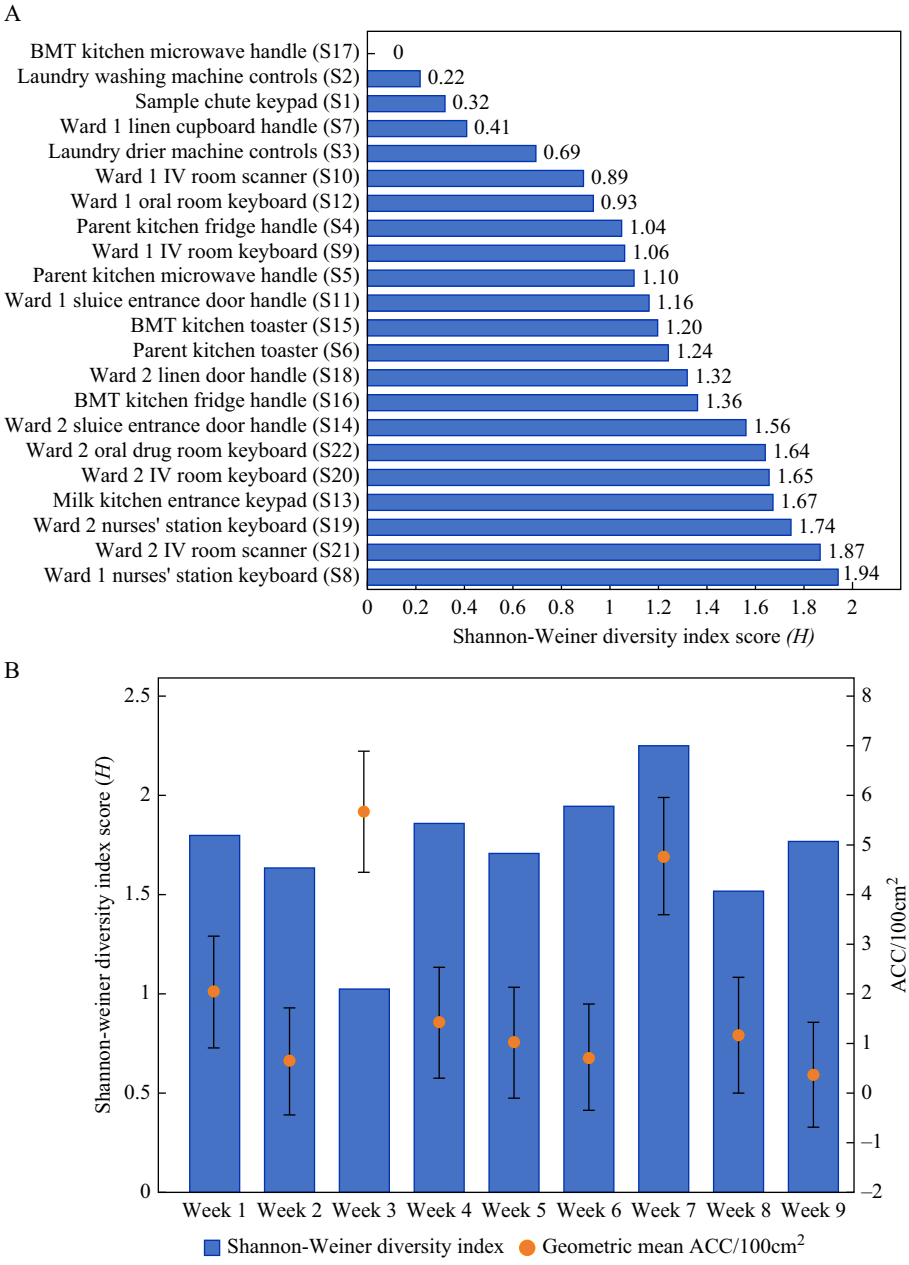

Figure 4. Shannon–Weiner Diversity Index scores (*H*) for sample sites investigated. Diversity scores were calculated, reflecting the amount and abundance of micro-organisms present on a sample site. (A) Diversity scores for each sample site over the duration of the study were determined. (B) Diversity scores for each sampling week are plotted alongside mean aerobic colony counts (ACC)/100 cm^2 for each week. Error bars show ± geometric standard error of the mean (GSE).

$H = 0.099$. Conversely, intermediate transmission link sites ($N = 328$) had $H = 1.20$, while contact transmission link sites ($N = 1031$) had $H = 1.10$.

Sample sites associated with parent user groups are predominantly occupied by environmental microbes (Figure 6). A similar trend can be seen for sample sites associated with sample processing (91.59%) when grouped by sample site activities. In this grouping, sites associated with intravenous medication preparation were predominantly occupied by skin microflora (82.07%), while other grouping categories displayed more balance between skin and environmental microbiota.

Discussion

This study illustrates the dynamic nature of microbial contamination of touch surfaces in the clinical setting. Keyboards had the highest geometric ACC/100 cm^2 means, with high diversity scores compared to other sites. These results were obtained in the presence of enhanced environmental cleaning, indicating that keyboards were frequently recontaminated. Therefore, the high diversities observed may reflect a high usage rate relative to other surfaces investigated. Keyboards in the clinical setting are known to become contaminated with a wide

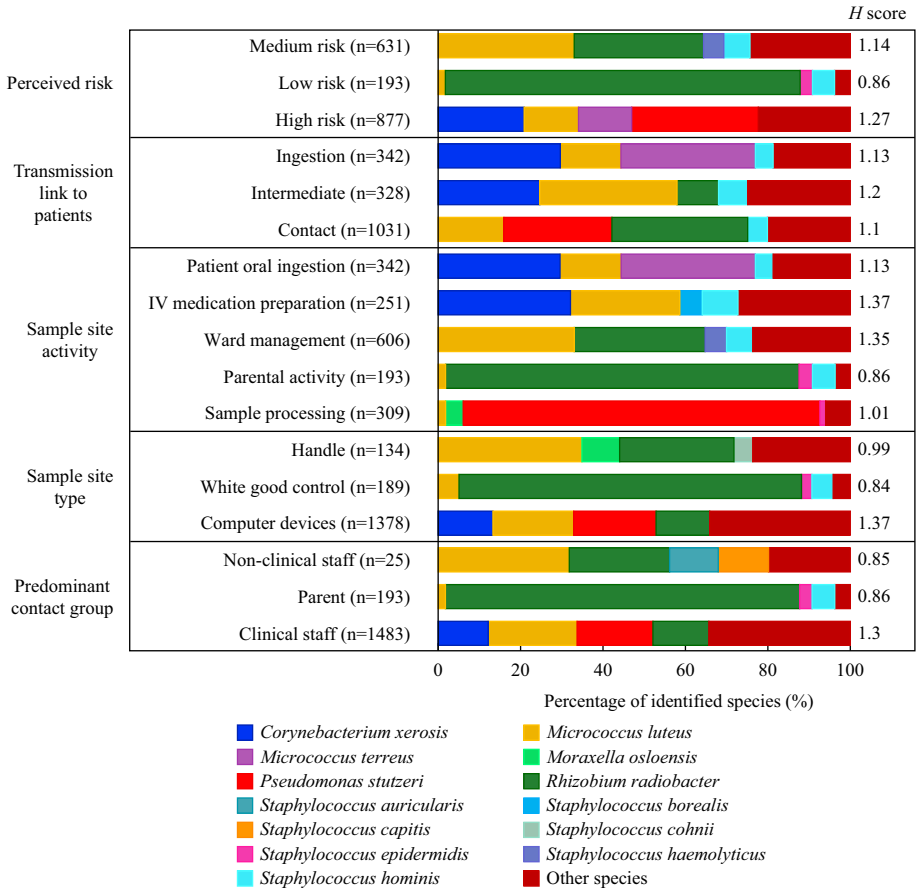

Figure 5. Distribution and diversity of identified micro-organisms on sample sites classified in each grouping category over the study duration. Sample sites were grouped into predominant contact group, sample site type, sample site activity, transmission link to patients, and perceived risk to patients. Micro-organisms were identified through either MALDI ToF MS or 16s rDNA sequencing. The species which comprised the four highest proportions of the identified population in each grouping are displayed. All other identified species are classified as 'Other species'. Shannon—Weiner Diversity Index scores (*H* scores) for each group within these categories were calculated. All grouping categories use all identified species ($N = 1701$).

range of micro-organisms [20]. Keyboards have been implicated in *Acinetobacter baumannii* dissemination in a paediatric burns unit, and have recently been shown to harbour a wide range of nosocomial pathogens [9,21]. This shows the propensity for keyboards to be reservoirs of HAI-associated pathogens and to harbour large, diverse microbial communities.

In this study, *Staphylococcus capitis* was identified on four keyboards sampled. *S. capitis* has previously been regarded as a contaminant; however, recently its role in neonatal bloodstream infection and nosocomial outbreaks has become clear [22]. Such outbreaks are often the result of the NRCS-A clone, which is known to persist in clinical environments [23,24]. *S. capitis* was also identified on the sample chute keypad: ward 1 and ward 2 sluice entrance door handles, milk kitchen entrance keypad, the BMT kitchen toaster controls and BMT fridge handle. While these isolates were not identified as the NRCS-A clone, this demonstrates how *S. capitis* can be a widespread environmental contaminant.

Micrococcus luteus and CoNS were consistently present across sample sites, while *R. radiobacter* was found in high quantities over fewer sites (Figure 5). CoNS are reported at species level here due to varied clinical risks associated with each species [25]. *M. luteus* and CoNS are constituents of the human skin microbiome and their continual presence on clinical surfaces is expected, likely reflecting transient contamination deposited from skin [26]. This is supported by metagenomic analysis of hospital surfaces and staff/patient skin microbiota, which suggested that environmental surfaces were seeded with ubiquitous skin microbes [27]. *M. luteus* and CoNS have been shown to cause HAI, and *R. radiobacter* is recognized as a rare cause of central line bloodstream infection [28—30].

Keyboards were observed to have high diversity indices and geometric mean ACC/100 cm^2 counts, while the laundry washing machine controls had high geometric mean ACC/100 cm^2 counts and low diversity index scores (Figures 2A and 4A). Similar trends were seen for different sample site groupings (Figure 5). Variations in microbial diversity on hospital surfaces have been reported previously. An investigation of hospital surface microbial communities across three hospitals found one to be dominantly contaminated with Enterobacterales, while the other two had significantly greater microbial diversities [31]. Variations in both microbial abundance and diversity have been shown between different hospitals, wards, and surfaces within the clinical space [32]. Additionally, high diversity has been shown in culturable micro-

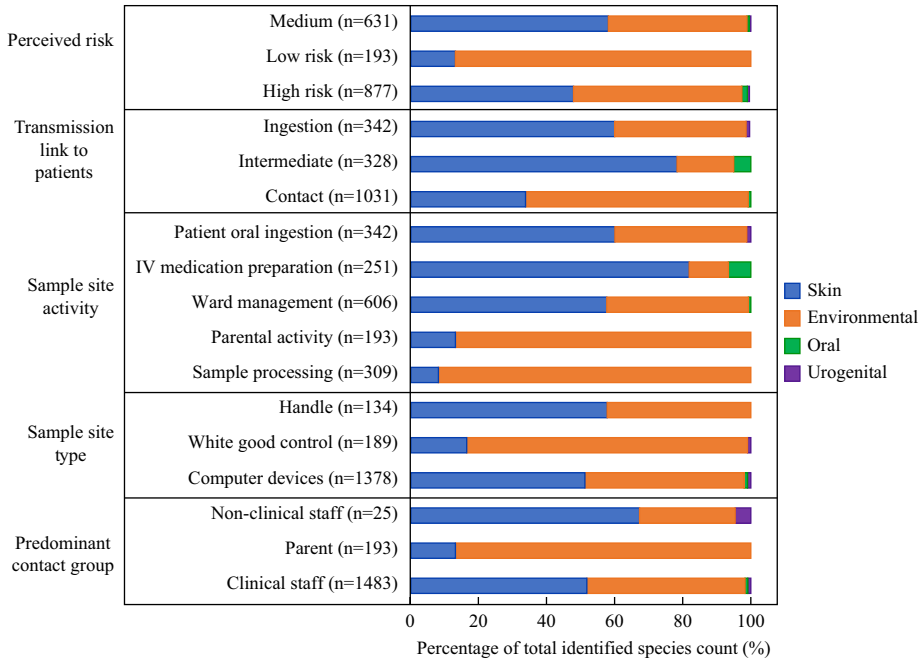

Figure 6. Distribution of identified species counts when classified by the ecological niches inhabited across each grouping category. Sample sites were grouped by predominant contact group, sample site type, sample site activity, transmission link to patient, and perceived risk to patients. Identified species were categorized as belonging to either skin, environmental, oral, or urogenital ecological niches. Percentages of each niche classification for each sample site grouping category were determined. Each grouping category represents the total number of identified organisms collected ($N = 1701$).

organisms recovered from near-patient surfaces [33]. Such differences could be caused by environmental cleaning, with cleaning known to significantly reduce microbial diversity [34]. Enhanced environmental cleaning was undertaken throughout this study, although evidence on cleaning efficacy was not collected. Housekeeping teams were responsible for cleaning kitchen and general ward areas, while keyboards and sample sites in medication preparation rooms were cleaned by nursing staff and audited by IPC. It is possible therefore that differences in cleaning efficacy over the study duration impacted the amount and diversity of micro-organisms recovered.

The microbiota of environmental surfaces within the clinical space is increasingly investigated due to the potential for surfaces to harbour HAI-associated pathogens. 16S rDNA sequencing of microbial communities on environmental surfaces within neonatal intensive care unit (NICU) settings has previously been conducted, with multiple genera associated with HAI identified including *Acinetobacter*, *Clostridioides*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, and *Stenotrophomonas* [10,14,15]. Equally, studies investigating microbial communities in both intensive care unit (ICU) and non-ICU settings have recovered HAI-associated pathogens from a range of environmental surfaces (*Staphylococcus*, *Streptococcus*, *Pseudomonas*, *Acinetobacter*, *Enterococcus* and *Klebsiella* spp.) [8,31,35]. Patterns in culturable microbial contamination have also been assessed on a range of hospital environmental surfaces, showing *Staphylococci* present on 81% of surfaces, with *Enterococcus* spp. (13%), *Acinetobacter* spp. (7.4%), *C. difficile* (4.2%), *P. aeruginosa* (0.9%), and *Klebsiella* spp. (0.5%) also detected [8].

While metagenomic evidence provides high-quality information on the total composition of surface microbial communities, it is not a feasible option for routine environmental monitoring. This is due to the technical complexity, cost, and lack of species-level identification resolution required to inform clinical risk assessments. Here, microbial contaminants were investigated using readily available methods and techniques. Limitations to this methodology exist, however. The use of an indirect sampling method may limit the recovery of all organisms present on the sample site, meaning true microbial quantification cannot be performed. Furthermore, swab sampling methodologies have been shown to be ineffective at recovering organisms from dry surface biofilms [9,36]. Despite these, the ease of use allows information on the microbiological safety of the hospital environment to be gathered in a practical manner by clinical teams using this methodology.

There are currently no legislative standards for microbial hygiene of clinical environmental surfaces, with 'visibly clean' being the current environmental surface cleanliness standard [37]. ACC/cm^2 counts (either 5.0 or 2.5 ACC/cm^2) have been suggested for microbial surface monitoring, with the presence of specific indicator organisms (including *S. aureus*, *C. difficile* and VRE) also assessed [16,17]. Such investigations require the usage of quantitative direct sampling methodologies (e.g. RODAC plates (replicate organism detection and counting)), and therefore such standards cannot be applied to results obtained here. The logic behind such standards is that the higher the microbial loading of environmental surfaces, the higher the risk to patients. This is not necessarily the case, however, as we have shown that high environmental loadings

can be the result of multiple or single micro-organism contamination. The question then is whether loading with a high diversity or low diversity confers greater risk to patients. Such methods also do not account for viral pathogens. Monitoring for the presence of viruses will require different environmental sampling and processing workflows, with different interpretation requirements. Currently there are no recommended standards for viral contamination levels on clinical environmental surfaces.

Higher diversity loading contains a wide array of micro-organisms in the clinical space, with species from human and environmental niches being present. The risk here is that species with high clinical consequence are present within these diverse communities and can be transferred throughout the clinical space. With keyboards often observed here to have this contamination profile, transfer of pathogens on the hands of healthcare workers may be a dissemination pathway for these organisms. In contrast, lower diversity loading indicates a single organism present in high numbers, as was observed here with the environmental micro-organisms *R. radiobacter* and *P. stutzeri*. Environmental microbes have been known to cause HAI, especially within this patient cohort, and high environmental loading may enhance the risk of transmission and patient acquisition [38,39].

Additional research is required here to further characterize microbes associated with each contamination pattern. Understanding which surfaces become contaminated with different microbial diversity patterns can help inform environmental infection control risk assessments. This could be achieved through understanding which surfaces pose greater risk to patients and targeting environmental IPC interventions accordingly, and through selecting appropriate surfaces for routine monitoring of micro-organism presence. This information will support the development of better results interpretation frameworks, and will help IPC interventions to be more effectively targeted and implemented sooner, supporting patient safety.

In conclusion, this study illustrates the dynamic nature of microbial contamination on environmental surfaces in healthcare settings. To be effective, routine microbial monitoring of the clinical space must be done regularly and cover a wide range of sites in order to effectively capture spatial/temporal trends in microbial surface contamination. Different patterns in microbial contamination can be seen with sites of different user groups and associated activities. High-loading contamination occurs with both high and low diversities, which are associated with different micro-organisms and clinical risks. Performing routine environmental surface monitoring of micro-organisms allowed these contamination trends to be identified, allowing for the microbial risks posed by different surfaces to be understood, supporting environmental IPC decision-making.

Acknowledgements

Many thanks to the microbiology department at Great Ormond Street Hospital for Children (GOSH), especially F. Yongblah for allowing access to required equipment. Thanks to V. Health for assisting with sample collection. Finally, many thanks to the staff of Fox and Robin wards and the infection prevention and control team at GOSH. Thanks to E. Tranfield from Bruker Daltonics for training and support with the MALDI Biotyper® Sirius.

Conflict of interest statement
None declared.

Funding sources
This work was supported by the Healthcare Infection Society Major Grant Scheme [MRG/2019/006].

References

[1] Guest JF, Keating T, Gould D, Wigglesworth N. Modelling the annual NHS costs and outcomes attributable to healthcare-associated infections in England. BMJ Open 2020;10:e033367.
[2] Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 2009;48:1—12.
[3] Azoulay E, Russell L, Van de Louw A, Metaxa V, Bauer P, Povoa P, et al. Diagnosis of severe respiratory infections in immunocompromised patients. Intensive Care Med 2020;46:298—314.
[4] Rowe JW, Fulmer T, Fried L. Preparing for better health and health care for an aging population. JAMA 2016;316:1643—4.
[5] Maki DG, Alvarado CJ, Hassemer CA, Zilz MA. Relation of the inanimate hospital environment to endemic nosocomial infection. N Engl J Med 1982;307:1562—6.
[6] Dancer SJ. Mopping up hospital infection. J Hosp Infect 1999;43:85—100.
[7] Suleyman G, Alangaden G, Bardossy AC. The role of environmental contamination in the transmission of nosocomial pathogens and healthcare-associated infections. Curr Infect Dis Rep 2018;20:12.
[8] Comar M, D'Accolti M, Cason C, Soffritti I, Campisciano G, Lanzoni L, et al. Introduction of NGS in environmental surveillance for healthcare-associated infection Control. Microorganisms 2019;7:708.
[9] Ledwoch K, Dancer SJ, Otter JA, Kerr K, Roposte D, Maillard JY. How dirty is your QWERTY? The risk of healthcare pathogen transmission from computer keyboards. J Hosp Infect 2021;112:31—6.
[10] Lax S, Gilbert JA. Hospital-associated microbiota and implications for nosocomial infections. Trends Mol Med 2015;21:427—32.
[11] Mitchell BG, McDonagh J, Dancer SJ, Ford S, Sim J, Thottiyil Sultanmuhammed Abdul Khadar B, et al. Risk of organism acquisition from prior room occupants: an updated systematic review. Infect Dis Health 2023;28:290—7.
[12] Dancer SJ, Adams CE, Smith J, Pichon B, Kearns A, Morrison D. Tracking *Staphylococcus aureus* in the intensive care unit using whole-genome sequencing. J Hosp Infect 2019;103:13—20.
[13] Price JR, Cole K, Bexley A, Kostiou V, Eyre DW, Golubchik T, et al. Transmission of *Staphylococcus aureus* between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. Lancet Infect Dis 2017;17:207—14.
[14] Hewitt KM, Mannino FL, Gonzalez A, Chase JH, Caporaso JG, Knight R, et al. Bacterial diversity in two neonatal intensive care units (NICUs). PLoS One 2013;8:e54703.
[15] Brooks B, Firek BA, Miller CS, Sharon I, Thomas BC, Baker R, et al. Microbes in the neonatal intensive care unit resemble those found in the gut of premature infants. Microbiome 2014;2:1.

[16] 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.
[17] Griffith CJ, Obee P, Cooper RA, Burton NF, Lewis M. The effectiveness of existing and modified cleaning regimens in a Welsh hospital. J Hosp Infect 2007;66:352—9.
[18] Galvin S, Dolan A, Cahill O, Daniels S, Humphreys H. Microbial monitoring of the hospital environment: why and how? J Hosp Infect 2012;82:143—51.
[19] Freiwald A, Sauer S. Phylogenetic classification and identification of bacteria by mass spectrometry. Nat Protoc 2009;4:732—42.
[20] Engelhart S, Fischnaller E, Simon A, Gebel J, Gemein S, Exner M. Microbial contamination of computer user interfaces (keyboard, mouse) in a tertiary care centre under conditions of practice. Hyg Med 2008;33:504—7.
[21] Neely AN, Maley MP, Warden GD. Computer keyboards as reservoirs for *Acinetobacter baumannii* in a burn hospital. Clin Infect Dis 1999;29:1358—9.
[22] Laurent F, Butin M. *Staphylococcus capitis* and NRCS-A clone: the story of an unrecognized pathogen in neonatal intensive care units. Clin Microbiol Infect 2019;25:1081—5.
[23] Heath V, Cloutman-Green E, Watkin S, Karlikowska M, Ready D, Hatcher J, et al. *Staphylococcus capitis*: review of its role in infections and outbreaks. Antibiotics 2023;12:669.
[24] Butin M, Dumont Y, Monteix A, Raphard A, Roques C, Martins Simoes P, et al. Sources and reservoirs of *Staphylococcus capitis* NRCS-A inside a NICU. Antimicrob Resist Infect Control 2019;8:157.
[25] Hitzenbichler F, Simon M, Salzberger B, Hanses F. Clinical significance of coagulase-negative staphylococci other than *S. epidermidis* blood stream isolates at a tertiary care hospital. Infection 2017;45:179—86.
[26] Khayyira AS, Rosdina AE, Irianti MI, Malik A. Simultaneous profiling and cultivation of the skin microbiome of healthy young adult skin for the development of therapeutic agents. Heliyon 2020;6:e03700.
[27] Lax S, Sangwan N, Smith D, Larsen P, Handley KM, Richardson M, et al. Bacterial colonization and succession in a newly opened hospital. Sci Transl Med 2017;9(391):eaah6500.
[28] Zhu M, Zhu Q, Yang Z, Liang Z. Clinical characteristics of patients with *Micrococcus luteus* bloodstream infection in a Chinese tertiary-care hospital. Pol J Microbiol 2021;70:321—6.
[29] Weiner-Lastinger LM, Abner S, Edwards JR, Kallen AJ, Karlsson M, Magill SS, et al. Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: summary of data reported to the National Healthcare Safety Network, 2015—2017. Infect Control Hosp Epidemiol 2020;41:1—18.
[30] Hartman RE, Freyer CW, Athans V, McCurdy SR, Frey NV. Central line-associated *Rhizobium radiobacter* bloodstream infection in two allogeneic hematopoietic cell transplant recipients. J Onc Pharm Pract 0(0):10781552231161826.
[31] Yano R, Shimoda T, Watanabe R, Kuroki Y, Okubo T, Nakamura S, et al. Diversity changes of microbial communities into hospital surface environments. J Infect Chemother 2017;23:439—45.
[32] Shobo CO, Alisoltani A, Abia ALK, Mtshali PS, Ismail A, Zishiri O, et al. Bacterial diversity and functional profile of microbial populations on surfaces in public hospital environments in South Africa: a high throughput metagenomic analysis. Sci Total Environ 2020;719:137360.
[33] Cruz-López F, Villarreal-Treviño L, Morfin-Otero R, Martínez-Meléndez A, Camacho-Ortiz A, Rodríguez-Noriega E, et al. Microbial diversity and colonization patterns of two step-down care units from a tertiary care hospital. J Res Med Sci 2021;26:126.
[34] Ribeiro LF, Lopes EM, Kishi LT, Ribeiro LFC, Menegueti MG, Gaspar GG, et al. Microbial community profiling in intensive care units exposes limitations in current sanitary standards. Front Public Health 2019;7:240.
[35] Chen J, Ji L, Xiong G, Ning K. The distinct microbial community patterns and pathogen transmission routes in intensive care units. J Hazard Mater 2023;441:129964.
[36] Ledwoch K, Dancer SJ, Otter JA, Kerr K, Roposte D, Rushton L, et al. Beware biofilm! Dry biofilms containing bacterial pathogens on multiple healthcare surfaces; a multi-centre study. J Hosp Infect 2018;100:e47—56.
[37] NHS England. National infection prevention and control manual for England 2023. Available at: https://www.england.nhs.uk/publication/national-infection-prevention-and-control/ [last accessed February 2024].
[38] Yee-Guardino S, Danziger-Isakov L, Knouse M, Bingaman W, Sabella C, Goldfarb J. Nosocomially acquired *Pseudomonas stutzeri* brain abscess in a child: case report and review. Infect Control Hosp Epidemiol 2006;27:630—2.
[39] Attaway HH, Cantey JR, Freeman KD, John JF, Michels HT, Salgado CD, et al. Copper surfaces reduce the rate of healthcare-acquired infections in the intensive care unit. Infect Control Hosp Epidemiol 2013;34:479—86.