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Overall bioburden by total colony count does not predict the presence of pathogens with high clinical relevance in hospital and community environments

F.C. Widmer [a], R. Frei [b], A. Romanyuk [c], S. Tschudin Sutter [a], A.F. Widmer [a,*]

[a] Division of Infectious Diseases and Hospital Epidemiology, University Hospital and University of Basel, Basel, Switzerland
[b] Division of Clinical Microbiology, University Hospital and University of Basel, Basel, Switzerland
[c] Glas Trösch AG, Bützberg, Switzerland

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SUMMARY

Background: Healthcare-associated infections (HAIs) affect millions of patients, increasing morbidity and mortality. Pathogens of HAIs originate from both the patient's own flora and the environment, including multi-drug-resistant organisms.
Aims: To determine the bioburden on different types of high-touch surfaces, and to identify cultures to species level and stratify strains into those of low and high clinical relevance.
Design: Association between bioburden and presence of pathogens of high clinical relevance (PHCR) in a tertiary care centre and urban environment.
Methods: The overall bioburden measured by total colony count (TCC) was assessed using tryptic soy agar contact plates and two selective agars to improve detection of PHCR. Isolates were routinely identified to species level using matrix-assisted laser desorption/ionization – time of flight mass spectrometry (MALDI-TOF). The definition of PHCR was based on listings outlined by the Centers for Disease Control and Prevention.
Findings: In total, 1431 contact plates were processed from 477 surfaces: 153 from hospitals and 324 from publicly accessible institutions or devices. At least one PHCR was identified from cultures from 73 samples. TCC was found to be poorly correlated with the presence of PHCR.
Conclusion: TCC poorly predicted the presence of PHCR, rendering the results from environmental sampling difficult to interpret. MALDI-TOF enables the identification of large numbers of isolates from the environment at low cost. Further studies on environmental contamination should use MALDI-TOF to identify all pathogens grown.

Introduction

Despite progress over the last decades, healthcare-associated infections (HAIs) affect millions of patients and remain a tremendous challenge for healthcare institutions [1]. It is estimated that nosocomial pathogens causing an HAI likely originate from the patient's endogenous flora in 40–60%

* Corresponding author. Address: Division of Infectious Diseases and Hospital Epidemiology, University of Basel and University Hospital Basel, Petersgraben 4, Basel 4031, Switzerland. Tel.: +41 61 265 38 51; fax: +41 61 265 38 54.
E-mail address: andreas.widmer@usb.ch (A.F. Widmer).

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of cases, from contaminated hands of healthcare workers (HCWs) in 20–40% of cases, and from contamination of the environment in approximately 20% of cases [2]. Eight studies have demonstrated that patients occupying rooms previously occupied by patients with vancomycin-resistant enterococci (VRE), meticillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile* and *Acinetobacter baumannii* infection or colonization have, on average, a 73% increased risk of acquiring the same pathogen than patients not occupying such rooms [3]. Contact with a contaminated environment may result in a similar risk of contamination of HCWs' hands, independent of contact with intact skin of colonized patients or their environment [4]. Similarly, the environment was considered as a source of infection with *C. difficile,* with 23% of cases linked to a ward-based inpatient innate source [5]. In addition, cleaning the environment proved to decrease the rate of infection with *C. difficile* in several studies, including one large randomized clinical trial [6]. Enterococci are very commonly cultured from the surfaces of healthcare institutions [7], particularly aminopenicillin-resistant *Enterococcus faecium* and VRE [8]. Similarly, Enterobacterales harbouring plasmids encoding extended-spectrum beta-lactamases or carbapenemases can also be transmitted by the environment [9]. Furthermore, Datta *et al.* found a significant reduction in the acquisition of MRSA and VRE by improvement in environmental cleaning [10].

The association between contamination of the environment and HAIs has previously been hampered by the fact that many studies have focused on the total number of colony-forming units (cfu) per area under study (cfu/cm^2). Dancer *et al.* suggested that contaminated surfaces in contact with the hands of HCWs should be lower than 5 cfu/cm^2 [11].

However, there is limited correlation between the total bioburden and the subset of pathogens of high clinical relevance (PHCR) [12]. The Centers for Disease Control and Prevention (CDC) published a list of PHCR that attempts to distinguish non-pathogenic bacteria from bacteria that are potentially harmful to humans. To detect PHCR, previous studies used selective culture media in addition to standard media to assess the total bioburden and presence of multidrug-resistant organisms (MDROs) [13].

Routine identification of isolates grown from environmental samples (e.g. from contact plates) was very laborious and expensive in the past, and therefore was not undertaken or was only performed in a subset of specimens or colonies. Recently, matrix-assisted laser desorption/ionization – time of flight mass spectrometry (MALDI-TOF) has revolutionized the identification of bacteria and fungi to species level. The advantages of this new identification technique are not only speed and accuracy, but also low cost [14].

Accordingly, identification of all the microbial flora cultured from high-touch surfaces from both hospital and community environments became feasible. Touchscreens (e.g. at railway stations and airports) may be an unrecognized source for acquiring PHCR. Therefore, this study screened different types of surfaces from healthcare institutions and from the community, and identified cultures to species level using MALDI-TOF, in addition to the assessment of total colony count (TCC). The aim of this study was to compare the total bioburden determined by TCC with the presence of PCHR in hospital and community environments.

Methods

High-touch surfaces within hospitals suspected of being contaminated with PHCR were chosen for sampling [15]. Surfaces from publicly accessible high-touch surfaces in the community were selected according to a predefined protocol. Based on a literature analysis, 25–35 'hot-spots' were chosen per location [15–17]. Restroom areas were excluded as bias towards bacteria originating from the digestive system, and further bias due to compliance with hand hygiene were expected.

Samples were taken from the selected high-touch surfaces in 16 different locations: five hospital locations, three public transportation locations (local airport, train and tram stations), locations in three local supermarkets and five public service providers (two libraries, migration office, post office and teller machines). Permission to take samples was granted by the local institutions or representative authorities.

Sampling protocol

Three different Replicate Organism Detection and Counting agar plates were applied sequentially. Samples were taken as close as possible to the target location, while avoiding repetitive sampling from the same location. Plates were applied for 5 s on an area of approximately 25 cm^2, if feasible, for each surface. Samples of difficult-to-assess areas (e.g. hand rails, buttons, handles) were taken as close as possible to 25 cm^2, but complete coverage of the agar surface was not always feasible. Representative sampling areas were photographed, and data were collected in a standardized case report form.

Microbiology

Agars were chosen in accordance with the current recommendations of the European Pharmacopoeia and US Pharmacopoeia. Tryptic soy agar, MacConkey agar and Baird-Parker agar (Heipha AG, Eppelheim, Germany) were used. Tryptic soy agar was used as a universal medium to determine the overall bioburden by TCC, MacConkey agar was used to grow Gram-negative bacteria selectively, and Baird-Parker agar was used to grow *S. aureus*.

Samples were transported in insulated cooling bags and were processed within 6 h of sampling. All samples were incubated for $\geq$48 h at 35°C. TCC per plate was assessed by counting the number of cfu using a laboratory colony counter. If TCC exceeded 100 cfu per plate, TCC was assessed semi-quantitatively, categorized in steps of 50 cfu.

Visually differentiable cfu, grown on any of the three plate types, were subcultured on blood agar, incubated for $\geq$24 h, and isolates were analysed by MALDI-TOF (MALDI Biotyper, BRUKER DALTONICS GmbH, Faellanden, Switzerland). The MALDI-TOF procedure was performed according to the manufacturer's recommendations. Scores of $>$1.6 were considered as sufficient for genus identification and scores of $\geq$2.0 for species identification, but a score of $>$2 was common. Each MALDI-TOF identification was performed in duplicate. Colonies resembling coagulase-negative staphylococci were classified visually, and were checked periodically by MALDI-TOF. If any PHCR was found, the investigated surface was marked positive for contamination with PHCR.

Table I
Correlation of total colony count (TCC) with presence of pathogens with high clinical relevance

TCC	1–50	51–100	>100
Overall			
N	334	85	26
PHCR	53	17	3
Mean TCC ± 2 SD[a]	26 ± 33	72 ± 36	291 ± 234
Correlation coefficient (rho)	0.22	−0.11	0.03
Hospital			
N	96	19	8
PHCR	16	6	2
Mean TCC ± 2 SD[a]	25 ± 32	68 ± 31	235 ± 147
Correlation coefficient (rho)	0.14	−0.33	−0.07
Community			
N	238	66	18
PHCR	37	11	1
Mean TCC ± 2 SD[a]	27 ± 74	73 ± 38	316 ± 252
rho	0.25	−0.02	0.32

PHCR, pathogens of high clinical relevance; SD, standard deviation. TCC (colony-forming units per plate) shows very low correlation (Spearman's rho = 0.13) with the presence of PHCR. TCC should not be used as a reliable predictor for the presence of PHCR.
[a] Values rounded to nearest whole number.

On Baird-Parker plates, an agglutination test (Pastorex Staph Plus, Bio-Rad Laboratories, Cressier, Switzerland) was performed to screen for *S. aureus*. *S. aureus* isolates were tested for meticillin resistance by the standard disk diffusion method (30 μg cefoxitin, 35 ± 1°C) using guidelines and breakpoints outlined by the European Committee on Antimicrobial Susceptibility Testing (www.eucast.org, breakpoint tables, 2016).

Definition of PHCR

There is no uniformly accepted definition for PHCR. This study used the definition outlined by the CDC National Healthcare Safety Network (NHSN). The list of micro-organisms designated as 'top' bacteria was used, representing the pathogens reported most frequently (https://www.cdc.gov/nhsn/xls/2016-NHSN-Organisms-List-Validation.xlsx, accessed January 2016), and was further refined by excluding any known skin contaminants based on CDC's list of skin contaminants (https://www.cdc.gov/nhsn/XLS/Common-Skin-Contaminant-List-June-2011.xlsx, accessed January 2016). This list was subsequently updated in 2017 (https://www.cdc.gov/nhsn/xls/master-organism-com-commensals-lists.xlsx).

Statistical analysis

Unless otherwise noted, statistical tests and boxplots were performed with standard parameters in Matlab (MATLAB and Statistics Toolbox Release 2017b, The MathWorks, Inc., Natick, MA, USA). Unless noted otherwise, correlations were performed using Spearman's rho test, crosstabulation significance tests were performed using Fisher's exact test, and TCC comparisons were performed using the Mann–Whitney *U*-test.

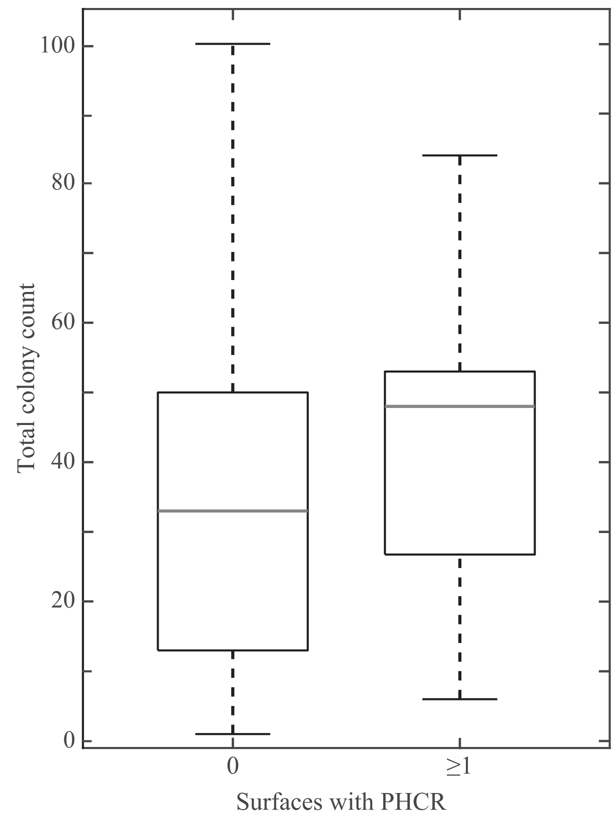

Figure 1. Total colony count (colony-forming units per plate) obtained from surfaces without the presence of pathogens of high clinical relevance (PHCR) and surfaces with at least one PHCR; no significant difference was found ($P = 0.67$).

Results

In total, 1431 contact plates were processed from 477 sampling areas: 153 surfaces from hospitals and 324 surfaces from publicly accessible locations in the community. At least 1 cfu grew from 445 of 477 (93.3%) sampling areas, and at least one PHCR was identified from 73 of 477 (15.3%) sampling areas. The presence of PHCR showed a low, non-linear correlation with TCC ($r_{overall} = 0.13$, Table I), which was similar when stratified by hospital and publicly accessible locations. Moreover, there was no significant difference between TCC with and without the presence of PHCR ($P = 0.67$, Figure 1).

Overall, lower TCC was found on plates within hospitals (median 9 cfu) compared with publicly accessible locations in the community (median 43 cfu, $P < 0.01$). PHCR were less common in hospitals [18/153 (11.8%)] than in publicly accessible locations in the community [55/324 (17.0%)], but no significant difference was found [$P = 0.17$, odds ratio (OR) 0.65, 95% confidence interval (CI) 0.37–1.15].

Overall, 1747 isolates were identified from the sampling areas: 1656 bacteria and 91 fungi. Overall, fewer Gram-negative bacteria ($N = 316$) were found compared with Gram-positive bacteria ($N = 1340$). Fewer Gram-negative bacteria [30/353 (8.5%)] were found on hospital surfaces compared with publicly accessible surfaces in the community [286/1303 (21.9%)], and the difference was significant with respect to Gram-positive bacteria ($P < 0.01$, OR 0.33, 95% CI 0.22–0.49).

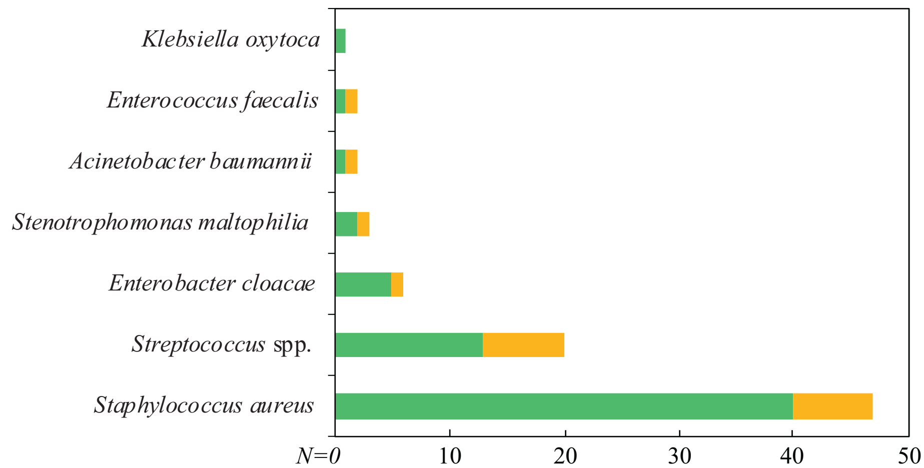

Figure 2. Number of identified species of pathogens of high clinical relevance ($N = 81$) found on surfaces of publicly accessible locations in the community (green bars) and surfaces within hospitals (yellow bars). The list of 'top organisms' issued by the Centers for Disease Control and Prevention is available at https://www.cdc.gov/nhsn/xls/2016-NHSN-Organisms-List-Validation.xlsx.

In total, 81 PHCR were identified on 73 surfaces. *S. aureus* was the most common pathogen among isolated PHCR ($N = 47$), followed by streptococci ($N = 20$), *Enterobacter* spp. ($N = 6$), *Stenotrophomonas maltophilia* ($N = 3$), *Acinetobacter* spp. ($N = 2$), *Enterococcus faecalis* ($N = 2$) and *Klebsiella* spp. ($N = 1$) (Figure 2). No *S. aureus* isolates were MRSA.

Discussion

To the authors' knowledge, this is one of the largest studies to date to use universal and selective agar contact plates for routine characterization of the bacteria to species level using MALDI-TOF.

Only low correlation ($r = 0.13$) was found between TCC and the presence of PHCR. A similar study focusing on MRSA alone did not find a correlation between TCC and the presence of PHCR [18]. Measuring environmental contamination as a surrogate marker for the presence of PHCR would ease checking patient rooms after terminal cleaning. Anderson *et al*. showed that patients exposed to rooms previously occupied by patients colonized with MDROs can acquire such organisms from their environment [19]. Measuring the bioburden before and after cleaning has been used to monitor the quality of terminal cleaning. Routine cleaning and disinfection of the hospital environment is associated with low bioburden, and this could be interpreted as low probability of the presence of PHCR [20]. However, the present data suggest that a high bioburden does not necessarily predict the presence of PHCR or even MDROs. Targeted approaches are suitable during outbreaks if the pathogen being transmitted is known. However, in the endemic setting with multiple potential pathogens, screening using conventional biochemical identification is not a financially reasonable option.

The introduction of MALDI-TOF allows large numbers of bacteria to be identified at low cost (approximately 0.35–0.79 US$ reagent cost per isolate) [21]. The transmission of PHCR might be common but has likely been unrecognized in the past, as appropriate, inexpensive and rapid diagnostic tools were unavailable. This study did not find MDROs such as MRSA; this pathogen has been found rarely at the study institution in the past and nosocomial transmission has been rare [22].

Samples were also taken outside of the hospital to improve the external validity of the study results generated in the healthcare setting. The results remained unchanged in these samples (Table I), with the environment outside the hospital being contaminated with PHCR more frequently than hospitals. High-touch surfaces such as touchscreens are disinfected routinely and frequently in hospitals, which potentially explains the higher frequency of contamination with PHCR in environments outside hospitals, where disinfection is less frequent or may never be performed.

This study has a few limitations. Firstly, the definition of PHCR by CDC was not developed for the purpose of environmental contamination and risk of HAIs. However, there is no uniformly accepted definition, and the authors considered the CDC definition to be the best available source to define PHCR. Secondly, the total cfu of PHCR was not evaluated because the study was not designed to answer this question. However, the bacterial density of nosocomial pathogens is generally in the range of <1 to 100 cfu/cm^2 on surfaces, and small numbers have been reported to be sufficient to cause infection in experimental lesions [23]. Thirdly, visual inspection of colonies on the agar may have missed some PHCR, but two experienced staff members at the microbiology laboratory did select all potentially different colonies for MALDI-TOF. Therefore, it is considered that few PHCR isolates were missed. Finally, the sample size was limited by financial and staff constraints.

In conclusion, overall bioburden was found to be poorly correlated with the presence of PHCR using MALDI-TOF for rapid, inexpensive and reliable identification of PCHR. Extension from targeted sampling to untargeted sampling may provide insights into the transmission of pathogens that were missed by selective screening plates.

Conflict of interest statement
None declared.

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