

Colonization of Hospital Staff Pens - Unmasking Relevant Employee Groups with Inadequate Hygiene Training

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ABSTRACT

Background: Healthcare-associated infections remain a significant burden, and hospital hygiene algorithms play a central role in their prevention. Medical staff hand hygiene is often in the center of investigations, however, much less attention has been paid to the role of everyday utensils as possible indicators for weak points of the own system.

Aim: To assess the degree of colonization of pens of using simple methods in the hospital setting to uncover hygienic vulnerabilities and identify neglected employee groups.

Methods: Collection of 200 pens in the hospital that were either publicly accessible or could be assigned to a specific employee group (doctors, nurses or admissions clerks) and inoculation in a nutrient broth. This was followed by sonication of the broth and plating on different agar plates as well as pathogen identification and resistance testing.

Findings: Overall, 99% of the pens showed bacterial (but no fungal) growth, with polymicrobial colonization found in 70% of cases. Of the 417 detectable microorganisms, 96.2% were gram-positive and 3.8% were gram-negative, with only one isolate being classified as multi-resistant (meticillin-resistant *S. aureus*). Analysis of the origin of the pens and the assignment to the respective professions showed at least two neglected employee groups in our hospital (physicians and admission clerks), which are completely underrepresented concerning hygiene training.

Conclusion: The study employed a simple methodical approach to investigate the colonisation of staff pens, revealing deficiencies in hygiene training among specific employee groups. This emphasizes the need to thoroughly question even established algorithms within the own hygienic strategy.

Keywords: Hospital Hygiene; Infection Control; Colonization; Clinical Microbiology; Nosocomial Infections

Abbreviations: MRSA: Meticillin-Resistant Staphylococcus aureus; VRE: Vancomycin-Resistant enterococci; ESBL: Extended-Spectrum Betalactamase-Producing Bacteria

Introduction

Preventing nosocomial infections is a key task in hospital hygiene, focusing transmission routes of relevant microorganisms. In this context environmental examinations in hospital settings consistently reveal that surfaces, both near and far from the patient, can harbour

a significant amount of microbial contamination [1-3] with hand hygiene often being a weak point [4,5]. Less attention is given to everyday items far from the patient as vectors for microorganisms. Our study examined the colonisation of pens used by different employee groups to assess whether their origin or surface properties reflect the hospital's hygiene management.

Methods

Sample Collection:

Between January and March 2023, a total of 200 ballpoint pens from different clinical departments of the University Hospital Salzburg were examined. A distinction was made as to whether the pen was in the personal use of one person (n= 100 pens, usually carried in the pocket of the work cloth) or whether it was accessible to several persons (n= 100 pens). Furthermore, it was documented from which staff group the respective person originated (physicians, nurses, administration clerks), where the pen was predominantly used (nursing station, patient registration, examination room, physicians' room, intensive care unit) and of which material the surface of the pen was made of (plastic or rubber grip). Upon collection the private pens were placed directly into 9 ml thioglycolate nutrient broth (Oxoid®) by the respective user. Pens of publically access were placed in the broth by hygienists from the hospital hygiene team after hand disinfection and using nitrile gloves. Efforts were made to insert the pens as a whole into the nutrient solution. In case this was not possible due to the size, the pen was disassembled, and the components were added to the broth.

Sample Preparation:

Collected pens were treated by sonication similarly to the procedure outlined for explanted prosthetic joints and other foreign items to separate microbial biofilm from the surface and so increase the accumulation of microorganisms [6]. For this, broth tubes containing the pens were sonicated for one minute at a cleaning frequency of 35 kHz in an ultrasonic bath (Bandelin® SONOREX SUPER RK 510 H). In the preliminary phase of the study, either 10µl of the sonicate was applied to different agar plates to detect microbial growth (see below). Alternatively, the sonicated tubes were pre-incubated for 24h at 35°C in aerobic conditions and then plated in the same way. As it became clear from the first plating's that only preincubated broth tubes showed significant bacterial growth, only this method of processing was chosen for all further samples.

Sample Cultivation and Identification of Microorganisms:

Turbidity of the broths after 24h of incubation indicated microbial growth. Subsequently, the samples were homogenized by vortexing and each 10µl applied to a universal agar (tryptic soy agar enriched with 5% sheep blood, Biomérieux) and selective chromogen agars concerning the detection of Meticillin-Resistant *Staphylococcus Aureus* (MRSA) (Brilliance® MRSA 2, Thermo Scientific), Vancomycin-resis-

tant enterococci (VRE) (chromID® VRE, Biomérieux), and Extended-Spectrum Betalactamase-producing bacteria (ESBL) (Brilliance® ESBL, Thermo Scientific). Cultivation of the plates was performed for a maximum of 48 h at 35°C under aerobic conditions including an initial assessment for microbial growth after 24h in a proven manner. In the case of microbial growth, microorganisms were identified using the Maldi-TOF mass spectrometry (matrix assisted laser desorption ionization-time of flight, VITEK MS®, Biomérieux) method. Testing of antimicrobial susceptibility interpretation of results was carried out using the Vitek 2 XL® automated antimicrobial susceptibility testing system (Biomérieux) in accordance with the current EUCAST recommendations (European Committee for Antimicrobial Susceptibility Testing). Ten unused pens (ten each in a cardboard box from the hospital's warehouse) from the group with a rubber or plastic grip acted as negative controls and were treated in the same way as the test pens.

Results

Preliminary Experiments and Impact of Pre-Incubation of the Sonicates

In preliminary tests on 48 samples, pre-incubating sonicates for 24 hours significantly increased pathogen detection compared to immediate plating. Without pre-incubation, bacterial growth was found in 27 samples (56.3%) with 10-90 CFU/ml, while pre-incubation led to growth in 47 samples (97.9%). This difference was particularly evident in detecting *Staphylococcus spp.* (including *S. aureus*), *Enterococcus spp.*, and gram-negative bacteria, which were undetectable without pre-incubation. Given the substantial impact of this method on microorganism detection, pre-incubation was implemented for all subsequent pen samples to ensure more comprehensive bacterial recovery and accurate analysis of microbial contamination.

Microbiologic Growth

Overall, 99% of the pens (n=198) exhibited bacterial growth, with no diagnostic focus on yeasts (no corresponding enrichment or agar plates). The two pens without growth were non-personalized, publicly accessible, from the "physicians' room" and "patient registration." There was no significant difference concerning microbiological growth between shared (98%) and private pens (100%). Polymicrobial growth occurred in 70% of pens (n=140) (Figure 1): 40.5% had two bacteria, 23% had three, 4.5% had four, and 2% had five different microorganisms. Monomicrobial growth was found on 29% (n=58) of pens. One negative control pen, featuring a rubber handle, displayed positive bacterial growth with *Bacillus spp.* detected.

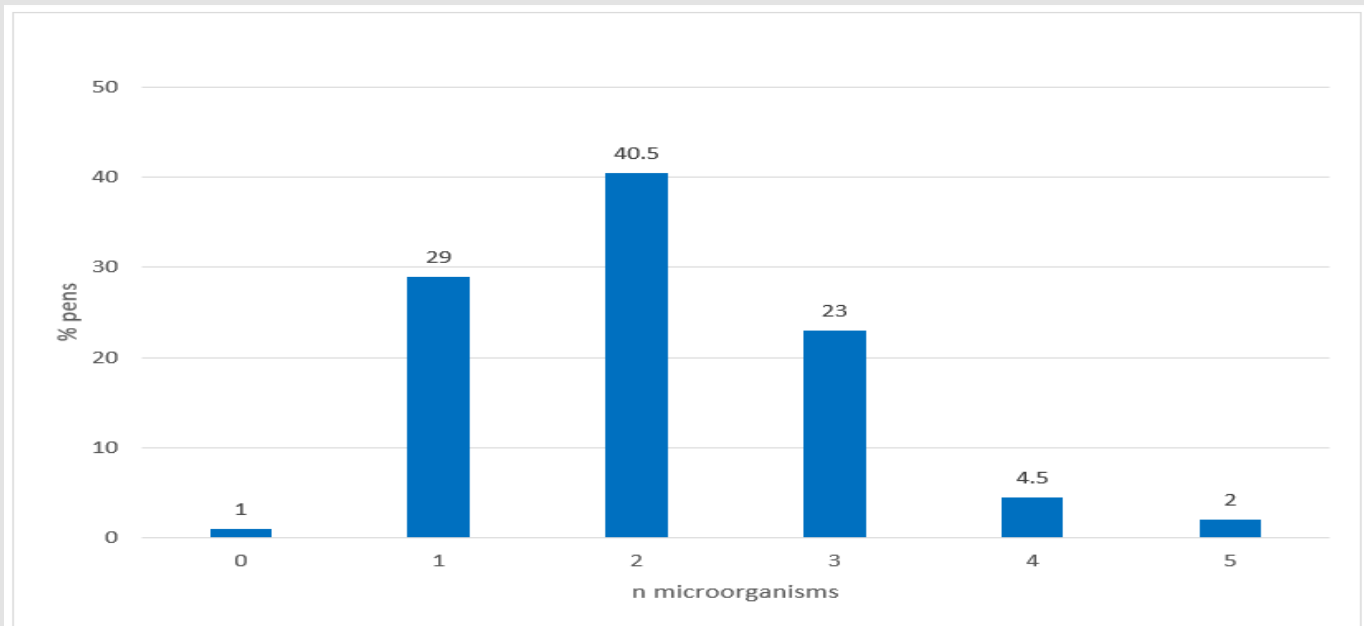


Figure 1: Amount of microorganisms for each pen.

Detected Microorganisms

A total of 417 microorganisms were detected in 200 pens examined, with gram-positive bacteria making up the vast majority at 96.2% (n=401), while gram-negative bacteria accounted for 3.8% (n=16) of the pathogens. Overall, gram-positive bacteria were detected on 198 pens (99%) with coagulase-negative staphylococci representing the largest bacterial entity. They were detected on 152 pens (76%), closely followed by *Bacillus spp.* which were detected on 141 pens (70.5%). The gram-positive microorganisms were subsequently divided into *Enterococcus spp.* (19%, n=38 pens), viridans streptococci (17.5%, n=35 pens), *Micrococcus spp.* (1.5%, n=3 pens) and *Streptococcus agalactiae* (1%, n=2 pens). *S. aureus*, the most pathogenic gram-positive microorganism here, was detected on 29 ballpoint

pens (14.5%), with one isolate being a Meticillin-resistant *S. aureus* (MRSA), which corresponds to an MRSA rate of 3.5%. This strain was found on a physician's personal pen in the intensive care unit (rubber grip). Gram-negative bacteria were detected on 13 pens (6.5%), and none of them was declared multiresistant. Among the gram-negative bacteria, *Pseudomonas spp.* was the largest entity with detection on six pens (1.4%) (all non-*P. aeruginosa* isolates), followed by *Pantoea agglomerans* (n=4), *Klebsiella oxytoca* (n=3) and *Enterobacter cloacae* complex, *Moraxella osloensis* and *Paracoccus yeei* (n=1 each), which together accounted for 2.4% of all microorganisms. Multi-resistant gram-negative bacteria and fungi were not detected in this study.

(Figure 2) provides a detailed overview of all microorganisms detected.

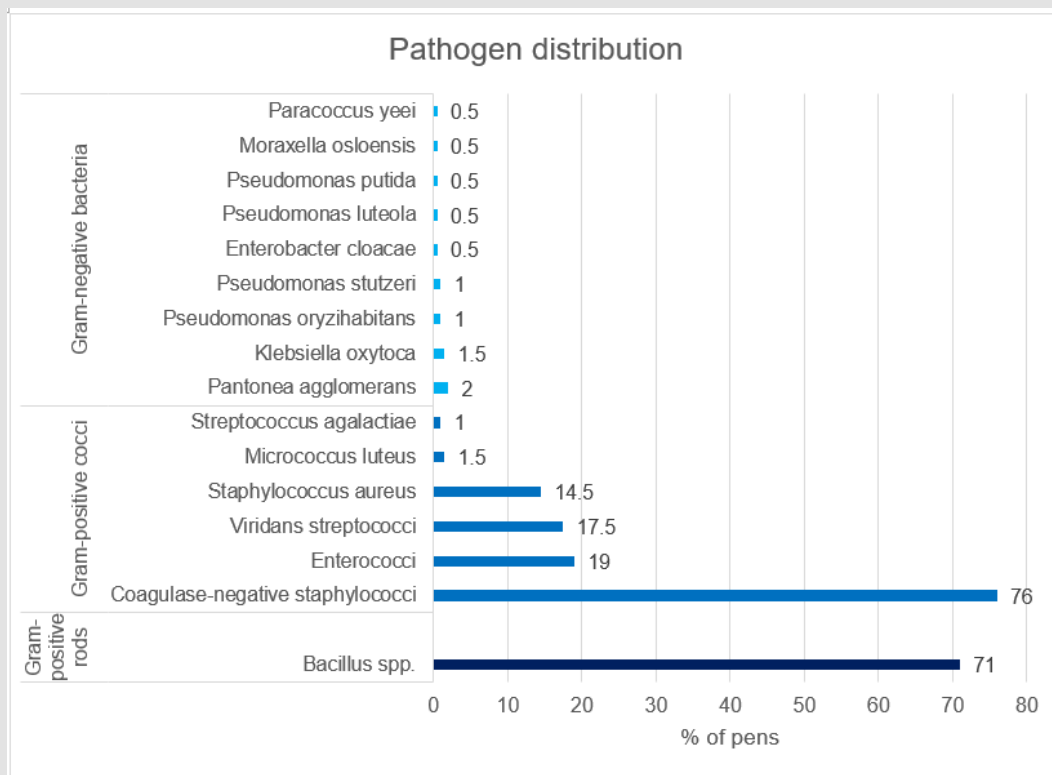


Figure 2: Detected microorganisms on hospital pens.

Colonization Rates of Pens

No significant differences were found between publicly available and personalized pens in terms of total microbial growth: 98% and 100% of the pens were colonized. An almost equal colonization rate

of 98% and 100% of the pens was also shown between the types of grips. This pattern was also seen in the subgroups of the origin of the pens (Table I). There were also no significant differences in colonization among the microorganisms of interest.

Table 1: Bacterial growth by pen category

Category/ origin of pens		Bacterial growth	
	n total	n	% of category
Total	200	198	99
Shared use	100	98	49
Private use	100	100	50
Plastic grip	100	98	49
Rubber grip	100	100	50
Patient registration			
Total	40	39	97.5
Shared use	20	19	47.5
Private use	20	20	50
Plastic grip	20	19	47.5
Rubber grip	20	20	50

Examination room			
Total	40	40	100
Shared use	20	20	50
Private use	20	20	50
Plastic grip	20	20	50
Rubber grip	20	20	50
Physician's room			
Total	40	39	97.5
Shared use	20	19	47.5
Private use	20	20	50
Plastic grip	20	19	47.5
Rubber grip	20	20	50
Nursing station			
Total	40	40	100
Shared use	20	20	50
Private use	20	20	50
Plastic grip	20	20	50
Rubber grip	20	20	50
Intensive care unit			
Total	40	40	100
Shared use	20	20	50
Private use	20	20	50
Plastic grip	20	20	50
Rubber grip	20	20	50
Control group			
Total	20	1	5
Plastic grip	10	0	0
Rubber grip	10	1	10

Microorganisms of Interest

Almost all pens in our study were colonized, primarily by bacteria from the physiological skin flora. Consequently, we focused our analyses on pens with detected *S. aureus* or gram-negative bacteria due to their potential pathogenicity in the hospital environment.

Staphylococcus Aureus

In total, *S. aureus* was detected on 29 pens (14.5%). Here, 13 of the isolates (45%) originated from pens with plastic handles and 16 isolates (55%) from those with rubber handles – the latter including the only MRSA isolate. The pens associated with *S. aureus* primarily originated from patient administration and physicians' rooms (62%), with only 10% from the nursing ward, 21% from the examination room, and 7% from the ICU (Figure 3). Of the pens, 55% were personalized and 45% were publicly used. In terms of user profession, 50%

of *S. aureus*-associated pens belonged to physicians, while 25% were used by nurses and admission clerks each (Figure 4).

Gram-Negative Bacteria

A total of 13 pens (6.5%) were colonized with gram-negative bacteria, with 46% having rubber grips and 54% having plastic grips. Rubber-handled pens showed higher levels of *Pseudomonas spp.* and *Pantoea spp.*, while plastic pens contained *Enterobacteriales*, including *Klebsiella oxytoca* and *Enterobacter cloacae*-complex (data not shown). A distribution pattern comparable to that of *S. aureus* could also be demonstrated here: Most pens came from the patient administration (38%) and physicians' rooms (31%), with fewer from the examination room (23%) and ICU (8%). No pens were from the nursing station (Figure 3). For personalized pens, 62.5% were linked to physicians, while one pen (12.5%) belonged to a nurse and two (25%) to admission clerks (Figure 4).

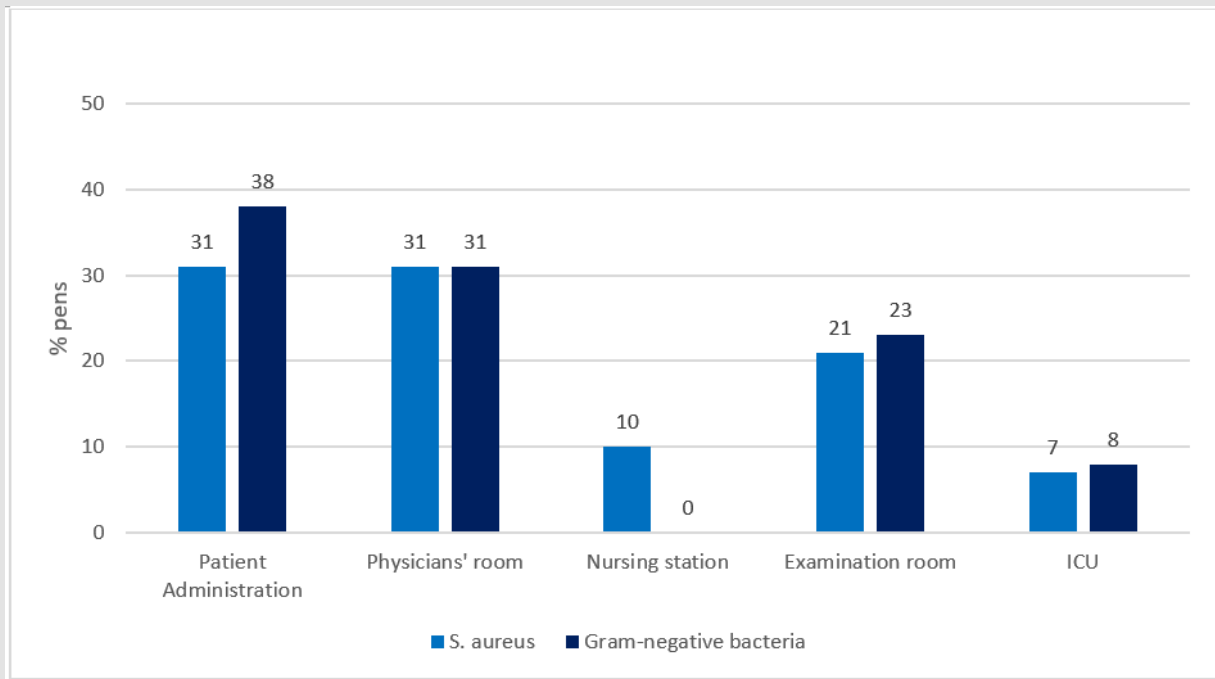


Figure 3: Allocation of pens colonized with bacteria of interest.

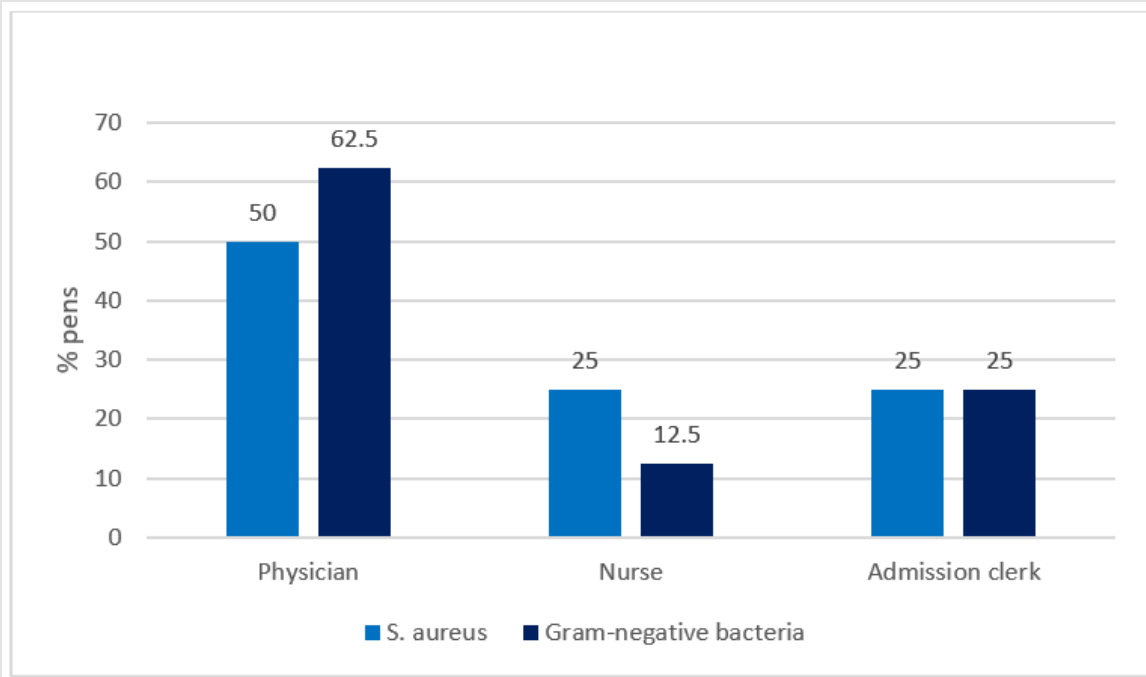


Figure 4: Assignment of personalized pens by profession.

Discussion

Environmental hygiene in hospitals plays a central role in the prevention of hospital-acquired infections. It includes technical and personal hygiene as well as employee-related aspects such as hand disinfection [2]. It is essential to identify the weaknesses in one's own system in order to implement consistent improvements, provide consistent training and take appropriate countermeasures. Using a simple method of analysing the bacterial colonisation of pens from different professions, we were able to show that physicians and admission clerks are a neglected staff group with regard to hygienic teaching algorithms and surveillance. Our study builds on the research by Zhang, et al. [7] on contamination in healthcare pens, but introduces important variations. We used sonication to detect bacteria in potential biofilm, followed by pre-cultivation of the sonicate for 24 h to improve bacterial recovery. The use of selective and universal agar plates allowed us to identify a wider range of bacteria.

Detected Microorganisms

Colonization with gram-positive and gram-negative bacteria was found in 99 % of the pens. Polymicrobial growth predominated (70 % of the pens) and we focused on *S. aureus* and gram-negative bacilli as microorganisms of interest. *S. aureus* was detected in 14.5% of cases, and gram-negative bacilli in 6.5% of cases. In contrast to the group of Zhang, et al. [7] who had detected numerous MDR microorganisms on the pens (with MRSA, VRE and MDR-*Acinetobacter baumannii* as dominant bacteria in 41%, 25%, and 23% of cases respectively) [7], only one MRSA strain could be detected in our investigation - although we used both selective and non-selective culture media in order to achieve a broad pathogen detection. One explanation could lie in the fundamentally completely different prevalence of MDR pathogens between Austria and China, with an Austrian MRSA rate of 6.6% in 2021 [8] compared to that from China with 50-70% among *S. aureus* isolates in some regions [9]. The situation is similar with *A. baumannii*, which plays a subordinate role in Austria with 87 documented invasive isolates in 2021, while this pathogen is responsible for a high number of infection-associated deaths in China [10]. Furthermore, we did not detect any differences regarding in the surface of the pens.

Identifying Neglected Staff Groups

S. aureus and gram-negative bacilli were defined as the microorganisms of greatest relevance with pens of physicians and admission clerks showing the most frequent colonisations. Regarding colonisation with *S. aureus*, the majority of pens (62%) originated from the patient administration area or a physician's room (31% each). Taking into account the personalised pens located with the physicians, a corresponding picture emerged, showing that 50% of the pens colonised with *S. aureus* originated from this staff group; 25% each came from the pens of either a nurse or an admission clerk. A similar pattern was observed for pens colonized with gram-negative bacteria. The

majority of these pens (69.5%) came from either the patient administration area (38%) or the physicians' rooms (31%). With regard to personalized pens, those assigned to physicians were again the most relevant subgroup (62.5%) followed by the group of admission clerks (25%, n=2). However, only a single pen assigned to the nurses' group (12.5%) showed such colonisation. In this study, writing utensils from different professions and hospital areas were intentionally examined as an element of environmental hygiene. Although it is not possible to draw direct conclusions about the hand hygiene of the individuals involved, the results of the study can be linked to known phenomena regarding hygiene compliance among different staff groups, with physicians consistently being the least compliant with hygiene guidelines [11].

According to van Dijk et al. [12] this appears to be even more pronounced in teaching hospitals, which should be setting the standard [12]. However, administration staff could be revealed in the present study as a previously unnoticed staff group in our hospital concerning hygienic teaching and algorithms. This enabled us to identify a significant gap in the hygiene training provided at our clinic. While nursing staff receive 30 hours of hygiene training and regular refresher courses, physicians and admissions personnel receive only minimal hygiene training: physicians receive a four hours lecture during their medical studies, whereas admissions staff receive only brief initial training. Furthermore, admission clerks are not restricted in their use of nail varnish, nail extensions or finger rings, as is the case for other clinical employees with patient contact. The hospital hygiene team addressed the issue of neglected staff training to the hospital management, leading to the introduction of a hygiene session for all new employees on Welcome Day, including e-learning. Targeted hygiene training for admission clerks with patient contact is also being developed. Nudging interventions are planned to be tested as it is known that these contextualisations, which are sometimes low-threshold and feasible, can lead to increased compliance with hygiene recommendations [13,14].

Conclusion

This study used a low-tech approach to identify clinically relevant microorganisms on pens, particularly those of physicians and admissions staff. It highlights the neglect of staff in hospital hygiene protocols and may encourage other hygiene teams to critically evaluate established practices and identify weaknesses using simple methods for effective countermeasures.

Conflict of Interest Statement

None declared.

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