

Effectiveness of Disinfection in Bacteriology Laboratories in Togo, 2021

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Abstract

Background: Laboratory personnel is at risk of biological contamination leading to laboratory-acquired infections (LAIs). The use of disinfection products is essential in the prevention of these infections. This work aimed to evaluate the effectiveness of antimicrobial agents used in disinfection in bacteriology laboratories in Togo. Methods: This was a cross-sectional study conducted from June to December 2021 in all bacteriology laboratories in Togo. Swabs taken before and after disinfection of surfaces and staff hands were immediately plated on agar media. Counting and identification of isolated colonies were done after 24 hours of incubation. The ANOVA test was used to compare calculated means, prevalence ratio (PR) and 95% confidence intervals (CI) to compare bacterial frequencies. Results: A total of 393 samples were taken, of which 41.2% were from hands. Before disinfection, surfaces were more contaminated than hands with respectively 40.4% and 29.6% (PR = 1.3; CI 95% = [0.9 - 1.9]). After surface disinfection with 0.5% of chlorine solution, bacterial elimination was total, but partial on hands washed with soap, with residual contamination of 3.7%. A total of 108 strains were isolated before disinfection of which Klebsiella spp. 38.9% and Staphylococcus spp. 25.0%; after disinfection 4 strains were isolated of which: Staphylococcus spp. 75.0% and Klebsiella spp. 25.0%. Conclusion: Surface disinfection was more effective than hand washing with soap and water. We recommend proper hand washing.

Keywords

Effectiveness, Antimicrobial Agents, Disinfection, Hands, Surfaces

1. Introduction

Medical laboratories are one of the professional sectors most exposed to infectious risks. Microbiology is particularly concerned since all biological agents are likely to be the subject of diagnostic examinations [1] [2]. Microorganisms from the environment or from the samples handled can contaminate surfaces (laboratory benches, equipment, floors) and the hands of operators. Infections acquired by personnel handling these pathogenic microorganisms in biological laboratories have been described in the literature since the middle of 1930 years and a study carried out in 2016 in Canada in 1352 laboratory areas, described a total number of 46 incidents of exposure to micro-organisms with an incidence of 3.4% [3]. Contaminated surfaces are an important potential source of healthcare-associated infection (HAI) transmission due to many pathogens [4]-[9]. Among the potential sources of pathogens that cause HAI, the most common are the patient's microbiota and the hands of healthcare workers [10]. Many studies have shown the role of hands in the transmission of nosocomial infections in Benin [5] [11] [12] [13]. In Togo, many studies have been conducted, but few have been published, hence the lack of available information. Hygiene, cleaning and disinfection of hands are prerequisites for hygiene management in hospitals and the food industry in order to minimize the risk of healthcare and foodborne infections [11] [13]. Making laboratory operations safe means controlling the risks at different stages of sample processing [1] [2]. One of the means used to protect oneself, the community and the environment of pathogens is disinfection. It is therefore necessary to implement procedures for cleaning and disinfecting surfaces [12]. Many commercial products are available for disinfecting and sanitizing surfaces in health care facilities and food premises such as restaurants and factories [14] [15]. Antimicrobial agents, whether disinfectants or antiseptics, were used empirically until Pasteur demonstrated the responsibility of microorganisms in infectious diseases and laid the scientific foundation for antisepsis and disinfection [16]. The use of these products aims to reduce or eliminate microorganisms. These chemicals are widely used in medicine, whether as a curative measure (wounds) or, above all, as a preventive measure for humans (body hygiene, hand hygiene, pre-operative skin preparation) or for the environment (cleaning, disinfection of premises, surfaces and equipment) [16]. The implementation of these preventive measures makes it possible to reduction of expositions frequency. These expositions can result in a failure, the absence of appropriate protection, or non-compliance with procedures.

It is therefore important to monitor and evaluate the effectiveness of the disinfection procedures implemented in laboratories. This study aimed to measure the effectiveness of hand washing of laboratory staff with water and mild soap and surfaces disinfection with sodium hypochlorite in bacteriology laboratories in Togo in 2021.

2. Methods

2.1. Study Design

This was a cross-sectional and descriptive study conducted in all medical bacteriology laboratories in Togo.

Togo is located in West Africa with an area of 56.600 km². Its population was estimated at 7.886.000 in 2021 [17].

The health system is organized according to a pyramid structure with three levels (central, intermediate and peripheral). In terms of the availability of public medical bacteriology laboratories, they are much more at the central and intermediate levels of the health pyramid. At the central level, there are four bacteriology laboratories: one per teaching Hospital (Campus, Kara and Sylvanus Olympio) and one at the Institut National d'Hygiène. At the intermediate or regional level, each of the six health regions has a bacteriology laboratory. At the peripheral level, only the districts of Lacs (Aného), Kloto (Kpalimé) and the Hospital of Bè have bacteriology laboratories. Private or confectional bacteriology laboratories (NNL) for the confirmation of epidemic prone diseases was created on the 25th October 1998 by Order n°113/98/CAB establishing the NNL. This network includes the national reference laboratory, regional laboratories and district laboratories.

2.2. Study Period

The study was conducted from June to December 2021.

2.3. Sampling, Population and Study Materials

All public and private bacteriology laboratories of Togo (21) were included.

Laboratory staff was chosen by convenience (two laboratory technicians, one laboratory assistant and one secretary). The hands of this laboratory staff were sampled before and after disinfection by washing with water and mild soap. On each work surface (laboratory bench, the doors' handles, the sink, the microscopes' mechanical stage, stages controls, adjustments, the staff mobile phone, and the inside of the autoclave) a swab was taken. On the laboratory benches, samples were taken before and after disinfection.

2.4. Disinfection Protocol

2.4.1. Surfaces Disinfection

Surfaces are disinfected using a 0.5% of sodium hypochlorite solution prepared

from concentrated solutions. The surfaces are disinfected before and at the end of each operation using a paper towel. The surface is first wiped to remove organic matter and dust before the 0.5% bleach solution is applied [18].

2.4.2. Hand Washing with Water and Mild Soap

Washing should be carried out before each handling and before wearing gloves, as well as after handling and removing gloves [18].

- Wet your hands with water and then apply the soap;
- Hands are rubbed;
- Rinse with water and dry.

2.5. Variables of Interest

Several variables were exploited. The microbiological quality of surfaces and staff's hands: presence of germs (Staphylococci, Enterobacteriaceae, Pseudomonas, Streptococci, Enterococci and yeasts) before and after disinfection of the sites. The average number of colonies present on the different surfaces and staff's hands in colony-forming units (CFU).

2.6. Data Collection Technique and Tools

Data collection was done by interview, observation of practices, and laboratory measurements.

A questionnaire was designed by the investigators for this study. It was tested during a pilot survey in Greater Lomé. This questionnaire contains 5 sections with 13 items. See the questionnaire in the appendix.

Optical microscopes were used for direct examination and after Gram staining.

2.7. Sample Collection and Processing

Two types of samples were taken using sterile swabs:

Sterile swabs moistened with sterile normal saline (0.9%) were used to collect samples from the palms of both hands of the staff, rotating them momentarily over the entire palm surface and between the fingers. For laboratory technicians and assistants, samples were taken before hands washing and gloves wearing, after removing gloves and washing hands at the end of the manipulation. For the secretary, swabs were taken before and after hands washing with water and mild soap.

Sterile swabs moistened with sterile normal saline (0.9%) were used to sample some at risk surfaces before and after disinfection with bleach (0.5% active chlorine). The rest of the surfaces, were swabbed only before disinfection.

In each laboratory, the swabs collected were immediately plated onto Oxoid, UK branded agar media (Blood Agar, Mannitol Salt Agar, Sabouraud Chloramphenicol Agar, Mac Conkey Agar and Brilliance Chromogenic Medium[™] UTI). The plates were incubated at 37°C for 24 hours. The identification of the isolated germs was carried by using the morphological characteristics and the Gram control with microscope. The conventional biochemical tests and the Brilliance UTI chromogenic medium were used for the presumptive diagnosis [19]. Isolated colonies were counted on both blood agar and UTI medium with a magnifying glass. The results were expressed as Colony Forming Units (CFU)/swab [20].

The quality of the media was ensured by sterility and fertility tests using *E. coli* ATCC 25922 reference strains.

2.8. Statistical Analysis

Frequencies and mean colony numbers were calculated. The ANOVA test was used to compare the mean numbers of colonies and the prevalence ratio and 95% confidence intervals were used to compare the frequencies.

2.9. Ethical Consideration

A clearance of ethical comity of the Ministry of Health of Togo was obtained prior to the study ($N^{\circ}027/2021/CBRS$ of 25/06/2021).

3. Results

The samples were collected in a total of 21 bacteriology laboratories distributed in the country.

A total of 393 samples were taken, from which 41.2% were from staff's hands. Among them, 188 from the maritime region, 91 from kara region, 57 from central region, 38 from plateaux region and 19 from savanes region. **Figure 1** shows the distribution of the different bacteriology laboratories visited throughout the country.

3.1. Microbiological Quality of Surfaces and Staff's Hands before and after Disinfection in Bacteriology Laboratories in Togo, 2021

3.1.1. Proportion of Contaminated Sites before and after Disinfection in Laboratories

Table 1 illustrates the proportions of contaminated sites before and after disinfection in the laboratories. Before disinfection, the most contaminated sites were sinks (66.6%), laboratory benches (61.9%), refrigerator door handles (47.6%), and technicians' mobile phones (42.8%). After disinfecting the benches with bleach, no bacterial growth was observed on their surfaces. However, the hands washed with soap and water showed persistent bacteria.

The remaining contamination after disinfection of benches and hands is 2.9% (3/102). The hands washed with mild soap and water showed persistent bacteria 3.7% (3/81).

The surfaces were more contaminated than staff's hands. **Table 2** illustrates the contamination proportions of hands and surfaces in the laboratories.

The laboratory benches were twice more contaminated than staff's hands with 61.9% and 29.6% respectively, PR: 2.0 (1.2 - 3.3).



Figure 1. Map of Togo showing regions with bacteriology laboratories visited.

Table 1.	Proportions of	of contamination	before and af	ter disinf	ection on	different sites	sampled in bac	teriology la	boratories in T	0-
go, 2021										

	Before I	Disinfection	After D	visinfection	Remaining Contamination
Sampling Sites	Collected	Positive (%)	Collected	Positive (%)	(%)
Hands of technician 2	21	8 (38.1)	21	1 (4.8)	
Hands of technician 1	21	7 (33.3)	21	0	3/81 (3.7)
Hands of the secretary	18	5 (23.8)	18	1 (5.5)	
Hands of the laboratory assistant	21	4 (22.2)	21	1 (4.8)	
Sinks in the manipulation room	21	14 (66.6)	-	-	
Laboratory bench	21	13 (61.9)	21	0 (0)	
Reagent fridge door handle	21	10 (47.6)	-	-	
Staff mobile phone	21	9 (42.8)	-	-	
Inside the autoclave (before decontamination)	21	9 (42.8)	-	-	
Microscope stage controls and mechanical stage	21	7 (33.3)	-	-	
Sampling room handle	21	6 (28.5)	-	-	
Manipulation room handle	21	6 (28.5)	-	-	
Microscope (fine and coarse) adjustments	21	6 (28.5)	-	-	
Bleach tray for soiled objects	21	5 (23.8)	-	-	
Total	291	109 (37.4)	102	3 (2.9)	

- means sampling not carried out.

Period	Sites Sampled	Number of Samples	Positive n (%)	PR; IC 95%		
	Surfaces	210	85 (40.4)	12.00 10		
Before	Hands	81	24 (29.6)	1.5; [0.9 - 1.9]		
Disinfection	Laboratory benches	21	13 (61.9)	2.0; [1.2 - 3.3]		
	Hands	81	24 (29.6)			

Table 2. Proportions of contamination of hands and surfaces in bacteriology laboratoriesin Togo, 2021.

3.1.2. Distribution of Germs Isolated before and after Disinfection in the Laboratories

Before disinfection, the proportion of *Klebsiella spp* was 44.3% on surfaces and that of *Staphylococcus spp* 75.0% on staff's hands. After hands washing, two types of strains were persistent: *Staphylococcus spp* 75.0% and *Klebsiella spp* 25%. **Table 3** illustrates the distribution of germs before and after disinfection of surfaces and hands in the laboratories.

Before the disinfection the proportion of enterobacteria (*Klebsiella spp.* and *E. coli*) represented 51.8% and *Staphylococcus spp.* 25.0%. After the hand washing the proportion of the persistent germs was *Staphylococcus spp.* 75.0% and *Klebsiella spp.* 25.0%.

3.2. Efficacy of Antimicrobial Agents Used for Hands and Work Surfaces Disinfection

On the laboratory benches, after disinfection with 0.5% bleach solution, there was no growth on the inoculated media.

The staff's hands remained contaminated despite the washing with soap and water. Before washing, the average number of colonies was 16.7 (min: 0; max: 1000) and after washing this number was 13.6 (min: 1; max: 1050). The difference between the number of colonies observed before and after washing was not statistically significant (p = 0.8). The residual contamination was 3.7%.

4. Discussion

This study aimed to measure the effectiveness of hand washing of laboratory staff with water and mild soap and surfaces disinfection with sodium hypochlorite in bacteriology laboratories in Togo. The results showed that the laboratory benches and sinks were the most contaminated surfaces in the laboratories and *Klebsiella spp.* and *Staphylococcus spp.* were the most commonly identified germs. This result was not surprising since both germs are not to be distributed in the environment [21] [22]. The contamination of workers hands and mainly in microbiology laboratories was also showed by other authors [23].

The laboratory benches disinfection with a 0.5% of sodium hypochlorite solution led to an absence of bacterial growth in culture. The effect of sodium hypochlorite on bacteria growth is also well documented [24].

		After Disinfection				
Isolated Germs	Staff Hands n (%)	Surfaces n (%)	(Hands and Surfaces) before Disinfection n (%)	Staff Hands n (%)	Surfaces n (%)	
Staphylococcus spp.	15 (75.0)	12 (13.6)	27 (25.0)	3 (75.0)	0 (0.0)	
Enterobacteria (Klebsiella spp. and E. coli)	4 (20.0)	51 (57.9)	56 (51.8)	1 (25.0)	0 (0.0)	
Klebsiella spp.	3 (15.0)	39 (44.3)	42 (38.9)	1 (25.0)	0 (0.0)	
Escherichia coli	1 (5.0)	12 (13.6)	13 (12.0)	0 (0.0)	0 (0.0)	
Enterococcus spp.	1 (5.0)	12 (13.6)	13 (12.0)	0 (0.0)	0 (0.0)	
Acinetobacter spp.	0 (0.0)	9 (10.2)	9 (8.3)	0 (0.0)	0 (0.0)	
Streptococcus spp.	0 (0.0)	2 (2.3)	2 (1.8)	0 (0.0)	0 (0.0)	
Pseudomonas aeruginosa	0 (0.0)	2 (2.3)	2 (1.8)	0 (0.0)	0 (0.0)	
Total	20 (100.0)	88 (100.0)	108 (100.0)	4 (100.0)	0 (0.0)	

Table 3. Distribution of germs isolated before and after disinfection of surfaces and staff's hands in bacteriology laboratories in Togo, 2021.

4.1. Microbiological Quality of Surfaces and Staff's Hands before and after Disinfection in Bacteriology Laboratories in Togo, 2021

The bacteria on the surfaces before disinfection were mainly represented by 51.8% of enterobacteria including *Klebsiella spp*. Strains of *Klebsiella spp* are commensal enterobacteria of the digestive and respiratory tracts of animals including humans. In addition enterobacterial are ubiquitous [25]. They are common in faeces and can be an indicator of faecal contamination. The high proportion of enterobacteria is due to the sampling method, according to the literature, the wet swab technique appears to be better at to detect Gram-negative bacilli [26].

Mobile phones of staff had a significant contamination rate in our study. But this result is lower than those obtained in Ethiopia, France and Nepal with respectively 94.2% (213/226), 94.0% (49/52) and 97.0% (97/100) [27] [28] [29]. This difference could be explained by the fact that our work was carried out during the COVID-19 pandemic, when disinfection measures had been reinforced following awareness-raising and training. Laboratories workers mobile phones can be a potential source of contamination for themselves, the community, their families especially their children who touch their parent's phones and without washing their hands send them to the orifices (mouth, eyes, nose and ears). In addition, these mobile phones are a potential source of nosocomial infection [10].

The hand-carried flora was predominantly represented by *Staphylococci*. After disinfection by washing, we noted a persistence of these germs. Our results are consistent with those obtained in Greece by Tselebonis *et al.* in 2016 [30] and by Ravaoarisaina *et al.* (2019) in Madagascar [31] who found the same trend where *Staphylococci* represented more than 50% of the germs. This predomin-

ance of *Staphylococci* on the hands could be explained by the fact that they are commensal bacteria of the epithelia of humans and animals [32]. Furthermore, *Staphylococcus epidermidis* is a skin commensal in almost 100% of humans.

4.2. Effectiveness of Antimicrobial Agents Used for Hands and Work Surfaces Disinfection in Bacteriology Laboratories in Togo, 2021

During this study, it was noted that all the laboratory benches disinfected with the 0.5% bleach solution did not show any bacterial growth. These results confirm the effectiveness of this biocide or disinfectant, especially when used under the proper conditions as indicated by the manufacturer [12]. They are in agreement with those obtained in Madagascar by Ravaoarisaina *et al.* in 2019 who showed the sterility of the bleach disinfected benches [31].

The residual contamination of hands after washing was 3.7%. Our results are in agreement with those obtained in Ireland by Creamer *et al.*, in 2010 where Methicillin-resistant *Staphylococcus aureus* was isolated from 3% fingertips of healthcare workers' hands when hand hygiene was performed [33].

Our study showed less efficacy of hand washing compared to the study conducted in England by Burton *et al*, in 2011 in which the volunteers hand washing showed a significant reduction of germs (p < 0.001) [34]. These results may be explained by the fact that hand washing and drying techniques were not followed by all staff in our study.

For more than twenty years, most studies have consistently shown poor compliance with hand washing in all healthcare sectors, including sectors considered at risk of nosocomial infections such as intensive care units [32]. Overall, compliance calculated in these studies varies between 20% and 50%. Out of the 50% of people who wash their hands frequently, only 25% do so in accordance with the rules of the art by respecting the techniques [30].

Limitations of the Study

Laboratory surfaces (door handles, fridges, sinks, microscope coarse adjustment knobs and stages, and staff mobile phones) were not sampled after disinfection, as these surfaces are not routinely disinfected. Nevertheless, this study showed that the 0.5% sodium hypochlorite (bleach) solution was totally effective in disinfecting benches.

5. Conclusion

In this study, which assessed the effectiveness of disinfectants used in medical bacteriology laboratories in Togo, it emerged that sinks and benches were the most contaminated surfaces, with *Klebsiella spp.* and *Staphylococcus spp.* predominating. The use of 0.5% sodium hypochlorite solution to disinfect benches was effective. However, the risk of contamination by pathogenic bacteria persists because hand washing with water and mild soap is not sufficiently effective. It is therefore necessary to raise staff awareness of the need to comply with good mi-

crobiological practices, and specifically with appropriate hand-washing techniques.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix. Sheet for the Laboratories Data Collection

Sheet N[°]: .../

Section I. LABORATORY INFORMATIONS

Data collection date:/...../2021

Site name: District: Region: Manager surveyed:

Section II. INFORMATIONS ON DISINFECTANTS PRODUCTS

Product	Provider/Brand	Expiry date	Storage time	Storage conditions	Dilutions	
						_

Section III. SAMPLING CARRY-OUT DATA

		BEFORE DISINFECTION							AFTER DISINFECTION										
Sampling	g Sampling sites	ID	Cult			ture results				Colonies number			Culture results				Colonies number		
	Technicians' hands:		1	2	3	4	5	6	7			1	2	3	4	5	6	7	
	• Technician 1	P01								// /_	_///								
skin	• Technician 2	P02								// /_	_///								
	Laboratory assistant	P03								// /_	_///								
	Secretary's hands	P04								// /_	_///								
	Laboratory bench	P05								///_	_///								
	Manipulation room handle	P06								// /_	_///								
	Sampling room handle	P07								// /_	_///								
	Reagent fridge door handle	P08								// /_	_///								////
	Skins in the manipulation room	P09								// /_	_///								
Surfaces	Microscope (fine and coarse) adjustments	P10								_	_//_/								
	Mechanical stage and stage controls	P11								///_	_///								////
	Staff mobile phone	P12								// /_	_///								////
	Inside the autoclave																		
	• Before decontamination	P13								// /_	_///								
	• After decontamination	P14								// /_	_///								
	Bleach tray for soiled objects	P15								// /_	_///								

1: Staphylococci; 2: Enterococci/Streptococci; 3: Enterobacteria; 4: Pseudomonas; 5: Yeasts; 6: Others; 7: No growth.

Section IV. CULTURE RESULTS

BEFORE DISINFECTION	AFTER DISINFECTION	BEFORE DISINFECTION	AFTER DISINFECTION
P1		Р9	
P2		P10	
Р3		P11	
P4		P12	
P5		P13	
P6		P14	
P7		P15	
P8			

Section V. LIST OF GERMS ISOLATED IN THE LABORATORY DURING THE MONTH