HOSPITAL HYGIENE

Persistence of Biofilm-Forming and Multidrug-Resistant *Staphylococcus aureus* on High-Touch Hospital Surfaces Despite Routine Cleaning and Disinfection

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Keywords

Hospital surface contamination • *Staphylococcus aureus* • Antimicrobial resistance • Biofilm

Summary

Background. *Contaminated hospital surfaces play a key role in the transmission of healthcare-associated infections (HAIs), particularly those caused by antimicrobial-resistant pathogens. Despite routine cleaning and disinfection, high-touch surfaces may remain reservoirs for multidrug-resistant organisms, including biofilm-forming Staphylococcus aureus.*
Methods. *An environmental surveillance study was conducted in a single-bed room of an Internal Medicine ward in a hospital in Northern Italy. High-touch surfaces in the near-patient area and room furniture were sampled twice daily over one week, before and after routine cleaning/disinfection with chlorine-based agents (0.1-0.5% Cl). Cleaning effectiveness was evaluated using aerobic colony count (ACC) and detection of S. aureus as indicators of environmental hygiene, applying accepted microbiological benchmarks (ACC < 5 CFU/cm²; S. aureus < 1 CFU/cm²). S. aureus isolates were characterized by PFGE, spa typing, antimicrobial susceptibility testing, and biofilm production assays.*
Results. *Mean ACC decreased significantly after cleaning/disinfection (10.06 ± 15.67 vs 2.89 ± 5.52 CFU/cm²; p < 0.001), with a substantial reduction in non-compliant samples. However, residual contamination persisted on high-touch surfaces. S. aureus was detected in 12/238 samples, including post-cleaning samples from the near-patient area. Molecular analysis identified four distinct strains; notably, a spa type t032 (MLST ST22) isolate−methicillin-resistant, multidrug-resistant, and a strong biofilm producer−persisted on the bedside table handle both before and after cleaning.*
Conclusion. *Routine cleaning and disinfection significantly reduce environmental bioburden but may not reliably eliminate biofilm-forming multidrug-resistant S. aureus from critical hand-contact surfaces. These findings highlight the need for continuous microbiological surveillance and targeted IPC interventions to address environmental reservoirs of antimicrobial resistance in healthcare settings.*

Introduction

Healthcare-associated infections (HAIs) represent one of the major challenges in healthcare settings.
In the most recent report on hospital-acquired healthcare-associated infections (HAIs), referring to the period 2022-2023, the European Centre for Disease Prevention and Control (ECDC) estimated that approximately 4.3 million cases of HAIs occurred across the European Union and European Economic Area (EU/EEA), corresponding to a prevalence rate of 7.1% [1].
These infections cause significant patient morbidity and can prolong hospital stays, leading to substantial additional costs beyond those associated with the patient's primary condition [2, 3].
Furthermore, bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), carbapenem-resistant *Enterobacteriaceae*, and *Clostridioides difficile* are increasingly resistant to antibiotics, making infection prevention even more crucial today [4-6].

The hospital environment has been shown to serve as a reservoir for microorganisms responsible for infections [7-10]. It is also well-documented how easily pathogens can be transferred from colonized and/or infected patients to surfaces within the patient's room and from these surfaces to the hands of healthcare workers [11]. Nosocomial pathogens can persist on surfaces in hospital environments – such as patient rooms – for days, months, or even years, increasing the risk of transmission to susceptible individuals [12]. MRSA strains have been detected on 1-27% of sampled surfaces in patient rooms, with this figure rising to 64% in burn units and in the presence of MRSA-positive patients.
MRSA strains can remain viable for over 14 days on furniture surfaces and over 6-9 weeks on cotton fabrics. Additionally, it has been shown that pathogens, including some *S. aureus* strains, are able to form biofilms on surfaces. This enables them to proliferate, exchange antibiotic resistance genes with other bacteria, and persist for extended periods within the protective biofilm matrix.

In addition to hand hygiene, surface cleaning/disinfection are critical to reducing the transmission of pathogenic microorganisms and the risk of nosocomial infections [13, 14].

CDC guidelines for routine and terminal cleaning of hospital rooms emphasize the importance of disinfecting so-called "high-touch surfaces", those more likely to harbor and transmit microbial pathogens [15]. While conventional hospital environments such as restrooms, shared surfaces, and sinks are subject to frequent cleaning, high-touch surfaces are often inadequately cleaned despite their well-recognized role in cross-contamination [16-20]. The CDC recommends disinfecting high-touch surfaces in patient rooms at least once a day, while the cleaning of low-touch surfaces should follow hospital schedules and depend on the visibility of dirt [15]. Available studies suggest that daily disinfection of high-touch surfaces with detergents and/or disinfectants significantly reduces environmental contamination and the incidence of healthcare-associated infections [21]. This risk-based approach is essential to determine the frequency and method of cleaning across all patient care areas, thereby reducing the risk of infection transmission [22, 23].

Today, there are alternatives to traditional microbiology, such as ATP bioluminescence testing, which, although not a substitute for microbiological culture methods, is a useful tool for assessing cleaning effectiveness [24].

With regard to microbiological techniques for evaluating cleaning/disinfection efficacy, the scientific literature has proposed two complementary indicators: "the identification of an indicator organism of potential high-risk to patients in any amount, and second, the quantitative assessment of organisms found within a specified area, regardless of identity" [25]. So, in this study, *S. aureus* (<1 CFU/cm²) was selected as a marker of potential high risk to patients and second, the quantitative assessment of microbial load on hand-contact sites, regardless of the identity of the isolates, using the aerobic colony count (ACC). In this case, ACC <5 CFU/cm² is considered acceptable, whereas the finding of ≥5 CFU/cm² may indicate an increased risk of infection in that environment [25, 26].

As part of a surveillance activity assessing the implementation of Infection Prevention and Control (IPC) measures in a hospital in Northern Italy, a study was conducted to evaluate the effectiveness of cleaning/disinfection procedures targeting high-touch surfaces in an internal medicine ward. The evaluation was based on both the ACC and the detection of *S. aureus*.

A further objective, in case of *S. aureus* detection, was to assess the presence of clinically and epidemiologically relevant strains and to characterize them through biomolecular analyses.

Methods

Setting

The study was conducted in a single-bed inpatient room in the Internal Medicine department of a hospital in Northern Italy. During the study period, the room consecutively hosted alert, oriented, and ambulatory patients.

Sampling was carried out in a single-bed inpatient room; the patients were not found to be colonized by *S. aureus* in order to avoid confounding variables in the assessment of surface colonization.

During the sampling period, both healthcare personnel and cleaning staff were informed of the assessment being conducted.

Environmental disinfection

In the examined facility, after cleaning, daily environmental disinfection was performed on all room surfaces using chlorine-based disinfectants (0.1-0.5% Cl) and color-coded microfiber cloths, according to contamination levels and surface type.

Outsourced personnel were responsible for performing cleaning and disinfection activities. Daily disinfection was performed twice a day. Extraordinary cleaning was carried out immediately in the event of accidental contamination with biological fluids or other hazardous materials. Terminal cleaning was performed upon patient discharge or transfer. Each cloth was replaced after the disinfection of each patient unit and was not reused across different patient units.

The disinfectant was left on the surface for the recommended contact time of 5 minutes before drying or reuse.

Surface Sampling and Microbiological Analysis

High-touch surfaces were identified within the patient area, including furniture in the room, selected from those listed in the CDC checklist [27].

For the patient area, surfaces sampled in the vicinity of the patient included the bedside table, call button, headboard, footboard, and bed rails. Furniture surfaces sampled in the room included the table and chair. The surface of the bedside table was covered with non-porous plastic material, while the various components of the bed (footboard, headboard, and side rails) were made of metal and partially covered with non-porous plastic material, the same applied to the furniture. All surfaces were undamaged.

Sampling was performed twice a day, before and after sanitization, over the course of one week.

Where surface type permitted, a 10×10 cm area was sampled using a delimiting template; for irregular surfaces, the available area was sampled, preferably approximating 100 cm², and the sampled dimensions were recorded accordingly. Microbial concentration was expressed as CFU/cm².

Sampling was performed using swabs with internal shafts, rayon tips, and 10 mL of isotonic saline solution containing neutralizing agents to inactivate disinfectants and sanitizing agents.

Microbiological Analysis

Samples were immediately transported to the laboratory and processed to determine the ACC at 37°C and the

presence of *S. aureus* as an indicator of human-derived contamination.
Prior to inoculation, swabs were vortexed for approximately 1 minute to facilitate the detachment of microorganisms from the swab surface.

Determination of ACC at 37°C

To determine the aerobic colony count at 37°C, 1 mL of the solution contained in the swab vial was inoculated onto Nutrient Agar plates (ISO 6579, Liofilchem, Italy). The inoculum was spread over the plate using a sterile L-shaped rod to allow uniform distribution and facilitate colony counting after incubation.
Plates were incubated at 37°C for 48 hours. The final concentration in CFU/cm² was calculated considering both the inoculated volume and the sampled surface area.

Determination of *Staphylococcus aureus*

To detect *S. aureus*, duplicate 1 mL aliquots of the swab solution were plated onto blood agar and incubated at 37°C for 48 hours.
Suspected colonies grown on blood agar were subcultured onto selective Mannitol Salt Agar and subsequently identified using MALDI-TOF (Biomerieux, Marcy-l'Étoile, France).
The final concentration in CFU/cm² was calculated by considering the inoculated volume and the sampled surface area.
Two standard microbiological thresholds were used to assess cleaning effectiveness, as proposed by Dancer: <5 CFU/cm² regardless of species, and < 1 CFU/cm² for *S. aureus* [26].

Molecular Analysis via PFGE – *Staphylococcus aureus*

PFGE analysis of *S. aureus* was performed following the protocol described in "Pulsed-Field Gel Electrophoresis of *Staphylococcus aureus*" by Golding et al. [28].
Images were captured using a Gel Doc XR and analyzed using Image Lab Software and GelJ 2.2 software.

Molecular Analysis via spa Typing – *Staphylococcus aureus*

The procedure involved the preparation of pure *S. aureus* cultures on TSA (Trypticase Soy Agar II with 5% sheep blood), incubated for 16-18 h at 37°C.
Bacterial DNA was extracted by lysing cells with lysostaphin using the QIAGEN QIAamp DNA Mini Kit. DNA amplification was performed following the protocol described by Shopsin et al [29] and by Harmsen et al [30].
After purification of the amplified using the High Pure Product Purification Kit (ROCHE), the next step involved sequencing (Sanger – GATC Eurofins Genomics).
The analysis of the DNA sequence electropherograms, conducted using the FincTV Software, and the comparison with sequence repeats available in the RIDOM Spa server database (http://spaserver.ridom.de/) allowed the identification of the SPA Typing corresponding to each profile previously determined by PFGE technique.
Using the RIDOM Spa server database, it was also possible to assign a correspondence between the identified SPA Typing profiles and an MLST profile.

Evaluation of antibiotic resistance of *Staphylococcus aureus*

The identified colonies were tested for antibiotic resistance using the VITEK 2 Compact system (Biomerieux), with a library updated according to the most recent EUCAST breakpoint criteria.

Evaluation of biofilm formation

S. aureus isolates (PFGE profiles A, B, C, D) were cultured using a protocol designed to induce biofilm production in biofilm-forming strains, following the method described by Cramton [31].
Briefly, the isolates were first grown on blood agar plates for 24 hours. Subsequently, 1-2 colonies from each strain were inoculated into 2 mL of Tryptic Soy Broth (TSB) and incubated overnight at 37°C.
After incubation, the bacterial suspension was adjusted to a 1.0 McFarland standard, and after diluted 1:100 in TSB supplemented with 0.5% glucose.
The diluted bacterial suspension was then inoculated into sterile 96-well microtiter plates and incubated at 37°C for 24 hours, together with the negative control, in this case the control wells contained sterile broth.
Following incubation, the contents of each well were removed, and the plates were washed with Phosphate-Buffered Saline (PBS, pH 7.3). The wells were stained with 1% crystal violet, rinsed with water, air-dried, and the stained biofilm was then solubilized with 20% ethanol to detect the biofilm adhered to the bottom of the wells.
Each experiment was performed in independent replicates for each isolate to ensure the reliability and representativeness of the experimental data.
Biofilm density was classified according to the scheme of Stepanovic et al [32]. The cut-off value (ODc) for each microtiter plate was defined as three standard deviations above the mean OD of the negative control. Isolates were then classified into four categories based on ODc and average OD of the strain: strong biofilm producer (4ODc ≤OD); moderate biofilm producer (2ODc ≤OD ≤4ODc); weak biofilm producer (ODc ≤OD ≤2ODc); and non-biofilm producer (OD ≤ODc). For the biofilm formation assay, four wells per strain were used, and each test was repeated three times.
Biofilm growth was evaluated by measuring the optical density (OD) at 570 nm (EPOH, EPOCH Agilent BioTek, Santa Clara CA, USA)

Statistical analysis

Statistical analysis was carried out using STATA SE 19™ (StataCorp, College Station, TX, USA). Results were analysed in terms of descriptive statistics, expressed as means ± standard deviations for continuous variables, and as frequencies (percentages) for categorical variables.
To evaluate the effect of the intervention on contamination

rates, a 2×2 contingency table was constructed, and Fisher's exact test was used due to the small sample size, while comparisons between pre- and post-intervention groups were performed using the Wilcoxon signed-rank test for paired samples. A p-value < 0.05 was considered statistically significant.

Results

Aerobic colony count (ACC)

The mean values of ACC detected on all sampled surfaces before (phase 1) and after the cleaning/disinfection procedure (phase 2) were 10.06 ± 15.67 CFU/cm² and 2.89 ± 5.52 CFU/cm², respectively, showing a statistically significant reduction ($p < 0.001$).
An analysis of the ACC was then carried out based on the different areas in the two phases (1 and 2).
It was found that the ACC in the near patient area and furniture decreased from 10.41 ± 13.53 CFU/cm² and 10.86 ± 21.04 CFU/cm² (phase 1) to 2.88 ± 6.50 CFU/cm² and 1.96 ± 4.22 CFU/cm² (phase 2), respectively.
The difference in the mean ACC values across areas (near patient, furniture) between phases 1 and 2 was always statistically significant ($p < 0.001$).
However, considering the wide range of ACC values observed for each area, the percentage of non-compliant samples results (≥ 5 CFU/cm²) before and after cleaning/disinfection was also evaluated.
The results showed how the cleaning/disinfection procedure reduced the percentage of non-compliant results samples (CFU ≥ 5 CFU/cm²), dropping from 51.65% to 12.09%, 39.29% to 3.57%, for the near patient area and furniture respectively.
The reduction in non-compliance results was statistically significant both for all sampled surfaces and when grouped by area ($p < 0.01$).
Nevertheless, results showed that cleaning/disinfection never completely eliminated non-compliance results.

Staphylococcus aureus

A total of 12/238 samples tested positive for *S. aureus* (>1 CFU/cm²), of which 9 were detected in phase 1 (corresponding to 7.56% of the samples) and 3 in phase 2 (corresponding to 2.52% of the samples).
Cleaning/disinfection eliminated the microorganism from all surfaces except those in the patient area.

Biomolecular Analysis Results of *Staphylococcus aureus* Strains

A. Molecular Typing by PFGE

PFGE analysis identified a total of four different banding patterns. Figure 1 shows the PFGE similarity analysis for the samples examined.
Based on the profiles highlighted by the PFGE analysis, the samples were classified as follows:

- Samples 1, 2, 3, 5, 6, 7 and sample 11 were assigned to profile A;
- Samples 8, 9, and 10 were assigned to profile B;
- Sample 4 was assigned to profile C;
- Sample 12 was assigned to profile D.

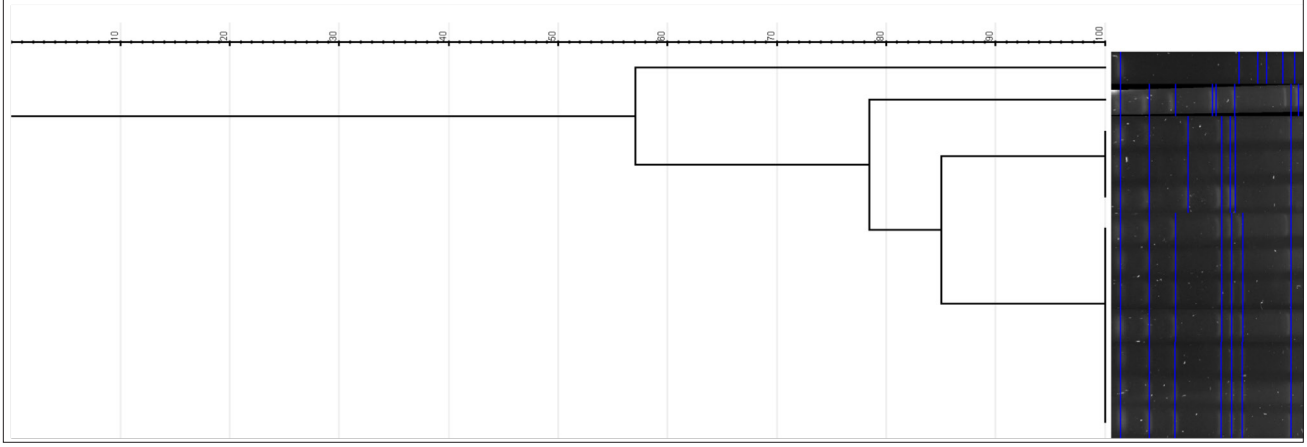
B. Molecular genotyping by SPA typing

For the four *S. aureus* profiles (A, B, C, and D) identified by PFGE, a further molecular genotyping analysis was performed using the SPA typing technique [29, 30].
The analysis of the DNA sequence electropherograms, conducted using FinchTV Software and comparison with the repeat sequences available in the RIDOM Spa server database (http://spaserver.ridom.de/), allowed determination of the SPA type for each profile previously identified by PFGE.
Using the RIDOM Spa server database, it was also possible to assign three of the identified SPA typing profiles to corresponding MLST profiles.
Table I reports, for each PFGE profile, the corresponding ST profile (Spa Typing with the DNA repeat code sequence) and, when available, the corresponding MLST profile.

Antibiotic Resistance Assessment

Table II reports the antibiograms of the *S. aureus* isolates,

Fig. 1. PFGE similarity analysis for the samples analyzed.



Tab. I. PFGE profile, ST profile (Spa Typing with DNA repeat code sequence), and MLST profile (when available) of Staphylococcus aureus isolates.

PFGE Profile	SPA Typing Repeat Sequence	SPA-Typing ST	MLST
A	26-23-17-34-17-20-17-12-17-16	t002	ST5; ST231
B	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	t032	ST22
C	04-20-12-17-17	t1312	-
D	26-23-17-34-17-20-17-12-17-16	t002	ST5; ST231

Tab. II. Evaluation of the antibiotic susceptibility profile of *Staphylococcus aureus* strains.

Antibiotics	A	b	c	D
Linezolid	S	S	S	S
Daptomycin	S	S	S	S
Teicoplanin	S	S	S	S
Vancomycin	S	S	S	S
Levofloxacin	I	**R**	I	I
Polymyxin	S	S	S	S
Tetracycline	S	S	S	S
Tigecycline	S	S	S	S
Gentamicin	S	S	S	S
Fusidic acid	S	S	S	S
Rifampicin	S	S	S	S
Trimethoprim / sulfamethoxazole	S	S	S	S
Erythromycin	S	**R**	S	S
Clindamycin	S	**R**	**R**	S
Cefoxitin	S	**R**	S	S
Benzylpenicillin	**R**	**R**	R	S
Oxacillin	S	**R**	S	S
Methicillin	S	**R**	S	S

S: Sensitive; I: Intermediate; R: Resistant.

indicating the susceptibility levels (sensitive, resistant, or intermediate) to the different antibiotics tested.

The analyses showed that strain D is sensitive to all tested antibiotics and intermediate to Levofloxacin; strain A is resistant to Benzylpenicillin and intermediate to Levofloxacin; strain C exhibits resistance to Benzylpenicillin and Clindamycin and is intermediate to Levofloxacin.

Strain B, on the other hand, shows resistance to seven antibiotics, including methicillin.

Biofilm production

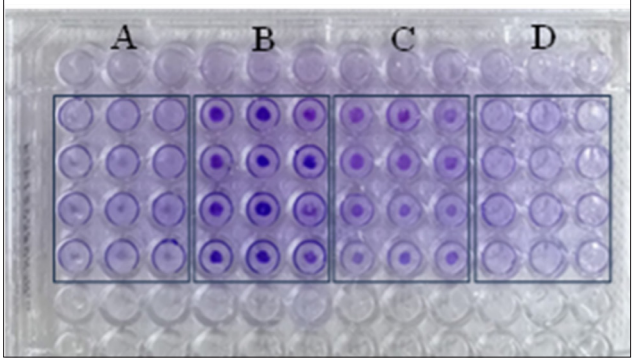
Biofilm growth assays revealed different mean absorbance values for each isolate, indicative of varying biofilm-forming capacities (Fig. 2).

Based on the tests performed, it emerged that the *S. aureus* isolates belonging to strains A, B, and C are strong biofilm producers; notably, strain B exhibited a biofilm production capacity more than twice that of strains A and C. Conversely, strain D was classified as a moderate biofilm producer.

Isolates belonging to strain B were identified in the near patient area, specifically on the bedside table handle, both before and after cleaning/disinfection.

Colonies of strains A, C and D were isolated only on furniture surfaces prior to cleaning/disinfection.

Fig. 2. Strains A, B, C, D and biofilms.



Discussion

Many studies have demonstrated the ease with which pathogens can be transferred from colonized and/or infected patients to surfaces within the patient's room and/or from surfaces to healthcare workers' hands.

Continuous evaluation and monitoring of cleaning/disinfection interventions to reduce the risk of transmission of environmental pathogens through defined procedures have been key elements of infection prevention and control practices in acute care hospitals for years [33].

The results of the present study showed that although

cleaning/disinfection procedures are effective in reducing microbial contamination, they do not always fully restore acceptable conditions, both regarding ACC and *S. aureus*, only partially reducing the risk of pathogen cross-transmission. In this study, microbiological identification was limited to *S. aureus*, as it is considered by the scientific literature to be a valid indicator of infection risk [25, 26]. Secondly, the study was conducted when there were no active infectious outbreaks or evidence of an increase in the prevalence of HAI that would require the research to be extended to other microorganisms. Moreover, this last assessment would have required the expenditure of economic resources, time, and personnel.

Regarding *S. aureus*, the results highlighted the persistence of a strain characterized as SPA Typing t032 MLST ST22, resistant to five classes of antibiotics, including methicillin. This strain, found in the near patient area (bedside table handle), proved to be a strong biofilm producer, more so than the other three strains, a characteristic that likely allowed it to survive the action of sanitizing agents.

Since no swabs were performed on the patients who occupied the beds, it was not possible to verify their colonization status or hypothesize the source of the strains found on the surfaces.

Spa Type t032 is widely distributed in Europe and is considered prevalent alongside two other spa types, t002 (also identified in this study) and t008. The scientific literature describes it as exclusively associated with MRSA and tightly linked to ST22. ST22, also known as the EMRSA-15 strain, is a widely spread, highly virulent clone in Europe and is significantly associated with hospital-acquired infections. It is also known for its rapid dissemination in healthcare environments [34]. In a study conducted in Portugal, ST22 accounted for 72% of nosocomial isolates and was able to replace previously circulating STs such as ST239 and ST247 [35].

According to Soliman et al. [36], ST22 possesses factors such as cna, sdrE, hlg, and ica, which confer colonization and virulence capabilities in hospital settings.

The spa type t002 ST5, belonging to clonal complex CC5, is historically associated with multidrug-resistant nosocomial strains, particularly in Asian countries and North America, although in our case the isolates were sensitive to several antibiotics (except for resistance to benzylpenicillin and intermediate sensitivity to levofloxacin).

It frequently harbors adhesion factors such as fnbA, cna, sdrE, hlg, and ica, which are associated with adhesive capacity, biofilm formation, and nosocomial virulence.

The ST5 allelic profile is very similar to that of ST231, also belonging to CC5. Both share a common evolutionary ancestry: ST231 is likely derived from ST5 via mutation or recombination in the yqiL gene [37]. They also display phenotypic similarities, as ST231 strains tend to resemble ST5 strains, particularly in their antibiotic resistance profiles, as evidenced by the findings of our study.

The persistence of MRSA (ST22) and the nonconformities in ACC detected in phase 2 underline the importance of microbiological monitoring of surfaces to promptly identify any risk conditions.

In hospitals, the presence of pathogenic microorganisms on surfaces is in itself a significant non-compliance, since even minimal quantities can pose a risk in conditions favorable to transmission.

For this reason, the results called into question the execution modalities of the cleaning/disinfection procedures. Therefore, after communicating microbiological results both to the ward and the hospital Infection Control Committee, a comprehensive improvement process was initiated through audits with cleaning staff, emphasizing the need to increase adherence to cleaning/disinfection techniques and, for all healthcare personnel, hand hygiene.

One aspect that must be taken into account with regard to hospital sanitation is that many hospitals (such as the one where the study was conducted) rely on outsourced companies, which often represent a significant operational constraint in terms of the possibility of changing the frequency, methods, and protocols of disinfection.

At the end of the improvement process, subsequent checks showed no nonconformities.

According to Dancer [26] the monitoring of microbial bioburdens on surfaces, compared to less expensive and faster methods such as ATP testing and other screening tools, should, be implemented as an integral part of environmental control protocols. While these conventional techniques may offer useful support for general assessments, they cannot replace more accurate quantitative and specific methods for the detection and identification of pathogenic microorganisms. For this reason, direct microbiological monitoring remains essential, particularly as a downstream component of a standardized control system that should consistently be in place.

Beyond confirming the efficacy of cleaning/disinfection, the collection of environmental surveillance data over time would allow predictive modeling in relation to healthcare-associated infection (HAI) rates and even enable forecasting of cross-infection episodes and epidemic outbreaks [26].

Conclusion

Improving the effectiveness of environmental disinfection in healthcare facilities must rely on a comprehensive strategy involving training and education of cleaning/disinfection and healthcare personnel, as well as implementation of verification systems to ensure the adequacy of disinfection.

A further development of this research could be the evaluation of timing and dynamics of surface contamination in patient wards to better define protocols for more effective cleaning/disinfection in reducing cross-infection.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

Conceptualization: MLC and MS; management of microbiological samples: SC and AMS; data collection: CD and SC; biomolecular analysis: GO, ES and CP; formal analysis: AC and MS; data interpretation: CD, MS, SB, ES and EP; writing–original draft preparation: MLC and GO; writing–review and editing: MLC, MS and CP; supervision: MLC.
All authors have read and agreed to the published version of the manuscript.

References

[1] European Centre for Disease Prevention and Control. Point prevalence survey of healthcareassociated infections and antimicrobial use in European acute care hospitals. Stockholm: ECDC; 2024. Available at https://www.ecdc.europa.eu/en/publications-data/PPS-HAI-AMR-acute-care-europe-2022-2023. Accessed on: 16/09/2025.

[2] Sartelli M, Bartoli S, Borghi F, Busani S, Carsetti A, Catena F, Cillara N, Coccolini F, Cortegiani A, Cortese F, Fabbri E, Foghetti D, Forfori F, Giarratano A, Labricciosa FM, Marini P, Mastroianni C, Pan A, Pasero D, Scatizzi M, Viaggi B, Moro ML. Implementation Strategies for Preventing Healthcare-Associated Infections across the Surgical Pathway: An Italian Multisociety Document. Antibiotics (Basel) 2023;12:521. https://doi.org/10.3390/antibiotics12030521.

[3] European Centre for Disease Prevention and Control. Healthcare-associated infections: surgical site infections. In: ECDC. Annual epidemiological report for 2021–2022. Stockholm: ECDC; 2025. Available at https://www.ecdc.europa.eu/en/publications-data/healthcare-associated-infections-surgical-site-infections-annual-2. Accessed on: 16/09/2025.

[4] Sticchi C, Alberti M, Artioli S, Assensi M, Baldelli I, Battistini A, Boni S, Cassola G, Castagnola E, Cattaneo M, Cenderello N, Cristina ML, De Mite AM, Fabbri P, Federa F, Giacobbe DR, La Masa D, Lorusso C, Marioni K, Masi VM, Mentore B, Montoro S, Orsi A, Raiteri D, Riente R, Samengo I, Viscoli C, Carloni R; Collaborative Group for the Point Prevalence Survey of healthcare-associated infections in Liguria. Regional point prevalence study of healthcare-associated infections and antimicrobial use in acute care hospitals in Liguria, Italy. J Hosp Infect 2018;99:8-16. https://doi.org/10.1016/j.jhin.2017.12.008.

[5] Ministero della Salute. Piano Nazionale di Contrasto dell'Antimicrobico-Resistenza (PNCAR) 2022–2025; Ministero della Salute: Roma, Italy, 2022. Available at: https://www.salute.gov.it/new/sites/default/files/imported/C_17_pubblicazioni_3294_allegato.pdf. Accessed on: 16/09/2025.

[6] Khan HA, Baig FK, Mehboob R. Nosocomial Infections: Epidemiology, Prevention, Control and Surveillance. Asian Pac J Trop Biomed 2017;7:478-82. https://doi.org/10.1016/j.apjtb.2017.01.019.

[7] Hota B. Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection? Clin Infect Dis 2004;39:1182-9. https://doi.org/10.1086/424667.

[8] Rutala WA, Weber DJ. Monitoring and improving the effectiveness of surface cleaning and disinfection. Am J Infect Control 2016;44:e69-76. https://doi.org/10.1016/j.ajic.2015.10.039.

[9] Facciolà A, Pellicanò GF, Visalli G, Paolucci IA, Venanzi Rullo E, Ceccarelli M, D'Aleo F, Di Pietro A, Squeri R, Nunnari G, La Fauci V. The role of the hospital environment in the healthcare-associated infections: a general review of the literature. Eur Rev Med Pharmacol Sci 2019;23:1266-78. https://doi.org/10.26355/eurrev_201902_17020.

[10] Weber DJ, Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, Clostridium difficile, and Acinetobacter species. Am J Infect Control 2010;38:S25-33. https://doi.org/10.1016/j.ajic.2010.04.196.

[11] Attaway HH 3rd, Fairey S, Steed LL, Salgado CD, Michels HT, Schmidt MG. Intrinsic bacterial burden associated with intensive care unit hospital beds: effects of disinfection on population recovery and mitigation of potential infection risk. Am J Infect Control 2012;40:907-12. https://doi.org/10.1016/j.ajic.2011.11.019.

[12] Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis 2006;6:130. https://doi.org/10.1186/1471-2334-6-130.

[13] Orlando P, Cristina ML, Dallera M, Ottria G, Vitale A, Badolati G. Surface disinfection: evaluation of the efficacy of a nebulization system spraying hydrogen peroxide. J Prev Med Hyg 2008;49:116-9.

[14] Donskey CJ. Does improving surface cleaning and disinfection reduce health care-associated infections? Am J Infect Control 2013;41:S12-9. https://doi.org/10.1016/j.ajic.2012.12.010.

[15] Sehulster L, Chinn RY; CDC; HICPAC. Guidelines for environmental infection control in health-care facilities. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). MMWR Recomm Rep 2003;52:1-42.

[16] Dancer SJ. The role of environmental cleaning in the control of hospital-acquired infection. J Hosp Infect 2009;73:378-85. https://doi.org/10.1016/j.jhin.2009.03.030.

[17] Dancer SJ. Hospital cleaning in the 21st century. Eur J Clin Microbiol Infect Dis 2011;30:1473-81. https://doi.org/10.1007/s10096-011-1250-x.

[18] Cobrado L, Silva-Dias A, Azevedo MM, Rodrigues AG. High-touch surfaces: microbial neighbours at hand. Eur J Clin Microbiol Infect Dis 2017;36:2053-062. https://doi.org/10.1007/s10096-017-3042-4.

[19] Huslage K, Rutala WA, Gergen MF, Sickbert-Bennett EE, Weber DJ. Microbial assessment of high-, medium-, and low-touch hospital room surfaces. Infect Control Hosp Epidemiol 2013;34:211-2. https://doi.org/10.1086/669092.

[20] WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care Is Safer Care. Geneva: World Health Organization 2009.

[21] Guh A, Carling P. Environmental Evaluation Working Group. Options for Evaluating Environmental Cleaning. CDC, 2010. Available at: https://www.cdc.gov/infection-control/media/pdfs/Toolkits-Environmental-Cleaning-Evaluation-2010-P.pdf. Accessed on: 3/12/2025.

[22] Datta R, Platt R, Yokoe DS, Huang SS. Environmental cleaning intervention and risk of acquiring multidrug-resistant organisms from prior room occupants. Arch Intern Med 2011;171:491-4. https://doi.org/10.1001/archinternmed.2011.64.

[23] Hacek DM, Ogle AM, Fisher A, Robicsek A, Peterson LR. Significant impact of terminal room cleaning with bleach on re-

ducing nosocomial Clostridium difficile. Am J Infect Control 2010;38:350-3. https://doi.org/10.1016/j.ajic.2009.11.003.

[24] Sanna T, Dallolio L, Raggi A, Mazzetti M, Lorusso G, Zanni A, Farruggia P, Leoni E. ATP bioluminescence assay for evaluating cleaning practices in operating theatres: applicability and limitations. BMC Infect Dis 2018;18:583. https://doi.org/10.1186/s12879-018-3505-y.

[25] 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. https://doi.org/10.1016/j.jhin.2003.09.017.

[26] Dancer SJ. Hospital cleaning: past, present, and future. Antimicrob Resist Infect Control 2023;12:80. https://doi.org/10.1186/s13756-023-01275-3.

[27] Centers for Disease Control and Prevention (CDC). Environmental Cleaning Checklist. Available at: https://www.cdc.gov/hai/pdfs/toolkits/environmental-cleaning-checklist-10-6-2010.pdf. Accessed on: 3/12/2025.

[28] Golding GR, Campbell J, Spreitzer D, Chui L. Pulse Field Gel Electrophoresis. In Methods and Protocols; Jordan K, Dalmasso M, Eds.; Springer: New York, NY, USA, 2015; Volume 1301, pp. 85-93.

[29] Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN. Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. J Clin Microbiol 1999;37:3556-63. https://doi.org/10.1128/JCM.37.11.3556-3563.1999.

[30] Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, Vogel U. Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol 2003;41:5442-8. https://doi.org/10.1128/JCM.41.12.5442-5448.2003.

[31] Cramton SE, Gerke C, Götz F. In vitro methods to study staphylococcal biofilm formation. Methods Enzymol 2001;336:239-55. https://doi.org/10.1016/s0076-6879(01)36593-x.

[32] Stepanović S, Vuković D, Hola V, Di Bonaventura G, Djukić S, Cirković I, Ruzicka F. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS 2007;115:891-9. https://doi.org/10.1111/j.1600-0463.2007.apm_630.x.

[33] Carling PC, Huang SS. Improving healthcare environmental cleaning and disinfection: current and evolving issues. Infect Control Hosp Epidemiol 2013;34:507-13. https://doi.org/10.1086/670222.

[34] Asadollahi P, Farahani NN, Mirzaii M, Khoramrooz SS, van Belkum A, Asadollahi K, Dadashi M, Darban-Sarokhalil D. Distribution of the Most Prevalent Spa Types among Clinical Isolates of Methicillin-Resistant and -Susceptible Staphylococcus aureus around the World: A Review. Front Microbiol 2018;9:163. https://doi.org/10.3389/fmicb.2018.00163.

[35] Espadinha D, Faria NA, Miragaia M, Lito LM, Melo-Cristino J, de Lencastre H; Médicos Sentinela Network. Médicos Sentinela Network. Extensive dissemination of methicillin-resistant Staphylococcus aureus (MRSA) between the hospital and the community in a country with a high prevalence of nosocomial MRSA. PLoS One 2013;8:e59960. https://doi.org/10.1371/journal.pone.0059960.

[36] Soliman RS, Phillips G, Whitty P, Edwards DH. Distribution of meticillin-resistant Staphylococcus aureus spa types isolated from health-care workers and patients in a Scottish university teaching hospital. J Med Microbiol 2009;58:1190-95. https://doi.org/10.1099/jmm.0.010132-0.

[37] Viana AS, Tótola LPDV, Figueiredo AMS. ST105 Lineage of MRSA: An Emerging Implication for Bloodstream Infection in the American and European Continents. Antibiotics (Basel) 2024;13:893. https://doi.org/10.3390/antibiotics13090893.

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