



Bacterial load and pathogenic species on healthcare personnel attire: implications of alcohol hand-rub use, profession, and time of duty

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SUMMARY

Background: Since hand hygiene might prevent the risk of bacterial transmission from healthcare personnel attire (HCPA), the present study investigates the effect of alcohol-based hand rub (ABHR) use on bacterial load and pathogenic species on HCPA.

Methods: HCPA from doctors and nurses was investigated for bacterial contamination post duty. Samples from distinct areas of HCPA were obtained and analysed for bacterial load and species. A standardized questionnaire was performed regarding time of duty and profession, and ABHR from each ward was calculated according to a national standard.

Findings: Bacterial load on HCPA (700 samples from 200 HCPA) was found to be up to four-fold higher when wearing for more than one shift. Moreover, doctors had a lower bacterial load on attire compared to nurses. In a multivariate linear regression model, negative correlations with bacterial load on HCPA were found for ABHR ($t = -2.080$, $P = 0.0379$) and being a doctor ($t = -6.009$, $P < 0.0001$), and a positive correlation for the time of duty ($t = 10.572$; $P < 0.0001$). Detection of *Staphylococcus aureus* as the most prominent pathogen found on HCPA was influenced by the time of duty (odds ratio: 3.27; 95% confidence interval: 1.93–5.72; $P < 0.0001$) but not by ABHR (1.22; 0.30–3.42).

Conclusion: ABHR, profession, and time of duty significantly affect the bacterial load on HCPA. Since the time of duty has the strongest impact on bacterial load, a daily change of HCPA is recommended.

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Introduction

It is universally acknowledged that hand hygiene is one of the most cost-effective methods for the prevention of healthcare-associated infections by healthcare workers [1,2].

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It becomes even more significant as multidrug-resistant microbes emerge as a growing threat to hospital healthcare. Hand hygiene compliance can be evaluated by direct observation following the World Health Organization's five moments for hand hygiene, which are considered as the reference standard [3]. However, triggered by the Hawthorne effect, a >60% overestimation of hand hygiene compliance was suggested when hand hygiene compliance was evaluated under direct observation [4]. An alternative surrogate parameter for hand hygiene compliance is the magnitude of the consumption of alcohol-based hand rub (ABHR) in relation to patient-days: this

tool seems to be a useful method to characterize the frequencies of hand hygiene action and is independent of a Hawthorne effect [5].

An increase in alcoholic hand-rub consumption is associated with a decrease in patient incidences of multidrug-resistant bacteria and may also protect healthcare personnel from colonization with hospital pathogens such as *Staphylococcus aureus* [6,7]. With regard to *S. aureus*, nasal carriage and environmental colonization seem to be a potential risk for patient transmission [8]. Thus healthcare personnel attire (HCPA) may also serve as an 'environmental' vector for pathogen cross-transmission. However, only sparse data exist to estimate the risk of HCPA on transmission, and the impact of hand hygiene on the bacterial colonization of HCPA has not been investigated so far [9].

The present study aimed to investigate the importance of hand hygiene on the bacterial load and pathogenic species on HCPA of normal care staff in a tertiary care hospital in Germany: the study focused on the effect of alcohol hand-rub use together with influential factors such as profession and time of duty. Data on hand-rub consumption were obtained according to the guidelines of the German national 'Clean Hands' campaign ('Aktion Saubere Hände') and the protocol of HAND-KISS, an alcohol-based hand-rub consumption surveillance at the level of wards or functional areas [10].

Methods

Study design; inclusion and exclusion criteria

The present prospective, single-centre, observational study was performed within a tertiary care hospital (45,000 inpatients per year) in Germany. Bacterial loads from HCPA were obtained from nurses and doctors working in adult and paediatric inpatient wards.

Staff from intensive care units, intermediate care units, and from outpatient areas were excluded from the study.

Standardized questionnaire

A standardized questionnaire was used to gain information about the profession of study participants (nurse or doctor/working on a surgical ward or medical ward/care for adults or children), type of routine work at the wards (examination of patients, routine care such as washing of patients) and how long personnel attire was used (one shift normally 8 h, two shifts, or longer). Participation in the study was voluntary.

Use of healthcare personnel attire

The hospital-wide HCPA policy includes the recommendation to change personnel attire (i) routinely after two shifts, and (ii) immediately after an obvious contamination. Staff require additional protective clothing and gloves when having direct contact with an infectious patient, a patient colonized with multidrug-resistant microbes, and patients with open wounds. The work-wear policy in the study hospital does not recommend short sleeves for doctors since the evidence regarding short sleeves and infection prevention is uncertain.

Used personnel attire was laundered (chemothermally) by a professional external laundry service according to the official



Figure 1. Sampling sites for bacterial load on nurses' and doctors' attire: samples were obtained from front (1), coat pocket (2), front pocket (3) and sleeves (4; sleeves were only sampled from doctors' attire).

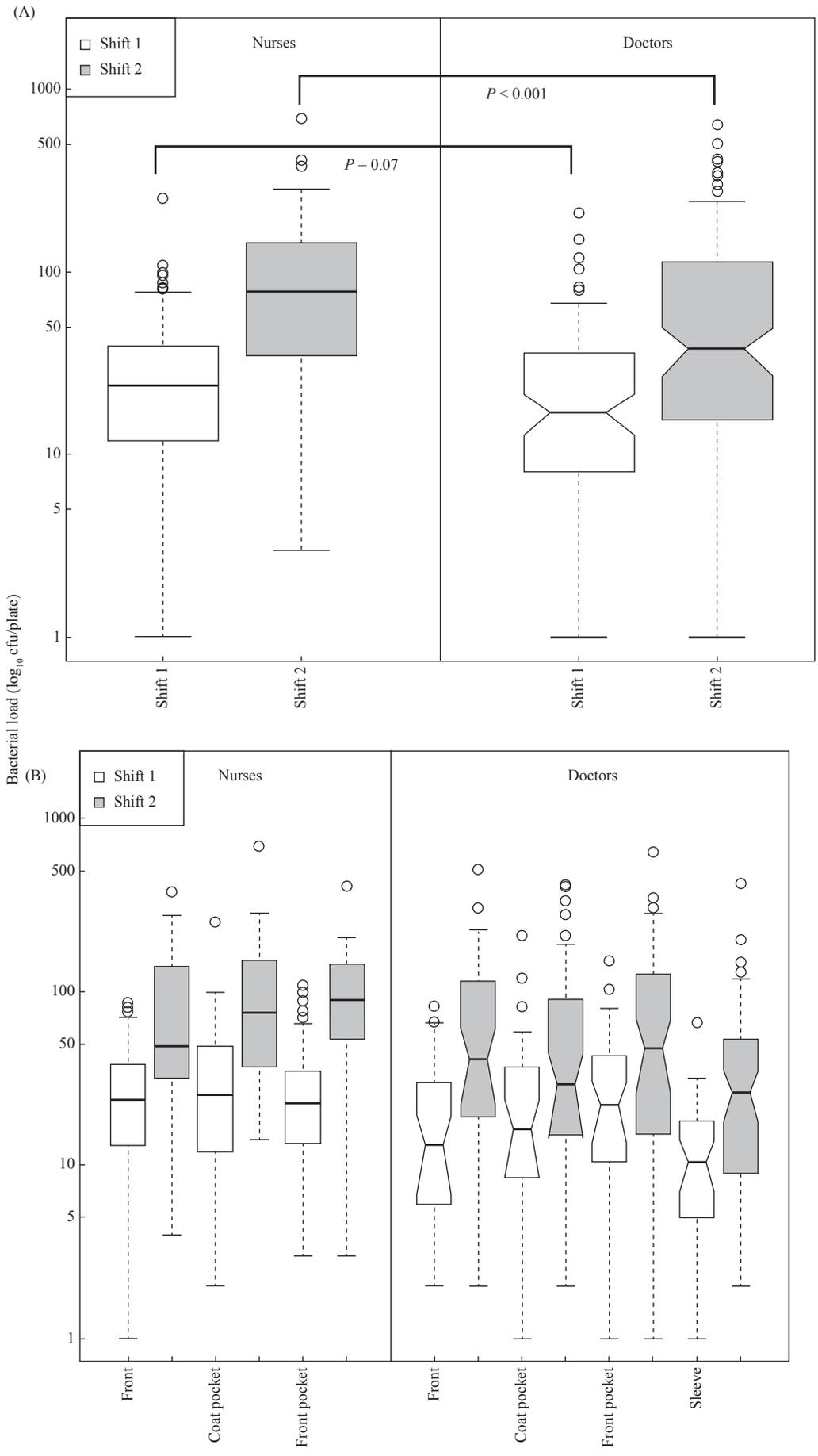
recommendation of the German Healthcare Services, then transported to the hospital in closed boxes and stored dust-free until use [11]. Maximum storing time of freshly laundered HCPA was one week.

Table I

Characteristics of participating medical wards ($N = 22$)^a

Ward no.	Surgery (S)/ non-surgery (Non-S) wards	Children/adult wards	Alcohol hand-rub use (mL/patient-days)	No. of participants (nurses/doctors)
1	Non-S	Adults	30	5/4
2	Non-S	Adults	36	5/5
3	Non-S	Adults	36	10/2
4	S	Adults	39	0/1
5	S	Adults	39	17/4
6	S	Adults	36	5/7
7	Non-S	Adults	27	4/8
8	Non-S	Adults	48	8/12
9	Non-S	Adults	45	4/2
10	Non-S	Adults	45	0/3
11	S	Adults	36	12/7
12	S	Adults	36	9/2
13	S	Adults	33	9/9
14	S	Adults	33	0/1
15	S	Adults	51	12/14
16	S	Adults/children	36	0/2
17	S	Adults	36	0/2
18	Non-S	Adults	51	0/2
19	Non-S	Adults	42	0/1
20	Non-S	Children	84	0/6
21	S	Children	48	0/4
22	S	Adults	27	0/2

^a Quality of hand hygiene for each medical ward was calculated according to the national KISS protocol and the 'Aktion Saubere Hände' [10].



Sampling for bacterial load

Sampling for bacterial load was performed by using RODAC contact plates (diameter 5 cm; area 25 cm²) according to the manufacturer's instructions (Oxoid, Basingstoke, UK). Standardized sampling sets of three (nurses) or four (doctors) areas of each HCPA (front pocket/coat pocket/front and sleeve (only doctors)) was performed at the end of the 8 h shift by one investigator (Figure 1). After overnight incubation at 37°C, plates were analysed for bacterial load (results were given as colony-forming units (cfu)/plate), and different colonies were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in the in-house microbiology laboratory.

To exclude significant bacterial contamination of HCPA before use, randomly selected HCPA were sampled according to the standard sampling procedure (see above) at the central output of the hospital laundry storage.

Quality of hand hygiene

The entire hospital is participating in the national surveillance of hand hygiene compliance organized by the National Reference Center for the Surveillance of Nosocomial Infections at the Institute for Hygiene and Environmental Medicine Charité – University Medicine Berlin. According to a standardized protocol, hand hygiene action was calculated from total alcohol hand-rub use (ABHR) per unit in relation to patient-days and used as a surrogate marker for hand hygiene quality [12]. The national surveillance for better hand-hygiene compliance includes: (i) the German nationwide 'Clean Hands' campaign ("Aktion Saubere Hände"), which contributes to improved hand hygiene compliance by recommendations; and (ii) the alcohol-based hand-rub consumption surveillance at the level of wards or functional areas (HAND-KISS).

Statistics

Statistical analysis was performed with the open source software R. Bacterial load was always considered on a decimal logarithmic scale. An increment of one was added to all bacterial load values before taking the logarithm in order to avoid log₀. Whenever odds ratios were specified, Fisher's exact test was performed to compute the corresponding *P*-value. For the comparison of bacterial load values, the Welch two-sample *t*-test was applied for the comparison of two samples. After the logarithmic transformation, distributions were not significantly skewed and the minimum sample size for the *t*-test was 35, so that departures from normality would not have a strong effect on the *t*-test. Replacement of this test by the Wilcoxon–Mann–Whitney test yielded similar results. In case of multiple comparisons, analysis of variance (ANOVA) with post-hoc Tukey's honest significance test was applied. For ANOVA, neither obvious departures from normality nor from variance

homogeneity were observed. The minimum sample size per group was 99. Values of bacterial load are given as mean (median, interquartile range (IQR)).

Results

Bacterial load on HCPA with regard to profession, time of duty, and site of sampling

Data from 700 contact plates from 200 HCPA (100 each from nurses and doctors) from 22 medical wards were obtained for statistical calculation (characteristics from participants and medical wards are given in Table I). Box plots representing bacterial loads according to sampling sites of HCPA and time of duty are given in Figure 2A,B. A total of 350 samples (nurses/doctors: 210/132) were obtained from white coats which were worn for only one shift, and the same number of samples from HCPA worn for a second shift (nurses/doctors: 90/268). In both professions, the median load was at least twice (for doctors and even three times for nurses) as high at the second shift when compared to the bacterial load obtained after one shift of duty (nurses and doctors: *P* < 0.001, respectively) (Figure 2A). No differences were found for bacterial load on HCPA of staff working at surgical or non-surgical wards (data not shown). Differences were not calculated for adult and paediatric wards since participants from only two paediatric wards were included.

Only six out of 350 samples after one shift (1.71%) were found to be negative for bacterial contamination, and one out of 350 (0.29%) after a second shift. Data from staff wearing HCPA for more than two shifts were excluded from statistics because of low numbers.

Bacterial load from nurses' HCPA compared to doctors' HCPA was increased with regard to the time of duty (Figure 2A): nurses' coats had a significantly higher bacterial load at the front site after one shift compared to doctors' coats (28 cfu/plate (median: 23; IQR: 23–37) vs 21 (12; 5–29); *P* = 0.02). After two shifts, load on coat pockets and front pockets of nurses' HCPA was significantly different compared to doctors' HCPA (coat pocket: 114 cfu/plate (75; 38–146) vs 66 (28; 14–89); *P* < 0.001 per front pocket: 102 (88; 52–142) vs 86 (46; 14–125); *P* = 0.009).

Focusing on doctors' attire, the lowest bacterial load was observed for sleeves (sleeves compared to front: *P* = 0.038; sleeves compared to front pocket: *P* = 0.005) (Figure 2B); ANOVA where each of the four locations on doctors' attires is considered as one group, *P*-values were given for the post-hoc test).

Sampling of HCPA before use (a total of 30 samples from 10 randomly selected HCPA were obtained) showed a nearly 'zero' bacterial load from the respective sampling sites without detecting pathogens (coat pocket/front pocket: 0 cfu/plate, respectively; front: 1 (0; 0–2)). Sleeves of unused HCPA were not sampled.

Figure 2. (A) Bacterial load on healthcare personnel attire according to profession (doctors/nurses) and time of duty (one shift/two shifts). Although for both doctors and nurses bacterial load increased significantly after two shifts of duty compared to one (*P* < 0.001, respectively), load was higher on nurses' compared to doctors' attire after each shift. (B) Bacterial load on nurses' (left slide) and doctors' (tailored boxes, right slide) attire according to sampling sites and time of duty (white boxes: after one shift; grey boxes: after two shifts).

Multivariate linear regression model estimating bacterial load

From the scatter plot with the regression line (95% confidence interval) shown in Figure 3A, it could be suggested that the bacterial load decreased with an increase in the quality of hand disinfection. Each scatter point represents the individual bacterial load from the sampling sites from staff HCPA, which were correlated with the corresponding ABHR from the respective ward where staff worked (ABHR data in Table I).

Indeed, in a multivariate linear regression model including profession (doctor or not (nurse)), time of duty (one shift of duty or a second shift), and quality of hand hygiene (ABHR as a continuous variable), bacterial load is estimated by the profession (a negative correlation was found for being a doctor; $P < 0.0001$), the time of duty (wearing for two shifts was positively correlated with bacterial load; $P < 0.0001$), and by the ABHR (ABHR was negatively correlated; $P = 0.0379$). Estimate, standard error (SE) and T -value are given in Figure 3B.

Detection of bacterial species on HCPA

With regard to non-pathogenic bacteria, up to 22 different species could be isolated from different locations on HCPA. However, mostly coagulase-negative staphylococci spore-forming *Bacillus* spp./*Paenibacillus* spp. and *Micrococcus luteus* were found. Ranges of incidences (species per 100 samples) for the most frequent species were given as follows: *S. hominis* 0.52–0.70, *S. epidermidis* 0.47–0.71 and *S. haemolyticus* 0.18–0.36; incidences for *Bacillus* spp. were 0.27–0.53, for *Paenibacillus* spp. 0.15–0.21 and for *Micrococcus luteus* 0.14–0.22.

Focusing on pathogens, *S. aureus* was the leading species found on HCPA. Twenty-two out of 350 samples after one shift of duty (incidence: 0.06), and 63/350 after a second shift (incidence: 0.18) were positive for *S. aureus* leading to a 3.27-fold risk (95% CI: 1.93–5.72) for contamination of HCPA after two shifts of duty compared to one. No differences were found for *S. aureus* colonization regarding profession (nurses vs doctors after one shift: 17/200 and 5/140, $P = 0.115$; after a second shift: 20/90 and 43/260, $P = 0.265$) or localization (front/front pocket/coat pocket after one shift: 5/105, 7/105 and 9/105, $P = 0.542$, χ^2 -test; after a second shift: 17/95, 18/95 and 20/95, $P = 0.854$, χ^2 -test). Hand hygiene quality had no effect on colonization of HCPA with *S. aureus* (OR: 0.89; 95% CI: 0.52–1.51; $P = 0.702$).

Regarding Enterobacteriaceae, *Escherichia coli* was the most frequent species detected on HCPA (after one shift: 9/328 (incidence 0.03); after a second shift: 6/287 (0.02)). Incidences for further pathogenic bacteria on HCPA such as *Citrobacter* spp., *Enterobacter* spp., *Pseudomonas* spp., *Acinetobacter baumannii* complex, *Burkholderia*, and *Enterococcus faecalis* were <0.02 . Since the detection rates of the abovementioned pathogenic bacteria other than *S. aureus* were low, no statistical analysis was performed.

Discussion

The present data clearly show that a higher bacterial load on HCPA was associated with profession, the time of duty, and with the quality of hand hygiene as measured by alcohol hand-

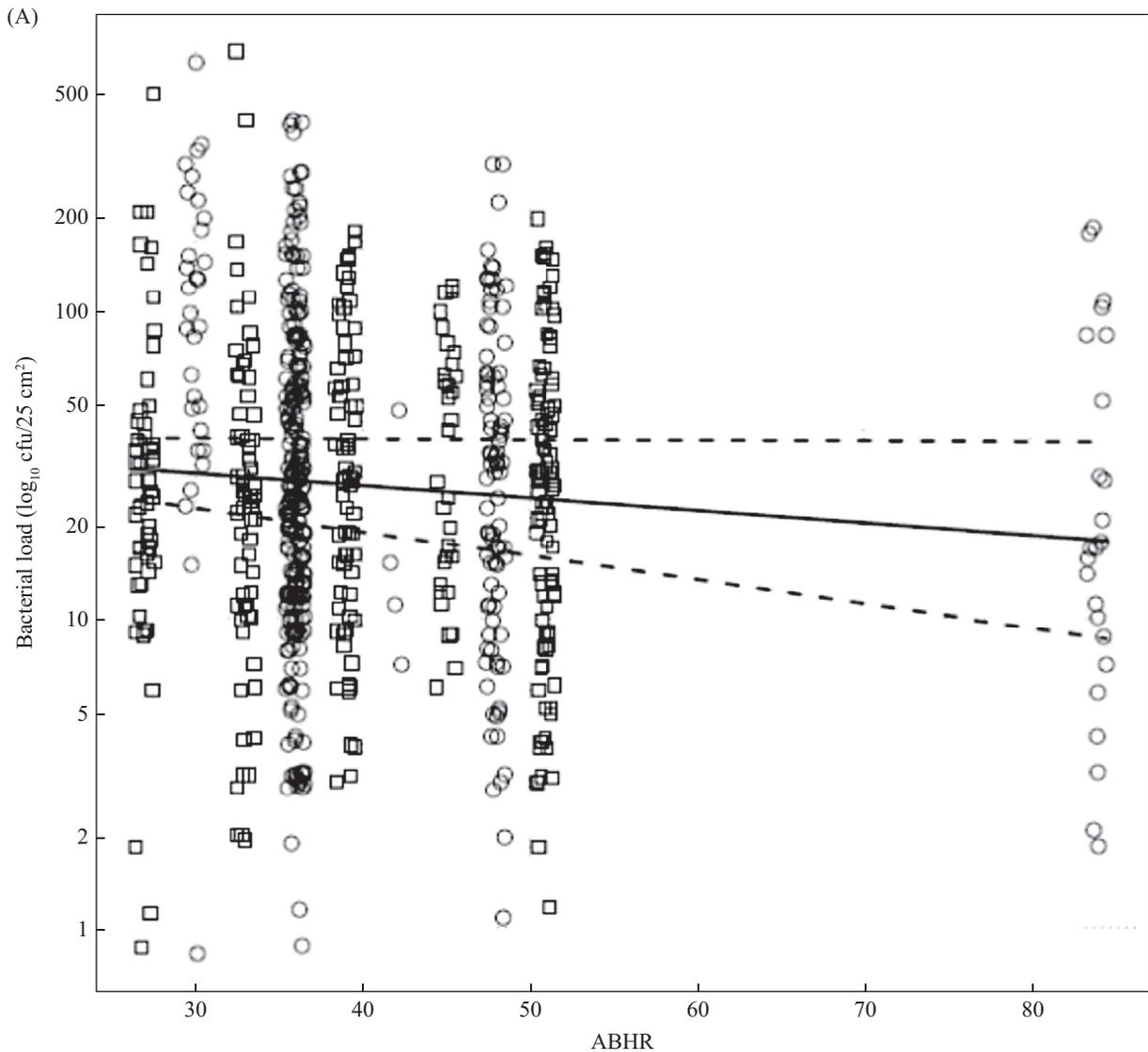
rub (ABHR) use. However, ABHR and profession had no effect on colonization with *S. aureus*, which was found as a major pathogen at all tested sites of HCPA: only time of duty increased the detection rate of *S. aureus* significantly from one shift to a second shift, from 6% to 18% of all sampling sites, leading to a significant OR of 3.27. Whereas other pathogens such as *E. coli*, *Enterobacter* spp., *Acinetobacter* spp., *Pseudomonas* spp., or enterococci were rare and found only in individual cases, non-pathogenic bacteria such as coagulase-negative staphylococci, spore-forming *Bacillus* spp./*Paenibacillus* spp. and *Micrococcus luteus* were present in up to 70% of sampling sites. In the present study, doctor's sleeves appeared to be the sampling site with the lowest bacterial load compared to front or pockets.

Several studies have been performed to investigate the potential significance of bacterial load on HCPA. The present data show that the mean bacterial load increased from the first to the second shift of duty by up to four-fold, regardless of profession and sampling site. However, the mean load on doctors' sleeves was lower compared to pockets or fronts. The latter is a surprising observation since long sleeves are considered to be at high risk for contact contamination during patient care [13,14]. However, our observation might be due to hand hygiene compliance above average within the study hospital: frequent hand disinfection includes disinfection of the wrist and might have a potential impact on the bacterial load of long sleeves.

In the present study, laundering and storage procedures as well as recommendation for usage of HCPA were available. Therefore, we are surprised by Burden *et al.*'s study finding that mean colony counts of newly laundered uniforms were higher than in our study after one or two shifts of duty [15]. The sampling procedure of both studies was similar, but no data were given by Burden and colleagues concerning the laundering procedure and the storage of coats. In another study by Burden's group, the bacterial load of scrubs before use was reported as <10 colonies, which is in line with our findings and according to the official recommendations for freshly laundered HCPA in Germany [11,16,17]. For a better standardization, laundering of HCPA by a professional laundry service is recommended following the professional associations for medical staff for the prevention of infection (Technical Rules for Biologicals, No. 250) [18]. This is in contrast to other countries, where home washing of workwear is frequent but difficult to standardize [19–21].

However, we did observe a higher bacterial load in nurses' compared to doctors' HCPA, which is most likely explained by the more frequent contact of nurses with patients during a shift; Azim *et al.* calculated a three-fold more frequent opportunity of hand hygiene for a nurse compared to a physician, which is indeed a good indicator for the contact frequencies [22]. In addition, a recent study investigating bacterial contamination of nurses' scrubs has shown that the level of contamination depends on the kind of work being higher when providing care for wounds or giving a bath [23].

As shown by our linear regression model, alcoholic hand-rub use is a significant factor associated with bacterial load on HCPA: it is possible to postulate that good hand hygiene compliance should stop the transfer of microbes to HCPA during care activities by hand contact and vice versa; the alcoholic hand-rub consumption of the present hospital was high and ranged within the top 10th percentile compared to other



(B)

Variable	Estimate	SE	<i>t</i> -value	<i>P</i>
Intercept	1.54048	0.072	20.84	<0.0001
ABHR	-0.004	0.002	-2.080	0.0379
Time of duty (two shifts)	0.4116	0.039	10.572	<0.0001
Profession (being a doctor)	-0.239	0.039	-6.009	<0.0001

Figure 3. (A) Hand hygiene quality was correlated with bacterial load, suggesting an effect of hand hygiene quality on bacterial load of healthcare personnel attire (HCPA). Each scatter point represents the individual bacterial load of doctors'/nurses' attire and the alcohol-based hand rub (ABHR) from the respective wards to which staff belong (according to Table I). The regression line with the 95% confidence interval for the slope are shown by the solid and the dashed lines, respectively. The upper margin of the confidence interval has a negative slope. (B) Multivariate linear regression model estimating microbial load on HCPA: in this model including profession, time of duty, and ABHR (continuous variable), all variables correlated with the bacterial load significantly. Being a doctor is negatively correlated with microbial load; wearing attire for two shifts correlated positively with microbial load; in addition, a higher ABHR is slightly but also negatively correlated with the microbial load. The intercept of 1.504 indicates that the estimated bacterial load of coats of nurses after the first shift without using any ABHR would be at $10^{1.504} = 31.92$ cfu/plate.

German hospitals participating in the National Surveillance System [24]. However, up to now only a few studies have investigated a direct link between bacterial growth on healthcare workers' hands and coats or scrubs: in the study by Munoz-Price performed at different intensive care units (ICU), only contamination of providers' hands with *Acinetobacter baumannii* was associated with contamination of physicians' white coats, whereas no such association was found for nurses' scrubs (because of different laundering procedures) or for *S. aureus* or enterococci [25]. Furthermore, Morgan et al. have found the strongest association between *Acinetobacter baumannii* on ICU healthcare workers' protective clothing and a positive environmental culture [26]. Contamination with MRSA, VRE, and *Pseudomonas* spp. was also found on protective clothing and gloves and was correlated with performing physical examination, contact with the ventilator, and duration in the patient room for >5 min. A positive correlation with the culture of 'any organism' on hands and scrubs was also found.

Although *S. aureus* was the most frequent pathogen on HCPA found in our study, no association was observed between hand-rub consumption and the detection rate of *S. aureus* on HCPA. With regard to the study of Morgan, our findings may suggest that appropriate hand hygiene practices alone before and after patient care are insufficient to halt transmission of hospital pathogens to HCPA [26]. However, we could not exclude self-contamination of HCPA with staphylococci in cases of a positive carrier status. For more detailed analyses, carrier status of staff should be analysed as a source, and direct observations of compliance with hand hygiene according to the 'five moments' would be necessary [3].

From our data, obtained from a German tertiary care teaching hospital, it is clear that pathogens other than *S. aureus* were very rare on HCPA: in three other studies from emerging and developing countries – India, Zambia, and Tanzania – the incidences of *Pseudomonas* contamination on white coats were 0.037, 0.040, and 0.068, which were higher compared to our study's incidence of 0.02; in another study from India, incidences of *Klebsiella* spp., *Pseudomonas* spp., and *E. coli* were 0.075, 0.184, and 0.478, which were much higher compared with the present data (*Klebsiella* spp.: none; *Pseudomonas* spp.: 0.02; *E. coli* (after a second shift of duty): 0.03) [27–30]. However, none of the abovementioned studies specified details about the hygiene management of infectious patients, patients with open wounds, and patients colonized with multidrug-resistant bacteria. It is possible that the low incidence for pathogens other than *S. aureus* found on HCPA in our study might be due to wearing protective clothing while caring for these patients.

With regard to detection of the so-called non-pathogens, diversity of germs on HCPA was high: more than 22 different species after a second shift – notably coagulase-negative staphylococci, potentially spore-forming *Bacillus* spp./*Paenibacillus* and *Micrococcus luteus* – were identified, and only 1.71% of samples after one shift of duty and 0.28% after a second shift of duty were found to be totally negative for any bacteria. With regard to hospital infections, coagulase-negative staphylococci (*S. epidermidis*, *S. hominis*, *S. haemolyticus*) are significant pathogens developing evasion immune mechanisms involved in infections with indwelling biomaterials and in skin and soft tissue infections [31,32]. Spore-forming bacilli were ubiquitous, and *Bacillus cereus* is present on surfaces and human skin. *Bacillus* could induce systemic infections in immunocompromised patients and

premature neonates as shown recently by Gasset et al. [33]. We wonder about the high incidence of *Bacillus* spp. (up to 0.53) in our study: an increased selection of spore-forming bacteria in medical wards characterized by a high ABHR cannot be excluded because of the known 'sporocidal gap' of alcohol.

Some limitations of the present study should be mentioned: data were obtained by a monocentric cohort study which generally limits the generalization of our findings. In addition, wearing time of HCPA was obtained from staff only by a questionnaire; veracity of the answers could not be assured. Furthermore, several further covariates potentially influence bacterial load on HCPA (sampling method/hygiene management with infectious patients and open wounds/laundry conditions/*S. aureus* carrier status) which could not be taken into account within the present data because of the limited scope of samples and resulting statistical problems.

In conclusion, our data strengthen the demand for an HCPA policy for inpatient care in healthcare facilities. This policy may include a 'bare below the elbows' recommendation for the use of HCPA with short sleeves since the risk of long sleeves for transmission of pathogens is not excluded by our data. In addition, the need for a daily change of HCPA also for hospital staff working in normal care units was underlined, since bacterial load and *S. aureus* colonization is greatly increased after a second shift of duty. Finally, hand hygiene compliance should be optimized in the clinical setting since a significant impact on the bacterial load on HCPA was also shown.

Conflict of interest statement

None declared.

Funding sources

None.

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