

Resistance Profile of Bacteria Isolated from Surfaces and Staff's Hands in Bacteriology Laboratories in Togo, 2021

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ABSTRACT

Background: Antimicrobial resistance involves the ineffectiveness of one or more antibiotics against an infection. Antibiotic-resistant bacteria kill 25,000 people per year in Europe.

Objective: The aim of this study was to describe the resistance profile of bacteria isolated from surfaces and staff's hands in bacteriology laboratories in Togo.

Methods: We conducted a descriptive cross-sectional study that took place from June to December 2021 in all bacteriology laboratories in Togo. Swabs taken from the surfaces and the hands of the staff were immediately inoculated onto the agar media. After 24 hours of incubation, the isolated germs were identified by conventional biochemical tests. Antimicrobial susceptibility test was carried out for each bacterial isolate using Kirby–Bauer disc diffusion method. The frequencies of antibiotic sensitivity and resistance were calculated.

Results: A total of 112 strains were isolated from 291 samples of which, 27.8% were taken from staff's hands. The predominant bacteria were *Klebsiella* spp 38.4%, *Staphylococcus* spp 26.8%, *E. coli* 11.6% and *Acinetobacter* spp 8%. *Klebsiella* spp strains were resistant to amoxicillin/clavulanic acid (20.9%), ceftazidim (11.6%), imipenem (2.3%) and ciprofloxacin (11.6%). *Staphylococcus* spp were resistant to penicillin G (90%), ceftazidim (30%), gentamicin (33.3%) and norfloxacin (20%). All staphylococci were sensitive to vancomycin. *E. coli* strains were resistant to amoxicillin-clavulanic acid (38.4%), ceftazidim (15.4%) and ciprofloxacin (30.7%). *Acinetobacter* spp were resistant to piperacillin (11.1%), piperacillin-tazobactam (11.1%), ceftriaxone (11.1%) and ciprofloxacin (100%). *Pseudomonas aeruginosa* were resistant to ticarcillin (100%), ceftazidim (100%), imipenem (100%), ciprofloxacin (100%) and sensitive to amikacin (100%).

Conclusion: Strains of *Pseudomonas aeruginosa* and *Acinetobacter* spp isolated from laboratory surfaces were highly resistant to ciprofloxacin, an antibiotic commonly used in treatment of infections.

Keywords

Bacteria, Laboratories, Profile, Resistance, Surfaces, Togo.

Introduction

Biological risks are a particular problem in medical biology laboratories because of the diversity of the biological products processed, the variability of their infectious potential and the ways in which they are exposed [1,2]. Microorganisms from the environment or from handled samples can contaminate surfaces (benches, equipment, floors) and the hands of operators. Infections acquired by staff handling these pathogenic microorganisms in biology laboratories have been described in the literature since the mid-1930 years. In Canada in 2016, a study revealed that the incidence of exposure to microorganisms in the laboratory was 3.4% [3]. Some bacteria can persist on environmental surfaces in hospitals and on the hands of healthcare workers, even after cleaning and disinfection [4-6]. These bacteria, which can be multiresistant, can easily be transferred from hospital surfaces to the hands of healthcare workers and then may spread to vulnerable patients in other parts of the hospital [7,8]. In recent years, a large number of studies have been devoted to antibiotic resistance in clinical isolates [9,10]. However, antibiotic resistance in isolates from hospital environments has received less attention [6]. The emergence of multiresistant bacteria is a major public health treat worldwide. Physicians can face considerable difficulties in treating patients infected with these pathogenic bacteria [11-13].

Togo is one of 10 African countries trained by the WHO in June 2017 in the development and implementation of action plans to control antimicrobial resistance [14]. In 2018, following the example of all countries in the world, Togo adopted a national plan to control antimicrobial resistance for the period from 2019 to 2023 [15]. Many studies have been carried out, but few have been published, resulting in a lack of available information. It is important to monitor antimicrobial resistance (AMR) in order to limit it and ensure effective antibiotic therapy. This study looked at the resistance profile of bacteria isolated from surfaces and staff's hands in bacteriology laboratories in Togo, 2021.

Materials and Methods

Study Design and Period

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 [16]. The health system is organized according to a pyramid structure with three levels (central, intermediate and peripheral). In terms of the availability of medical bacteriology laboratories, they are found 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 confessional bacteriology laboratories also exist in all the health regions of Togo. A national network of 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 (Institut National d'Hygiène), the teaching hospitals laboratories, regional laboratories and district laboratories. The study was conducted from June to December 2021.

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).

Two types of samples were taken in the laboratories. Swabs were taken on work surfaces at risk frequently touched by staff (laboratory bench, door's handles, sinks, the microscopes, mechanical stage, stages controls, adjustments, the staff mobile phone, and the inside of the autoclave). The laboratory staff's hands were also swabbed. Isolated bacteria were identified using conventional biochemical tests, and susceptibility was assessed using the Kirby-Bauer method, in accordance with the recommendations of the Antibigram Committee of the French Society of Microbiology EUCAST/CASFM for the current year [17].

Variables of Interest

Several variables were analysed. The microbiological quality of surfaces and staff's hands: presence of bacteria (Staphylococci, Enterobacteriaceae, Pseudomonas, Streptococci, and Enterococci) on the sites was analysed. The antibiotic susceptibility test results: sensitivity, intermediate sensitivity and resistance to the families of antibiotics (beta-lactam, aminoglycosides, macrolides, fluoroquinolones and vancomycin) were used.

Data Collection Technique and Tools

Data were collected by using a questionnaire and laboratory measurements.

Sample Collection and Processing

Two types of samples were taken using sterile swabs: Sterile swabs moistened with sterile physiological water (0.9%) were used to take samples from the palms of the staff's hands, rotating them momentarily over the entire surface of the palm and between the fingers. Sterile swabs moistened with physiological water (0.9%) were used to take samples from the surfaces.

In each laboratory, the swabs taken were immediately inoculated onto agar media (Fresh Blood Agar, Mannitol Salt Agar, Sabouraud Chloramphenicol Agar, Mac Conkey Agar and Brilliance™ UTI chromogenic medium) from Oxoid, UK. Plates were incubated at 35 ± 2°C for 20 ± 4 hours. Isolated bacteria were identified using the morphological characteristics of the colonies, the Gram control, conventional biochemical tests and the Brilliance UTI chromogenic medium, which enabled a presumptive diagnosis to be made [18]. The isolated strains were taken to the bacteriology laboratory of

the Institut National d'Hygiène (INH) for susceptibility testing. The Kirby-Bauer method was used for antibiotic susceptibility testing, following the recommendations of the French AntibioGram Committee EUCAST/CASFM, 2021 [19] for interpretation of the results. The production of extended-spectrum beta-lactamase in enterobacteria was carried out using the disc synergy method (one disc of Amoxicillin + clavulanic acid between two discs of third-generation cephalosporins: Ceftriaxime or Ceftriaxone) with the presence of a champagne cork image. The Cefoxitin disc was tested to detect the resistance to meticillin in Staphylococci strains. A reference strain of *Escherichia coli* ATCC 25922 was used to ensure quality control of the antibiogram.

Statistical Analysis

The frequencies of contamination of surfaces, staff's hands and of resistance of isolated bacteria were estimated.

Results

A total of 291 samples were taken, of which 27.8% were taken from the hands of staff, and 112 strains were isolated from the various samples taken. Figure 1 shows the distribution of the laboratories surveyed throughout the country.

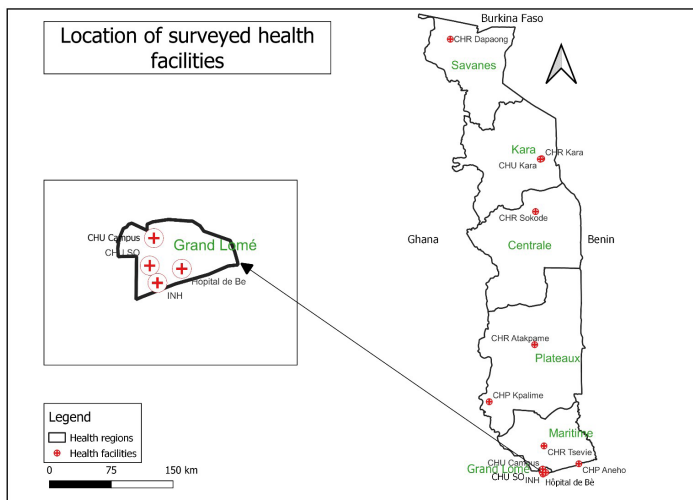


Figure 1: Map of Togo showing regions with bacteriology laboratories visited.

Proportion of Contaminated Sites in the Bacteriology Laboratories in Togo, 2021

Of 210 surfaces samples collected, 40.5% (88/210) were contaminated compared with 29.6% (24/81) of staff's hands. Sinks and benches were the most contaminated with 66.6% and 61.9% respectively. Figure 2 illustrates the proportion of contamination of surfaces and staff's hands in bacteriology laboratories in Togo.

Bacteria Isolated from Surfaces and Staff's Hands in Bacteriology Laboratories in Togo, 2021

Of 112 strains isolated, *Klebsiella spp* accounted for 44.3% (39/88) on surfaces and *Staphylococcus spp* for 75.0% (18/24) on staff's hands. Table 1 shows the distribution of bacteria isolated

from work surfaces and staff's hands in bacteriology laboratories in Togo.

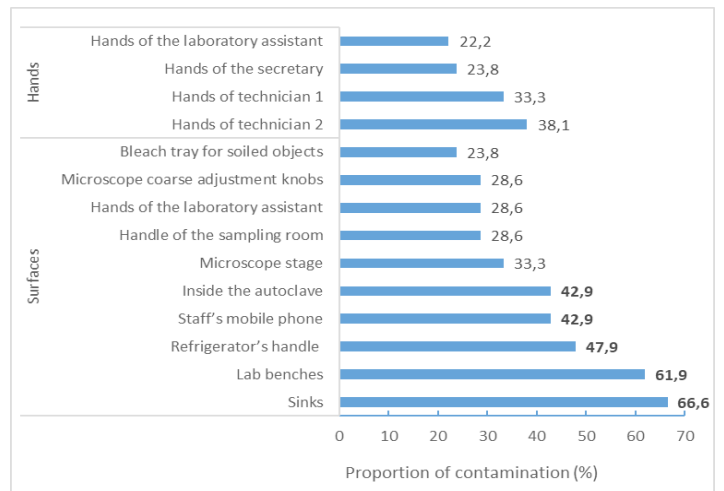


Figure 2: Proportion of contamination of surfaces and staff's hands in bacteriology laboratories in 346 Togo, 2021.

Table 1: Distribution of germs isolated from surfaces and staff's hands in the bacteriology laboratories in Togo, 2021.

Isolated bacteria	Staff's hands n (%)	Surfaces n (%)	Total n (%)
<i>Klebsiella spp</i>	4 (16.6)	39 (44.3)	43 (38.4)
<i>Staphylococcus spp</i>	18 (75.0)	12 (13.6)	30 (26.8)
<i>Escherichia coli</i>	1 (4.2)	12 (13.6)	13 (11.6)
<i>Enterococcus spp</i>	1 (4.2)	12 (13.6)	13 (11.6)
<i>Acinetobacter spp</i>	0 (0.0)	9 (10.2)	9 (8.0)
<i>Streptococcus spp</i>	0 (0.0)	2 (2.3)	2 (1.8)
<i>Pseudomonas aeruginosa</i>	0 (0.0)	2 (2.3)	2 (1.8)
Total	24 (100.0)	88 (100.0)	112 (100.0)

Table 2: Resistance profile of Gram negative bacilli isolated from surfaces and staff's hands in 360 bacteriology laboratories in Togo, 2021.

Antibiotics discs tested	<i>Klebsiella spp</i> (n=43)	<i>E. coli</i> (n=13)	<i>Pseudomonas aeruginosa</i> (n=2)	<i>Acinetobacter spp</i> (n=9)
Ticarcillin (TIC)	15 (34.9%)	6 (46.1)	2 (100.0)	1 (11.1)
Ticarcillin + clavulanic acid (TCC)	-	-	2 (100.0)	1 (11.1)
Amoxicillin (AMX)	15 (34.9)	6 (46.1)	-	-
Amoxicillin + clavulanic acid (AMC)	9 (20.9)	5 (38.5)	-	-
Ceftriaxon (CRO)	5 (11.6)	2 (15.4)	-	1 (11.1)
Ceftazidim (CAZ)	5 (11.6)	2 (15.4)	2 (100.0)	1 (11.1)
Cefepim (FEP)	5 (11.6)	2 (15.4)	2 (100.0)	1 (11.1)
Imipenem (IMP)	1 (2.3)	0 (0)	2 (100.0)	0 (0)
Ertapenem (ERT)	1 (2.3)	0 (0)	-	-
Chloramphenicol (CHL)	5 (11.6)	4 (30.8)	-	-
Amikacin (AK)	0 (0)	0 (0)	0 (0)	-
Tobramycin (TOB)	-	-	0 (0)	-
Gentamicin (GM)	2 (4.6)	1 (7.7)	-	2 (22.2)
Norfloxacin (NOR)	5 (11.6)	4 (30.8)	-	-
Ciprofloxacin (CIP)	5 (11.6)	4 (30.8)	2 (100.0)	9 (100.0)

« - » : means: Antibiotic disc not tested

Resistance Profile of Bacteria Isolated from Surfaces and Staff's Hands in Bacteriology Laboratories in Togo, 2021

Penicillin G resistance was 90% in *Staphylococcus spp.* and ciprofloxacin resistance was 100% in strains of *Pseudomonas aeruginosa* and *Acinetobacter spp.* Tables 2 and 3 illustrate the sensitivity profiles of Gram-negative bacilli and Gram-positive cocci isolated from surfaces and staff's hands in bacteriology laboratories in Togo, 2021.

Table 3: Resistance profile of Gram-positive cocci isolated from surfaces and staff's hands in 365 bacteriology laboratories in Togo, 2021.

Antibiotics discs tested	<i>Staphylococcus spp</i> (n=30)	<i>Enterococcus spp</i> (n=9)	<i>Streptococcus spp</i> (n=2)
Penicillin G (TIC)	27 (90.0)	-	0 (0.0)
Ampicillin (AMP)	-	0 (0.0)	-
Cefoxitin (FOX)	9 (30.0)	-	-
Imipenem (IMP)	-	9 (100.0)	-
Gentamycin (GN)	10 (33.3)	1 (7.7)	0 (0.0)
Erythromycin (E)	12 (40.0)	2 (15.4)	1 (50.0)
Chloramphenicol (CHL)	10 (33.3)	0 (0.0)	1 (50.0)
Norfloxacin (NOR)	6 (20.0)	1 (7.7)	1 (50.0)
Vancomycin (VA)	0 (0.0)	0 (0.0)	0 (0.0)

« - » : means: Antibiotic disc not tested

Discussion

The study showed that 40.5% of surfaces were contaminated, compared with 29.6% on staff's hands. Sinks and benches were the most contaminated, with 66.6% and 61.9% respectively. A total of 112 strains were isolated. *Klebsiella spp* (44.3%) was the main strain isolated from surfaces, and *Staphylococcus spp* (75.0%) from hands. *Staphylococcus spp* were resistant to penicillin in 90% of cases, *Pseudomonas aeruginosa* and *Acinetobacter spp* were resistant to ciprofloxacin in 100% of cases. The main limitation of this study lies in the fact that only some of the high-risk surfaces were sampled in the laboratories surveyed. However, it was possible to estimate the profile of antibiotics resistance.

Proportion of Contaminated Sites in Bacteriology Laboratories in Togo

In this study, laboratory work surfaces were the most contaminated. Indeed, the sinks and work benches in the laboratories are usually in contact with most of samples handled and staff's hands. These samples often contain the bacteria that cause infections. The strains present on the surfaces were predominantly Enterobacteriaceae, including *Klebsiella spp* (44.3%). This rate is lower than that found in Benin in a study assessing the risk of infection in medical analysis laboratories by Hounsa *et al.* in 2015 where enterobacteria (*E. coli*) represented 83.3% [20]. Our results could be explained by the fact that the sampling in our study only concerned bacteriology laboratories, whereas in Benin, samples were taken from surfaces in all diagnostic units of the hospital. In addition, our study was carried out at the height of the COVID-19 pandemic, when preventive measures had been strengthened through awareness-raising and training. Manuported flora was predominantly represented by strains of *Staphylococcus spp*. This same trend was observed by Hounsa *et*

al. in Benin in 2015 [20]. *Staphylococcus* strains are ubiquitous bacteria present at several sites in the environment, particularly human skin. Metagenomic sequencing surveys and traditional culture methods have demonstrated that coagulase-negative staphylococci (CNS) are one of the most abundant colonisers of all skin sites [21,22]. The presence of germs on surfaces and staff's hands is a potential source of healthcare-associated infection (HAI) transmission [23-28].

Resistance Profile of Bacteria Isolated from Surfaces and Staff's Hands in Bacteriology Laboratories in Togo

The bacteriological profile of the bacteria isolated was marked by a predominance of Gram-negative bacteria. This trend has been confirmed by other studies [29,30]. Sensitivity data showed that *Staphylococcus spp* strains were highly resistant to penicillin G (90.0%). This result is in agreement with that of Gonsu Kamga in 2013 in Cameroon (92.8%) [30] and Ouédraogo in 2011 in Burkina-Faso (76.5%) [29]. The *Staphylococcus spp* strains resistance to Gentamycin were 33.3% which result is consistent to the result found by Firesbhat (34.1%) in Ethiopia [31]. The high frequencies of resistance of enterobacteriaceae (*Klebsiella spp* and *E. coli*) to amoxicillin (34.9% and 46.1%) and amoxicillin-clavulanic acid (20.9% and 38.4%) could be due to self-medication and over-prescription of antibiotics. The total resistance to ciprofloxacin (100%) in non-fermenting Gram-negative bacilli is very alarming, and may reflect the nosocomial nature of these strains. This resistance to fluoroquinolones (ciprofloxacin, levofloxacin) could be linked to the selection pressure exerted by the abusive use of these molecules in both outpatient and hospital settings. The *Pseudomonas aeruginosa* resistance in our work is in agreement with that of Wang (95.8%) [6].

Conclusion

The antimicrobial susceptibility of isolated bacteria in surfaces and staff's hands is low. The resistance of *Staphylococcus spp* were high to penicillin G and the ciprofloxacin was totally ineffective against non fermentative Gram negative bacilli (*Pseudomonas aeruginosa* and *Acinetobacter spp*). The presence of resistant bacteria to several antibiotics on surfaces and staff's hands can be a source of contamination and lead to serious infections, with longer and more expensive treatment, and sometimes even be life-threatening for the patient. It is therefore necessary to monitor the use of these molecules or to replace them with other molecules or combinations of molecules for effective treatment of infections caused by these pathogens.

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