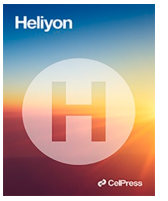
Heliyon 10 (2024) e25647

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Surface decontamination effectiveness at the "Université des Montagnes" Teaching Hospital: Monitoring in the biomedical analysis laboratory

O'Neal Dorsel Youté [a,b,*], Christelle Domngang Noche [a,b,c], Blandine Pulcherie Tamatcho Kweyang [d], Esther Guladys Kougang [a,b], Pierre René Fotsing Kwetche [a,b,c]

[a] Laboratory of Microbiology, Université des Montagnes Teaching Hospital, Bangangté-Cameroon
[b] School of Biomedical Sciences, Higher Institute of Health Sciences, Université des Montagnes, Bangangté-Cameroon
[c] School of Human Medicine, Higher Institute of Health Sciences, Université des Montagnes, Bangangté-Cameroon
[d] Laboratory of Microbiology, University of Yaoundé I, Yaoundé-Cameroon

ARTICLE INFO

Keywords:
Bacteria
Decontamination
Hospital
Surfaces

ABSTRACT

Background: Many infections in healthcare facilities are associated with the microbiological quality of the work environment, generally due to poor sanitation.
Aim: In order to evaluate the effectiveness of a decontamination protocol (cleaning + disinfection) applied at the "Université des Montagnes" Teaching Hospital, the present study assessed the variation of bacterial loads on surfaces subsequent to decontamination. Susceptibility of bacteria to disinfectants was also evaluated in the same frame.
Methodology: This work was conducted with an adjusted bacterial detection/enumeration and susceptibility test protocols and standard bacterial identification protocols. Sampling on surfaces was performed by wet swabbing before cleaning, between cleaning and disinfection and after disinfection.
Results: Major findings revealed the predominance of *Staphylococcus* (75.5%) on target surfaces. High bacterial loads recorded on these surfaces before decontamination became undetectable after cleaning with the detergent "Pax lemon". The majority of isolates (98%) were susceptible to the disinfectants tested, (Surfanios® 0.25% and sodium hypochlorite 0.12%).
Conclusion: Overall, these findings indicated process effectiveness on the subjected bacterial populations and suggest the use of either Surfanios® (0.25%) or sodium hypochlorite (0.12%) for work surfaces hygiene, justifying the use of these products in this department for surface decontamination. Also, cleaning with the detergent "Pax lemon" and disinfection with sodium hypochlorite may be sufficient for the types of surfaces subjected in the present research.

* Corresponding author. Laboratory of Microbiology, Université des Montagnes Teaching Hospital, Bangangté-Cameroon
E-mail addresses: yout.oneal2@gmail.com (O.D. Youté), dockrystlnoche@gmail.com (C. Domngang Noche), tamatcho@yahoo.com (B.P. Tamatcho Kweyang), estherkougang@gmail.com (E.G. Kougang), prfotsingk@gmail.com (P.R. Fotsing Kwetche).

https://doi.org/10.1016/j.heliyon.2024.e25647
Received 29 July 2023; Received in revised form 29 November 2023; Accepted 31 January 2024
Available online 6 February 2024

1. Introduction

Healthcare facilities are potent reservoirs for large numbers of microorganisms which can contaminate, colonized and cause infection in human expecting care giving, or providing healthcare services [1–4]. These microorganisms may be infectious agents (professional and/or opportunistic pathogens) which often express multiple drug-resistance to antimicrobials and can disseminate through stochastic means/processes connecting human hosts and the environment [4–7]. Their presence indicates risk for healthcare-associated infection (HAI) or that of the spread of hospital germs through exposed communities [5–8]. A healthcare-associated infection can be referred to as a physiological dysfunction caused by or associated with the presence of healthcare setting-acquired microorganisms. Among the infectious agents and according to common literature, most frequently met in healthcare environments are Vancomycin-resistant *Enterococcus*, *Clostridium difficile*, multidrug-resistant Gram-negative rods such as *Pseudomonas* spp., *Acinetobacter* spp. as well as methicillin-resistant *Staphylococcus aureus* and norovirus [3–7].

The microbial presence and the risk of infection acquired in a healthcare facility depend, (though not limited to), on what use the premises serve, the environment microbial flora, the transmission pathways that is often poorly understood, the germ entry pathways in the host and the host's vulnerability to infection; acknowledging that the patient's vulnerability increases with prolonged stay in healthcare setting, his age and underlying disease, to name a few. Consequently, this presence and risk are not identical everywhere in a facility and varies from one setting to the other [1,5,7]. This is the reason why in a healthcare facility, aspects like quality of patient care, general premise management policy, equipment care and hand hygiene are contextual paramount challenges for HAIs prevention. In order to address these crucial issues throughout the world, healthcare facilities design, integrate, develop and reinforce the "hospital hygiene" concept, which contributes to the prevention, control and management of infectious risk [7,8]. This concept was regarded as an aesthetic necessity before the 1990s and was an easy target for cost savings in the absence of concrete scientific evidence of its involvement in HAIs [7]. In this concept, one of the options in force is the decontamination of spaces and tools using sole cleaning or in combination with disinfection or sterilization [9].

Nowadays, it has been established that infectious etiologies such as those listed above are easily transferred from environmental surfaces to the hands of healthcare providers and even to reusable medical equipment. If they are contaminated, these items serve as transmission channels for microbes from contaminated surfaces to patients or from one patient to another. Also, the risk of acquiring an infection is increased if a newly admitted patient is placed in a room previously occupied by a patient known to be colonized or infected with an infectious agent [3,4]. Similarly, it is accepted that adherence to good hygiene practices has a strong influence on mitigating the presence of infectious agents in the patient's environment thereby, reducing the risk of infection [4]. Otherwise, health facilities must ensure that cleaning staff follow decontamination procedures.

Throughout the world, decontamination processes are subject to debate about frequencies, methods, equipment, benchmarks, monitoring and standards [10,11]. Due to multiple disparities (such as environmental microbial flora, environmental materials and architecture), development of contextual protocols is though crucial to be encouraged [3]. Standardized scientifically-based evaluations of the effectiveness of hygiene protocols are necessary to formulate and advocate the most appropriate and cost-effective methods for hygiene in the healthcare setting [4,7,12].

At the "Université des Montagnes" Teaching Hospital (UdMTH), several investigations have been performed to monitor the local bacterial flora. The authors of these studies consistently reported various alerts of infectious risk based on bacterial loads that were often high as well as markers of dissemination from one place to the other, with emphasize on the need for sustainable local references [8,13,14]. The aim of the present survey was firstly, to assess the effectiveness of decontamination methods used at the UdMTH. Focused on UdMTH biomedical analysis laboratory, this work was carried out to determine the bacterial populations that were present before and after decontamination of work surfaces in this department with a previous analysis protocol [15]. Secondly, authors' focus was to determine the susceptibility/tolerance of isolates to the traditional disinfectants. The results thereof could help improve local microbiological safety, be disseminated to other healthcare facilities throughout Cameroon and/or guide effective contextual protocols in force in specific areas to improved health and sanitation.

2. Material and methods

2.1. Study design

This was a descriptive cross-sectional study carried out at UdMTH from May 4th through June 16th, 2020; supported by research authorization N°. 2020/052/AED/UDM/CUM issued by the UdMTH Head and the ethical clearance N°. 2020/147/UdM/PR/CIE provided by the "Université des Montagnes" board of ethics. The investigation sites consisted of work surfaces at the UdMTH biomedical analysis laboratory. Data collection, sampling and specimen analysis were conducted within the premises of the same laboratory.

2.2. Decontamination protocol

The decontamination protocol was organized in two steps: cleaning and disinfection. The main material used was absorbent paper (standard wide examination sheet; 50 × 34 cm), the concentrated Surfanios® disinfectant solution from ANIOS®, the detergent "Pax lemon" and the sodium hypochloride (2.4%) "la croix" from COLGATE-PALMOLIVE® Cameroon. These products were selected because they were the ones used at the UdMTH. Also, chlorinated water is mainly used by many facilities in Cameroon.

For protocol implementation, the cleaning was done with diluted detergent (1/20 with tap water), while disinfection was

performed with diluted disinfectant in tap water (either Surfanios®, 0.25% or sodium hypochlorite, 0.12%). The sheets of examination cloth were folded to have pieces 4 times smaller than the original. In this survey, the decontamination protocol was applied to a surface area of 900 cm^2 (30 × 30cm).

Summarily, the target surfaces were first rubbed with pieces of folded examination sheet impregnated with cleaning solution. Then, it was wiped with a dry piece of folded examination sheet. The wiped surface was rinsed with a piece of folded examination sheet soaked in tap water. After this rinsing step, the surface was wiped with a dry sterile piece of folded examination sheet. After that, the disinfectant was applied onto this surface with a sterile piece of folded examination sheet impregnated with the product. The detailed protocol is presented in Table 1.

2.3. Work surface selection

Four sampling flat surfaces were selected. All were work surfaces within the UdMTH Biomedical Laboratory, selected for the high frequency of activities and the material they were made of. Location 1 was a ceramic surface, location 2 a glass surface, location 3 a formica surface and, location 4 a leather surface.

2.4. Sampling, transport, storage

Early specimen collection was conducted in the morning before the day's activities and was performed as described by Fotsing Kwetché *et al.* [15]. The area to be sampled (square area of 25 cm^2) was calibrated with a sampling template duly designed. Specimens were then collected in series, three times per series: before decontamination, after cleaning (before disinfection) and within 15 min after the application of the disinfectant. Fifteen minutes' time was chosen with reference to the disinfectant manufacturers' instructions harmonized with work activities flux. However, sampling of the leather surface after the application of the disinfectant was done after 20 min because the surface could not dry before this time.

All series of specimen collection were performed three times for three consecutive days (one series per day) for each of the two variants of decontamination (with Surfanios® 0.25% and with sodium hypochlorite 0.12%). Subsequent to collection, specimens were immediately submitted to laboratory analytical procedures.

2.5. Bacteriological screening

2.5.1. Culture

For each specimen, the culture was performed according to the procedure described by Fotsing Kwetché *et al.* [15], with a few modifications. Inoculation was done on 6 agar plates (3 Eosin Methyl Blue Agars, and 3 Mannitol Salt Agars). These preparations were incubated at 37 °C for four days. No dilution was performed at this step.

2.5.2. Enumeration and identification

Subsequent to incubation and bacterial growth, enumeration and identification of the colonies were performed according to previous protocols [13–16] through macroscopy, microscopy, and further characterization with differential biochemical and enzymatic tests for target bacterial populations.

2.5.2.1. Macroscopic, microscopic and enumeration. After incubation, the colonies were described with reference to characteristics that basically include their color, size, shape, roughness, opacity and consistency. Then, a microscopic examination on Gram smear was performed. Simultaneously with the macroscopic characterization of colonies, a count of colony forming units per Petri dish (CFU/Petri dish) was conducted for each colony type. Enumeration was done according to Fotsing Kwetché *et al.* [15]. In this view, the actual bacterial loads (ABL) of Gram-positive cocci (GPC) and Gram-negative rods (GNR) on surfaces were calculated with reference to the calibration equation for bacteria enumeration on flat surfaces [15] displayed in Table 2. This calibration was performed with *S. aureus*

Table 1
Surface decontamination protocol.

Steps	Description
1	Soak one side of the folded examination sheet with 20 mL of the cleaning solution
2	Rub the surface to be cleaned with the moistened side of the sheet in a circular centrifugal direction (from the center of the area to be cleaned to the periphery) for 10 s, then turn the sheet over and rub the surface with the opposite side of the sheet to the moistened side in the same direction for the same length of time;
3	Repeat the above steps once again;
4	Wipe the cleaned surface with another piece of folded examination sheet;
5	Soak one side of a new piece of folded examination sheet with 20 mL of tap water;
6	Perform step 2;
7	Wipe the cleaned surface with a sterile piece of folded examination sheet;
8	Soak one side of a new sterile piece of folded examination sheet with 15 mL of disinfectant;
9	Rub the cleaned surface with the moistened side in the same direction as before for the same length of time;
10	Allow to dry and resume working after 15 min.

Table 2
Characteristics of the bacterial enumeration calibration curves and detection threshold for the method used [15].

Strains	Materials	Equation of the calibration curve[a]	R^2	95%CI of the slope (a) and constant (b)	Detection threshold[b] (CFU/cm^2)
***E. coli* ATCC 25922**	**Aluminum**	ABL = 2.614 × 10^3 × OBL+2 × 10^3	0.99924	a: [2.584 × 10^3–2.644 × 10^3] b: [-8 × 10^3–12 × 10^3]	7228
	Ceramic	ABL = 400 × OBL+63 × 10^3	0.97667	a: [364−436] b: [-128 × 10^3–254 × 10^3]	63,800
	Formica	ABL = 161 × OBL+32 × 10^3	0.98504	a: [153−169] b: [-20 × 10^3–84 × 10^3]	32,322
	Glass	ABL = 180 × OBL+4 × 10^3	0.99691	a: [176−184] b: [-5 × 10^3–13 × 10^3]	4360
	Leather	ABL = 721 × OBL+7 × 10^3	0.99342	a: [697−745] b: [-12 × 10^3–26 × 10^3]	8442
	Sanded plywood	ABL = 33.5 × 10^3 × OBL+12 × 10^3	0.98943	a: [32.1 × 10^3–34.9 × 10^3] b: [-63 × 10^3–87 × 10^3]	79,000
***S. aureus* ATCC 29213**	**Aluminum**	ABL = 261 × OBL+302 × 10^3	0.98793	a: [246−276] b: [-221 × 10^3–825 × 10^3]	302,522
	Ceramic	ABL = 3.13 × 10^3 × OBL+53 × 10^3	0.98996	a: [2.99 × 10^3–3.27 × 10^3] b: [-230 × 10^3–336 × 10^3]	59,260
	Formica	ABL = 39 × 10^3 × OBL+782 × 10^3	0.86131	a: [32 × 10^3–46 × 10^3] b: [-1118 × 10^3–2682 × 10^3]	860,000
	Glass	ABL = 1.73 × 10^3 × OBL+23 × 10^3	0.99428	a: [1.66 × 10^3–1.8 × 10^3] b: [-404 × 10^3–450 × 10^3]	26,460
	Leather	ABL = 5.3 × 10^3 × OBL+587 × 10^3	0.93152	a: [4.7 × 10^3–5.9 × 10^3] b: [-663 × 10^3–1837 × 10^3]	597,600
	Sanded plywood	ABL = 1.31 × 10^3 × OBL+826 × 10^3	0.84549	a: [1.06 × 10^3–1.56 × 10^3] b: [-524 × 10^3–2176 × 10^3]	828,620

[a] ABL: actual bacterial loads (CFU/cm^2); OBL: observed bacterial loads (CFU/cm^2); 95%CI: 95% confidence interval; OBL = 40xN/25 where N is the number of CFU/Petri dish, 40 = volume of extraction liquid/volume of inoculum, 1/25 is the conversion factor from CFU on 25 cm^2 to CFU on 1 cm^2.
[b] The detection threshold for the analysis method used was calculated for 1 CFU/Petri dish, i.e. about 2 CFU/cm^2 of OBL.

ATCC 29213 and *E. coli* ATCC 25922 (for GPC and GNR, respectively). The actual loads of Gram-positive rods (GPR) were determined with reference to the calibration conducted with *S. aureus* ATCC 29213 for their cell envelope similarity. These references strains served for quality control throughout the investigation.

2.5.2.2. Orientating tests for bacterial identification. Orientations continued with reference to the cell microscopy. For GPR and GNR, identification was limited to macroscopy and microscopy. For GPC, a progressive process was followed using the catalase, mannitol fermentation, free coagulase, DNase tests; as well as Voges-Proskauer (acetoin) test.

2.5.3. Disinfectant susceptibility test

After identification, susceptibility to disinfectant was performed. This test was conducted in liquid medium. It was undertaken in order to determine the effectiveness of disinfectants on bacterial, and was associated with their loads and reference to known detection thresholds [15] for surface bacteria enumeration (Table 2).

2.5.3.1. Preparation of the bacterial suspension. For each bacterium, a 24-h subculture of the bacterial isolate was performed by streaking the organism on nutrient agar for purity. From the resulting growth, a bacterial suspension was aseptically prepared by mixing 2 mL of sterile physiological saline (0.9% NaCl) solution in a test tube with the bacterial population in order to achieve a turbidity equivalent to the 0.5 standard of the MacFarland scale. The suspension was inoculated with a single streak onto nutrient agar with a sterile calibrated (10 μL) wire loop (this represented the "control streak").

2.5.3.2. Inoculation and incubation. Aseptically, 100 μL (1.5 × 10^7 CFU) of the above suspension was dispensed into 500 μL of the disinfectant. The mixture was then incubated at room temperature (26 °C) for 15 min. Upon completion of this incubation, the preparation was inoculated three times (in triplicate) on the same agar in 55 mm-diameter Petri dishes (test streaks) as the original bacterial preparation with a sterile wire loop (10 μL calibrated loop). The preparations were eventually allowed for aerobic incubation at 37 °C for 24 h.

2.5.3.3. Reading and interpretation. Upon completion of the 24 h of incubation, the culture reading was conducted by assessing the bacterial presence on the test streaks.

A disinfectant was referred to as "good" for disinfection when it reduced the bacterial population (with reference to the positive control) to less than 5 log of the initial load [17], in this case less than 12 CFU in 10 μL for an initial load of 2.5 × 10^5 CFU in the same volume. Thus, the isolate was said to be "susceptible" to disinfectants if there was no bacterial growth on the three test streaks. Otherwise, it was referred to as "tolerant" to the disinfectants.

2.5.3.4. Sterility control for the disinfectants. The sterility of the disinfectants was also checked. For this purpose, 100 μL of the disinfectants were streaked onto 3 Eosin Methyl Blue Agars and 3 Mannitol Salt Agars in Petri dishes. The streaked agar plates were incubated at 37 °C for a week. The sterility of the disinfectant or the absence of tolerant bacteria in the disinfectant was confirmed when there were no visible bacterial growths upon completion of incubation.

2.6. Data analysis

The target variables were the numbers and types of bacterial isolates recovered from the work surfaces, the bacterial loads found on the work surfaces, and the category of isolates associated to susceptibility or tolerance to disinfectants. All pieces of information recorded were treated with analytic tools provided by Microsoft Excel 2013. Target pieces of information included the isolation rate of various bacteria group, numbers of isolate per sampling site, calculated actual bacterial loads and susceptibility/tolerant rates of isolates to disinfectants.

3. Results

3.1. Bacterial populations on the surfaces

Subsequent to the culture of collected specimens, 184 bacterial isolates were recovered. They belonged to the Gram-positive cocci, Gram-positive rods and Gram-negative rods sets. Related overall distribution is illustrated as shown in Fig. 1 and the distribution of bacterial types per collection site was summarized as presented in Table 3.

This distribution (Fig. 1) highlights diverse profiles, dominated by Gram-positive bacteria (97.8%) and mainly by *Staphylococcus* spp. (75.5%). This distribution also reveals that the overwhelming proportion (>3/5) consist of non-*aureus* coagulase-positive *Staphylococcus,* followed by GPR which are twice as frequent as coagulase-negative *Staphylococcus*.

Overall, it appears (from Table 3) that the decontamination is applied on bacterial populations permanently dominated by members of the genus *Staphylococcus.* This indicates a daily predominance of this bacterial type in the investigation sites environments.

3.2. Effect of decontamination on the bacterial loads

The overall observation of identified bacterial loads during decontamination is shown in Fig. 2.

The Fig. 2 reveals that the cleaning step had an effect on bacterial presence, with the reduction of bacterial loads. Beside the fact that data after cleaning falls below the detection level of the analytical method, Fig. 2 also shows that the final bacterial load (after decontamination) was less than the initial one (before decontamination). Thus, it indicates that the decontamination protocol used reduced the bacterial loads to undetectable levels.

The bacterial affected by decontamination were varied based on the sampling points and population diversity. This diversity is presented in Table 4.

The Table 4 reveals that bacterial density was higher at locations 3 and 4, compared to locations 1 and 2 before decontamination. Overall, however, *Staphylococcus* spp. consistently exhibited higher bacterial loads; followed by GPR. In addition, and whatever the target site, bacterial load was affected by the two variants of the decontamination procedures (drastically reduced or disappeared after the decontamination stage).

3.3. Bacteria susceptibility to disinfectants

Susceptibility test to disinfectants generated pieces of information summarized in Fig. 3.

Overall findings reveal that the large majority of bacterial isolates recovered are susceptible to Surfanios® 0.25% and sodium hypochlorite 0.12% within the time of exposure to these products. For each of these disinfectants, effectiveness rate of 98% was recorded. Otherwise, similar effectiveness was observed for both disinfectants.

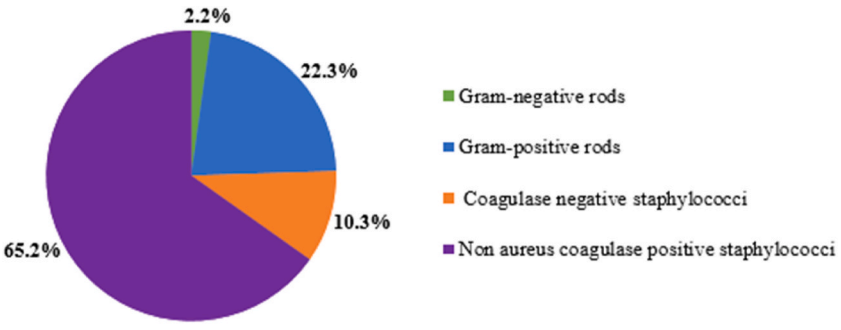


Fig. 1. Overall distribution of bacterial isolates.

Table 3
Bacterial isolates recovered from various work surfaces.

Decontamination protocols	Sampling locations	Sampling days	Bacterial type			
			GNR	GPR	CNS	naCPS
			Numbers	Numbers	Numbers	Numbers
Decontamination using Surfanios® 0.25%	Location 1	Day 1	0	5	0	4
		Day 2	0	0	2	8
		Day 3	0	3	1	6
	Location 2	Day 1	0	6	1	2
		Day 2	1	1	1	8
		Day 3	0	0	1	10
	Location 3	Day 1	2	3	2	0
		Day 2	0	2	0	1
		Day 3	0	0	2	10
	Location 4	Day 1	1	1	0	6
		Day 2	0	0	0	4
		Day 3	0	0	0	1
Decontamination using chlorinated water 0.12%	Location 1	Day 1	0	0	0	5
		Day 2	0	2	0	7
		Day 3	0	1	1	2
	Location 2	Day 1	0	3	0	5
		Day 2	0	0	1	8
		Day 3	0	2	2	2
	Location 3	Day 1	0	2	1	11
		Day 2	0	4	0	10
		Day 3	0	2	1	1
	Location 4	Day 1	0	2	2	6
		Day 2	0	0	0	3
		Day 3	0	2	1	0

GNR: Gram negative rods; GPR: Gram positive rods; CNS: Coagulase negative *Staphylococcus*; naCPS: non aureus Coagulase positive *Staphylococcus*, Location 1: Ceramic surface; Location 2: Glass surface; Location 3: Formica surface; Location 4: Leather surface.

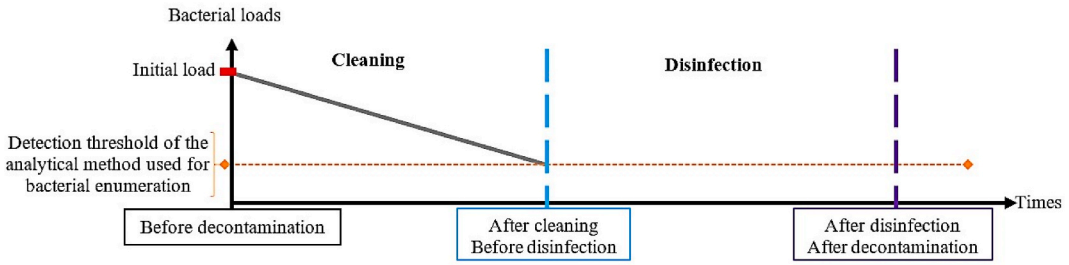


Fig. 2. Overall variation in bacterial loads following decontamination.

4. Discussion

The present investigation focused on effectiveness of a surface hygiene protocol at the UdMTH biomedical analysis laboratory. Variation of bacterial loads on target surfaces was assessed during and subsequent to decontamination; susceptibility tests to disinfectants was also investigated.

Data from collected specimens screening revealed that the work surfaces served as reservoirs for large numbers of bacteria, predominantly Gram-positive (*Staphylococcus* spp. (75.5%) and Gram-positive rods (22.3%)). Gram-negative rods were the least frequently recovered. This general distribution on surfaces also reflects the trend in the work environment previously reported by other authors in the same health facility [8,13,14], in other healthcare settings [14,18] or in non-hospital environments such as animal farms [19]. According to these authors, the predominance of Gram-positive bacteria in the environmental bacterial populations could be justified by the chemical composition of the cellular envelope, which provides resistance to adverse living conditions like draught and higher temperatures, compared to Gram-negative bacteria. In addition, some GPR like *Bacillus* spp., have the ability to develop spores which allow additional survival potentials in harsher environmental conditions. Further, these predominant populations are typically facultative aerobes. This ability for unchanged fitness in the presence or the absence of molecular oxygen is a special asset that strongly accounts for their frequencies and diversities in most settings. Accordingly, with the minority of GNR observed during the present investigation and in others [8,13,14], *Staphylococcus* spp. could effectively be used as biomarkers of microbiological quality control of healthcare environment, especially in settings where tools and other facilities for investigation are limited. The distribution of these organisms according to their loads and their density is globally very high on the work surfaces before decontamination and much more

Table 4
Evolution of bacterial loads during and after decontamination.

Sampling locations	Sampling days	bDe (bC)			aC/bDi aDe (aDi)
		ABL (CFU/cm^2)			
		GPR	GNR	*Staphylococcus* spp.	
Decontamination with 0.25% Surfanios®					
Location 1	1	1.0308×10^5	ND	8.43×10^4	Not detected (ND) = Absence of bacteria (actual bacterial load = 0 CFU/cm^2) or bacterial presence not detectable (actual bacterial load < detection threshold of the method used for bacterial enumeration of surfaces)
	2	ND		1.2812×10^5	
	3	1.2186×10^5		1.0934×10^5	
Location 2	1	6.106×10^4	ND	4.549×10^4	
	2	2.646×10^4	4.36×10^3	6.106×10^4	
	3	2.3×10^4	ND	6.798×10^4	
Location 3	1	1.406×10^6	3.9406×10^4	1.211×10^6	
	2	1.289×10^6	ND	8.6×10^5	
	3	ND		1.835×10^6	
Location 4	1	5.976×10^5	8.442×10^3	6.612×10^5	
	2	ND	ND	6.188×10^5	
	3			5.976×10^5	
Decontamination with 0.12% chlorinated water					
Location 1	1	ND	ND	8.43×10^4	Not detected (ND) = Absence of bacteria (actual bacterial load = 0 CFU/cm^2) or bacterial presence not detectable (actual bacterial load < detection threshold of the method used for bacterial enumeration of surfaces)
	2	6.239×10^4		9.369×10^4	
	3	5.926×10^4		6.865×10^4	
Location 2	1	3.338×10^4	ND	5.587×10^4	
	2	ND		5.414×10^4	
	3	3.684×10^4		3.684×10^4	
Location 3	1	9.77×10^5	ND	1.796×10^6	
	2	1.094×10^6		1.64×10^6	
	3	8.99×10^5		9.77×10^5	
Location 4	1	6.029×10^5	ND	7.407×10^5	
	2	ND		6.135×10^5	
	3	6.029×10^5		5.976×10^5	

GNR: Gram-negative rod; GPR: Gram-positive rod; ABL: actual bacterial load; ND: Not detected; CFU: Colony forming unit; bDe: before decontamination; aDe: after decontamination; bC: before cleaning; aC: after cleaning; bDi: before disinfection; aDi: after disinfection. Location 1: Ceramic surface; Location 2: Glass surface; Location 3: Formica surface; Location 4: Leather surface.

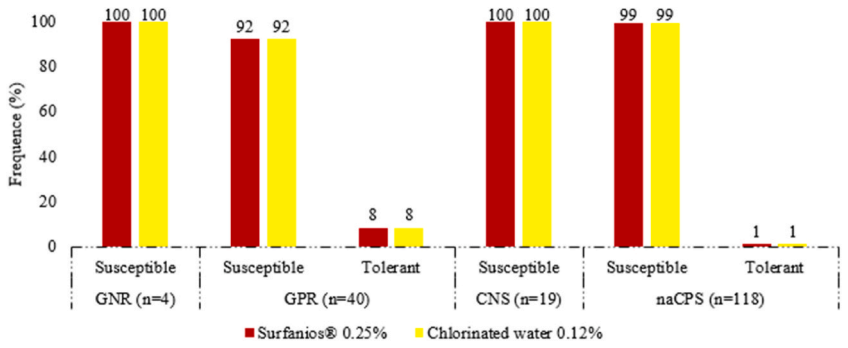


Fig. 3. Disinfectant susceptibility trends per isolate category.
GNR: Gram-negative rods; GPR: Gram-positive rods; CNS: coagulase-negative *Staphylococcus*; naCPS: non aureus coagulase-positive *Staphylococcus*.

on locations 3 and 4 that consist of formica and leather, respectively. About the decontamination process, data provided for the cleaning step revealed a reduction of bacterial load. This drop in bacterial loads subsequent to cleaning is consistent with previous reports which observed that this mechanical step is 80% effective [20]. From the microbiological point of view accordingly, cleaning makes disinfection more effective. During this process in fact, organic matters and other like-entities that interfere with the action of chemicals or physical agents are eliminated. In this case, they are Surfanios® (formulation of amino acid and quaternary ammonium) and chlorinated water [20,21]. Feliciano *et al.* observed that organic matters could reduce the effectiveness of sodium hypochlorite and quaternary ammonium prepared and used in food industries. For their evaluation, these authors used strains of murine norovirus 1, *E. coli* ATCC 29181 and *L. innocua* ATCC 33090 [21].

Cleaning also dislocates microbial biofilms (when it is present on surface), and provides better action for the disinfectant on the target surfaces admitting that this microbial configuration could also impair the effectiveness of hygiene protocols. The effectiveness of disinfectants on a biofilm varies according to their active principle, the biofilm density and architecture. Lineback *et al.* (2018)

observed, for instance, that hydrogen peroxide and sodium hypochlorite were more effective on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms than quaternary ammonium compounds [22]. These factors are reliable and could guide adjustment of the working volume to each product for a good cleaning and disinfection so that they are not used at concentrations lower than recommended by the manufacturer. In this way, bacteria will not be exposed to sub-lethal concentrations which are likely causes of resistant strains selection according to Condell *et al.* (2012) when compounding chemicals were tested individually. In turn, they observed low tolerance of these strains to formulations that combined several active compounds from benzalkonium chloride (a Surfanios® active compound) [23].

Still other factors might interfere with proper hygiene like low ambient temperature, shorter contact times and porous surfaces. It is therefore recommended that the ambient temperature, disinfection time and characteristics of the target surface should be taken into consideration in designing contextual protocols for effective disinfection [24].

Poor quality of water could also impede the effectiveness of the protocols if for instance, it is contaminated by microorganisms and/or organic maters. A study evaluating the microbiological quality of the water used in some health facilities of the Ndé Division (Western Cameroon) revealed that tap water was microbiologically safe at UdMTH [25].

The disinfectant susceptibility tests revealed high susceptibility rate (98%), underlining that Surfanios® (0.25%) and chlorinated water (0.12%) have good potentials on the target bacterial populations. Although it was not possible to assess the bacterial presence after the cleaning step due to the detection threshold imposed by the analytical method used [15], this isolates' susceptibility suggests that the disinfection was effective. In fact, due to the absence of a neutralization step that is used by some protocols, the results of the disinfectant susceptibility test in this work provided data on disinfectant effectiveness but did not provide a clearer view on the exact 15 min disinfection step. In fact, it was not possible to include a disinfectant neutralization step before or during the bacterial culture step in this work. However, these results can be inferred from this 15 min of exposure of bacteria to disinfectants during disinfection and from a potential length of residual exposure of bacteria to disinfectants after disinfection.

This high susceptibility rate was in line with above developments on the concentrations of chemicals used in the routine process. Still in addition, Surfanios® or chlorinated water are not generally used as antiseptics in the setting as already reported by other studies [26], and when they are used (for decontamination or disinfection), appropriate dosages are observed by the team in charge. Rouillon *et al.* [26] suggested that hospital strains such as *Pseudomonas* spp. and *Acinetobacter* spp. should be used for their specific characteristics and relevance in healthcare environment [5–7,26]. Still in line with findings from the present investigation, that suggestion will be applied in future like research initiatives.

Despite the low rate of disinfectant-tolerant bacteria observed (2%), their presence should draw attention on the necessity for proper hygiene that would further mitigate the spread of resistant traits through mobile genetic elements. The laboratory that served as study site is frequently visited by nurses and physicians who are in direct contact with patients. In the context of HAI prevention, it would be primordial to monitor as often as possible the likelihood of cross-resistance to antibiotics and disinfectants, especially for bacteria such as *Pseudomonas* spp. and *Acinetobacter* spp., which are members of the endogenous flora in healthcare environments [26], although this paradigm on cross-resistance remains subject for fierce debates. It is alleged that cross-resistance to antimicrobial that develops upon exposure and adaptation to a disinfectant occur when the disinfectant and an antimicrobial compound act on the same cellular target or have the same transport system or can be accommodated by the same resistance mechanism. It would also occur in instances where the genes contributing to disinfectant tolerance and antibiotic resistance are carried by the same mobile genetic elements. The efflux-pump resistance mechanism is one of the most recurrent in this anti-infective tolerance to disinfectants like benzalkomium chloride [23].

With a glance to susceptibility tests, Surfanios® 0.25% and sodium hypochlorite 0.12% can be indicated for disinfection during decontamination and could also be used alternately. For action sustainability, food industries and especially pharmaceutical groups recommend the regular change of active disinfectants used for decontamination of premises, to prevent the selection of resistant strains. Some authors like Rouillon *et al.* observed, however, that this alternation could be unnecessary when they reported permanent sustained potential of Surfanios® in most of healthcare facility strains for ten years [26]. Compared to chlorinated water, which only acts as disinfectant, Surfanios® has a combined detergent-disinfectant potential and would be the most suitable because its detergent potential would amplify that of the cleaning, thereby, increase the disinfectant action.

On the other hand, and with reference to resource availability and affordability, chlorinated water would be the best choice in most needy settings throughout the world.

Overall, data analysis revealed a good effectiveness of surface decontamination at the UdMTH Biomedical laboratory, regardless of surface material, though glass is most recommended for easy implementation of the protocols in force. Also, data analysis further brings out the fact that minimal available and affordable assets (Surfanios®, chlorinated water, tap water and "Pax lemon" detergent) could serve effectively. The contextual cost-effective solution choice should be adopted.

Future investigations should be conducted to confirm these results on other microbial communities in the work environment. At the same time, there is need to identify other contextual microbial biomarker for environmental contamination or hygiene monitoring that can be used at affordable in resource-limited settings (such as the one in this work). Further, and with emphasis on hygiene and sanitation based on microbial load, future research initiatives should be performed to improve the detection thresholds on surfaces.

5. Conclusion

The present survey revealed that, on the selected work surfaces, *Staphylococcus* was the predominant group of bacteria. The bacterial loads were high on all the target surfaces before decontamination but became undetectable after cleaning with the detergent "Pax lemon" during decontamination. The large majority of the recovered isolates were susceptible to Surfanios® (0.25%) and sodium

hypochlorite (0.12%). Overall, these findings indicated process effectiveness on the subjected bacterial populations and suggested the use of either Surfanios® (0.25%) or sodium hypochlorite (0.12%) for work surfaces hygiene. Otherwise in the present context, cleaning with detergent "Pax lemon" and disinfection with sodium hypochlorite might be sufficient for the types of surfaces that were targeted in the present survey.

Authors' contributions

Conceptualization and design of methodology: O.D.Y., P.R.F.K.; Validation: P.R.F.K.; Supervision: P.R.F.K., C.D.N.; Project administration, Investigation, Data curation and Formal analysis: O.D.Y.; Resources: O.D.Y., P.R.F.K.; Visualization and Writing – Original draft: O.D.Y.; Writing – Review and Editing: O.D.Y., E.G.K., B.P.T.K., C.D.N., P.R.F.K.

Funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data associated with this work were not deposited into a publicly available repository. Data included in article/supp. material/ referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Robert Soh Tchinda and Gisèle Benette Tchouela for the invaluable support provided along this survey. The authors thank Browndon Tchatchouang and Loïc Kamgaing for the help during the practical work. The authors also thank Pacome Tchuenkam for his contribution to the language editing. The authors also thank the "Université des Montagnes" and their teaching hospital for their support during this survey.

References

[1] O. Meunier, C. Hernandez, M. Piroird, R. Heilig, D. Steinbach, A. Freyd, Prélèvements bactériologiques des surfaces : importance de l'étape d'enrichissement et du choix des milieux de culture, Ann. Biol. Clin. 63 (5) (2005) 481–486 (French). https://www.researchgate.net/profile/Olivier-Meunier/publication/7536692_Bacteriological_sampling_of_surfaces_importance_of_the_step_of_enrichment_and_the_choice_of_the_culture_media/links/5549f9fa0cf26eacd6922944/Bacteriological-sampling-of-surfaces-importance-of-the-step-of-enrichment-and-the-choice-of-the-culture-media.pdf.

[2] P. Hartemann, M.-F. Blech, L. Simon, Les contrôles microbiologiques de l'environnement hospitalier, Rev. Fr. Lab. 291 (1997) 43–47, https://doi.org/10.1016/S0338-9898(97)80112-6 (French).

[3] M. Doll, M. Stevens, G. Bearman, Environmental cleaning and disinfection of patient areas, Int. J. Infect. Dis. 67 (2018) 52–57, https://doi.org/10.1016/j.ijid.2017.10.014.

[4] S.J. Dancer, Hospital cleaning in the 21st century, Eur. J. Clin. Microbiol. Infect. Dis. 30 (2011) 1473–1481, https://doi.org/10.1016/j.ijid.2017.10.014.

[5] A. Bertrou, J.C. Cetre, C. Chapuis, S. Guignement, J. Fabry, J. Hajjar, et al., Contrôles microbiologiques de l'environnement hospitalier, Vigilance environnementale. Hygiène 8 (3) (2000) 139–179. French, https://www.cpias.fr/nosobase/recommandations/cclin_arlin/cclinSudEst/2000_environnement_CCLIN.pdf.

[6] J.D. Cavallo, G. Antoniotti, N. Baffoy, S. Guignement-Coudrais, J. Hajjar, C. Horn, et al., Surveillance microbiologique de l'environnement dans les établissements de santé. Air, eaux et surfaces, Ministère chargé de la santé. DGS/DHOS, CTIN, 2002, p. 78 (French). https://sante.gouv.fr/IMG/pdf/recofin.pdf.

[7] S.J. Dancer, Controlling hospital-acquired infection: focus on the role of the environment and new technologies for decontamination – hospital cleaning and decontamination, Clin. Microbiol. Rev. 27 (4) (2014) 665–690, https://doi.org/10.1128/cmr.00020-14.

[8] S.H. Tchoukoua, P.R. Fotsing Kwetché, F. Njonga Tchami, S. Gamwo Dongmo, W.L. Nankam Nguekap, A.M. Yawat Djongang, J. Simo Louokdom, et al., Observance of guidelines towards mitigating the risk of hospital acquired infections in a university teaching hospital: preliminary findings from a pilot study towards healthcare quality improvement, WJAHR 2 (4) (2018) 204–212. https://www.researchgate.net/publication/339325890_Observance_of_guidelines_towards_mitigating_the_risk_of_hospital_acquired_infections_in_a_University_Teaching_Hospital_preliminary_findings_from_a_pilot_study_towards_healthcare_quality_improvement.

[9] J.G. Solon, S. Killeen, Decontamination and sterilization, Surgery 37 (1) (2019) 51–57, https://doi.org/10.1016/j.mpsur.2018.11.002.

[10] M. Dettenkofer, Healthcare environment decontamination, Healthc. Infect. 18 (2013) 47–48, https://doi.org/10.1071/HI13005.

[11] W.A. Rutala, D.J. Weber, The benefits of surface disinfection, Am. J. Infect. Control 32 (2004) 226–231, https://doi.org/10.1016/j.ajic.2004.04.197.

[12] L.F. White, S.J. Dancer, C. Robertson, A microbiological evaluation of hospital cleaning methods, Int. J. Environ. Health Res. 17 (4) (2007) 285–295, https://doi.org/10.1080/09603120701372433.

[13] P.R. Fotsing Kwetché, E.G. Kougang, C. Domngang Noche, O.D. Youté, S.H. Tchoukoua, A.M. Yawat Djogang, et al., Mitigating hospital infection risks at the "Université des Montagnes" teaching hospital: holistic policy to meet the challenge, World J. Pharm. Pharmaceut. Sci. 9 (3) (2020) 518–535. https://www.researchgate.net/publication/339658199_MITIGATING_HOSPITAL_INFECTION_RISKS_AT_THE_UNIVERSITE_DES_MONTAGNES_TEACHING_HOSPITAL_HOLISTIC_POLICY_TO_MEET_THE_CHALLENGE.

[14] S.A. Menteng Tchuenté, O.D. Youté, B.P. Tamatcho Kweyang, E.G. Kougang, P.B. Well à Well à Koul, P.R. Fotsing Kwetche, Strengthening environmental hygiene for healthcare-associated infections prevention in maternity ward: outstanding findings from a multisite survey in the Ndé division, west Cameroon, Microbiol Res J Int 33 (8) (2023) 25–35, https://doi.org/10.9734/mrji/2023/v33i81399.

[15] P.R. Fotsing Kwetché, C. Domngang Noche, O.D. Youté, B. Tamatcho Kweyang, W.L. Nankam Nguekap, V.I. Deumi Monthé, et al., Microbiological control in hospital environment: calibrating enumeration of bacteria on flat surfaces with wet swab, Int J Curr Microbiol App Sci 10 (2) (2021) 452–461, https://doi.org/10.20546/ijcmas.2021.1002.053.
[16] F. Denis, M.C. Ploy, C. Martin, E. Bingen, R. Quentin, Bactériologie Médicale, Techniques Usuelles, 2nd ed., Elsevier Masson SAS, Paris, 2011. French
[17] Société Française d'Hygiène Hospitalière, Produits de désinfection chimique pour les dispositifs médicaux, les sols et les surfaces, Guide pour le choix des désinfectants. Hygiènes 22 (6) (2015) 110 (French). https://www.sf2h.net/publications/le-choix-des-desinfectants.html.
[18] F.R. Tchapdie Ngassam, Megne Tantse, P.R. Fotsing Kwetche, D.P. Noukela Noumi, J. Kouamouo, J. Simo Louokdom, et al., Multicenter study on antibiotic susceptibility/resistance trends in the western region of Cameroon, Int J Biol Chem Sci 11 (1) (2017) 131–143, https://doi.org/10.4314/ijbcs.v11i1.11.
[19] A.M. Yawat Djogang, P.R. Fotsing Kwetché, J. Simo Louokdom, S. Gamwo Dongmo, W.L. Nankam Nguekap, S.H. Tchoukoua, et al., Antibiotic susceptibility profile of bacteria from farm wastes: findings in chicken excreta, food and water from four poultries versus trend in a non-exposed exposed community of West Cameroon, Int J Curr Res 10 (11) (2018) 75629–75638. https://www.researchgate.net/publication/336588124_ANTIBIOTIC_SUSCEPTIBILITY_PROFILE_OF_BACTERIA_FROM_FARM_WASTES_FINDINGS_IN_CHICKEN_EXCRETA_FOOD_AND_WATER_FROM_FOUR_POULTRIES_IN_A_NON-EXPOSED_COMMUNITY_OF_WEST_CAMEROON.
[20] R. Massicotte, Désinfectants et désinfection en hygiène et salubrité: principes fondamentaux, Santé et service sociaux, Québec, 2009, p. 77 (French).
[21] L. Feliciano, J. Li, J. Lee, M.A. Pascall, Efficacies of sodium hypochlorite and quaternary ammonium sanitizers for reduction of norovirus and selected bacteria during ware-washing operations, PLoS One 7 (12) (2012) e50273, https://doi.org/10.1371/journal.pone.0050273.
[22] C.B. Lineback, C.A. Nkemngong, S.T. Wu, X. Li, P.J. Teska, H.F. Oliver, Hydrogen peroxide and sodium hypochlorite disinfectants are more effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms than quaternary ammonium compounds, Antimicrob. Resist. Infect. Control 7 (1) (2018) 154–160, https://doi.org/10.1186/s13756-018-0447-5.
[23] O. Condell, C. Iversen, S. Cooney, K.A. Power, C. Walsh, C. Burgess, Efficacy of biocides used in the modern food industry to control Salmonella enterica, and links between biocide tolerance and resistance to clinically relevant antimicrobial compounds, Appl. Environ. Microbiol. 78 (9) (2012) 3087–3097, https://doi.org/10.1128/AEM.07534-11.
[24] Y. Jang, K. Lee1, S. Yun, M. Lee, J. Song, B. Chang, et al., Efficacy evaluation of commercial disinfectants by using *Salmonella enterica* serovar *Typhimurium* as a test organism, J. Vet. Sci. 18 (2) (2017) 209–216, https://doi.org/10.4142/jvs.2017.18.2.209.
[25] V.I. Deumi Monthé, M. Pahane Mbiada, P.R. Fotsing Kwetché, Qualité microbiologique de l'eau de consommation dans les hôpitaux du département du Ndé. [Dissertation for Bachelor's degree in Biomedical Sciences], Université des Montagnes. French, Bangangté-Cameroon, 2017,. Unpublished results.
[26] S. Rouillon, S. Ourdanabia, S. Jamart, C. Hernandez, O. Meunier, Étude de l'efficacité d'un produit détergent désinfectant pour sols et surfaces sur les souches bactériennes isolées à partir de l'environnement hospitalier, Pathol. Biol. 54 (6) (2006) 325–330, https://doi.org/10.1016/j.patbio.2006.01.011.French.