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Carriage and Antimicrobial Susceptibility Profile of Bacterial Strains Isolated in the Pediatric Departments of a Referral Hospital in Yaoundé, Cameroon

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ABSTRACT

Introduction: *Healthcare-associated infections (HAIs) represent a major public health threat, contributing significantly to antimicrobial resistance worldwide. In pediatric departments, patients are particularly vulnerable due to immature immune systems. The present study aimed to isolate and identify bacterial strains in the pediatric services at a referral hospital in Yaoundé, Cameroon, and determine their antimicrobial susceptibility profiles.*

Methods: *A descriptive cross-sectional study was conducted from February to March 2023 in the pediatric units of the Chantal Biya Foundation – Yaoundé Central Hospital. A total of 56 environmental samples were collected from various surfaces after equipement: and medical equipement, Swabbing was performed using sterile swabs moistened with normal saline solution, and samples were transported to the Bacteriology Unit of the Center for the Study and Control of Infectious Diseases for microbiological analysis. Bacterial identification was performed using API 20E and API 20NE strips, and antibiotic susceptibility testing was conducted using the Kirby-Bauer diffusion method following CA-SFM 2022 guidelines. Data were analyzed using SPSS version 26.0.*

Results: *The overall culture positivity rate was 43/56(77%). Beds (100%), intravenous poles (100%), pulse oximeters (100%), and soap dishes (100%) were the most contaminated items, followed by door handles (88%) and trolleys (88%). The cardiology-pulmonology unit and neuro-nephrology department showed the highest number of positive cultures. A total of 50 bacterial isolates were identified, comprising Gram-positive cocci, Enterobacteriaceae, and non-fermenting Gram-negative bacilli. Staphylococcus aureus was the most frequently isolated bacterium (n=13, 26%), followed by Raoultella ornithinolytica (n=9, 18%) and Enterobacter cloacae (n=5, 10%). Other isolates included Klebsiella pneumoniae (n=4), Burkholderia cepacia (n=4), Serratia phymuthica (n=4), and various Serratia, Citrobacter, and Enterobacter species. Antimicrobial susceptibility testing revealed high resistance rates. All S. aureus strains showed 100% resistance to penicillin G, oxacillin, ceftriaxone, levofloxacin, and ciprofloxacin. Among Enterobacteriaceae, R. ornithinolytica exhibited 100% resistance to amoxicillin-clavulanic acid, amoxicillin, ceftazidime, cefotaxime, gentamicin, and chloramphenicol. Enterobacter species were 100% resistant to amoxicillin-clavulanic acid, ceftazidime, and cefotaxime.*

Conclusion: *This study demonstrates high levels of bacterial contamination and high rates of antibiotic resistance. These findings highlight urgent gaps in hospital hygiene protocols and the critical need for reinforced infection prevention measures, including improved cleaning procedures and continuous microbiological surveillance to protect vulnerable pediatric populations from healthcare-associated infections.*

Keywords
Healthcare-associated infections, Antimicrobial resistance, Pediatric department, *Staphylococcus aureus*, Multidrug-resistant bacteria, Infection prevention and control, Environmental contamination, Cameroon.

Introduction
Healthcare-associated infections (HAIs) are a major public health problem, contributing to the global threat of antimicrobial resistance. At any given time, more than 1.4 million people worldwide suffer from infections contracted in hospitals. With an estimated 10 million annual deaths by 2050, this is set to become the world's leading cause of mortality [1].

High antibiotic consumption, overcrowding, and patient vulnerability make hospitals a focal point for the transmission of bacteria, particularly antibiotic-resistant strains [2]. The surfaces of the hospital environment play a role in these phenomena, serving as potential reservoirs and relays for the agents responsible for HAIs [3]. While several pathogens (parasites, viruses, fungi, and bacteria) can cause these infections, bacteria are the most frequent [4]. Various factors, such as the specific hospital ward, the immune status of patients, and antibiotic resistance, are decisive in the occurrence of HAIs [5].

A study conducted in Ivory Coast revealed that *Klebsiella pneumoniae* (38.98%) and *Staphylococcus aureus* (23.70%) were the primary bacteria found, followed by *Pseudomonas aeruginosa* (18.64%), as bacteria of medical interest in the care environment of the Anesthesia and Intensive Care Department at Treichville University Hospital [6]. In 2011, in Cameroon, the prevalence of nosocomial infections was 12% in the intensive care units of Laquitinie Hospital [7]. The germs responsible for HAIs can originate from the patient, the staff, or the hospital environment; the latter is considered less decisive than the first two sources. However, the environment can be contaminated by staff or patients and consists of medical equipment (various respiratory assistance and intravascular monitoring devices, instruments, tubing), surfaces (sinks, beds, mattresses, and linens), and ambient air [8].

The germs responsible for these infections show increased resistance to antibiotics. Antibiotic resistance is a global phenomenon, and the emergence of multidrug-resistant (MDR) bacteria is a public health emergency not only in healthcare facilities but also in everyday life, where these bacteria spread rapidly and become an even more significant source of serious infectious risk. Multidrug resistance thus represents a step toward therapeutic deadlock. Monitoring the microbiological quality of the hospital environment is one of the pillars of the policy to combat nosocomial infections [9]. To date, the exact role of the environment in the occurrence of HAIs is not precisely known. This lack of data is linked to methodological difficulties in establishing a causal link between environmental contamination and healthcare-associated infections. The objective of this study was to identify the germs present in the healthcare environment as well as their antibiotic sensitivity profile in various pediatric departments, with the aim to inform decision-making and better prevent the occurrence of healthcare-associated infections.

Materials and Methods
Study Setting
This descriptive cross-sectional study was conducted from February to March 2023 within the pediatric units of a referral hospital in Yaoundé, Cameroon.

Participants
The study focused on hospital surfaces and medical devices found across various pediatric units. Sampled sites were selected based on the high-risk contact with healthcare workers, patients and other users.

Sampling
Samples were collected from several pediatric sub-units: infectious diseases, pediatric emergency, pediatric intensive care, intermediate care (sickle cell unit), cardiology-pulmonology, nutrition, neuro-endocrinology, and neuro-nephrology. A total of 56 samples were collected from: Hand-wash basins (10), door handles (8), beds (11), soap dishes (1), trolleys (8), Intravenous poles (5), scales (2), pulse oximeters (1), oxygen extractors (2), stethoscopes (4), trays (3), and hands (1).

Sampling technique
Samples were collected early in the morning. Each sterile swab, pre-moistened with normal saline, was rubbed or swept across surfaces and medical equipment for approximately five seconds. The technique involved creating parallel, closely spaced streaks while slightly rotating the swab. The same area was then swabbed again using streaks perpendicular to the first ones. The swabs were then replaced in their protective cases and transported to the laboratory within fifteen minutes for bacteriological analysis.

Microbiological analysis
Samples were processed according to national standardized procedures.

Culture: On the first day post-collection, swabs were inoculated into brain-heart infusion broth and incubated for 18–24 hours. They were then sub-cultured on standard media: Eosin Methylene Blue (EMB) agar for the isolation of Gram-negative bacilli and Chapman (Mannitol Salt) agar for Gram-positive cocci.

Identification: Bacteria were identified using API 20E strips for *Enterobacteriaceae* and API 20 NE strips for non-fermenting Gram-negative bacilli. For the identification of *Staphylococci*, a coagulase test was performed.

Antibiotic susceptibility testing (AST): The AST was performed following the CA-SFM (2022 edition) guidelines, using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. Seventeen (17) antibiotic disks (OXOID) were tested: amoxicillin (30 µg), amoxicillin + clavulanic acid (30 µg), penicillin G (10 µg), imipenem (10 µg), oxacillin (1 µg), ceftazidime (30 µg), Cefotaxime (30 µg), ceftriaxone (30 µg), gentamicin (30 µg), amikacin (30

µg), ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), chloramphenicol (10 µg), erythromycin (15 µg), azithromycin (15 µg), and fusidic acid (30 µg).

Statistical analysis

Collected data were analyzed using SPSS 26.0 software.

Ethical Considerations

An ethical clearance was obtained from the Institutional Review Board of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1 under the number: No.0408/UYI/FMSB/VDRC/DAASR/CD.

Results

Microbiological analysis

The overall culture positivity rate was 77% (43/56). The most contaminated items included beds, intervenous poles, pulse oximeter, door handles, hand-wash basins and trolleys with 80-100% samples contaminated (Table 1).

Table 1: Culture positivity by sampling sites in the pediatric departments, Yaoundé, 2023 (n = 56).

Sampling site	Number of samples	Positive culture (n)	Frequency (%)
Hand-wash basins	10	8	80
Door handles	8	7	88
Beds	11	11	100
Hands	1	0	0
Soap dishes	1	1	100
Trolleys	8	7	88
Intravenous poles	5	5	100
Scales	2	0	0
Pulse oximeters	1	1	100
Respirators	2	1	50
Trays	3	2	67
Stethoscopes	4	0	0
Total	56	43	77

The number of positive cultures was highest in the Cardiology-Pulmonology (n=10) and Neuro-Nephrology departments (n = 7) (Figure 1).

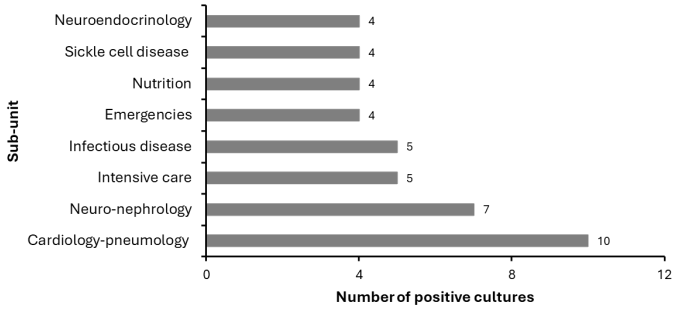

Figure 1: Positive cultures by sub-units of the pediatric departments, Yaoundé, 2023 (n = 43).

A total of 50 bacterial isolates were identified from the 43 positives cultures. The Gram-positive cocci *Staphylococcus aureus* (n = 13) was the most frequently identified bacterial isolate. Among the *Enterobacteriaceae*, *Raoultella ornithinolytica (n = 9)* and *Enterobacter cloacae* were the most frequently isolated (n = 5). *Burkholderia cepacia* (n = 4) and *Pseudomonas luteola* (n = 1) were the non-fermenting Gram-negative bacilli isolated (Figure 2).

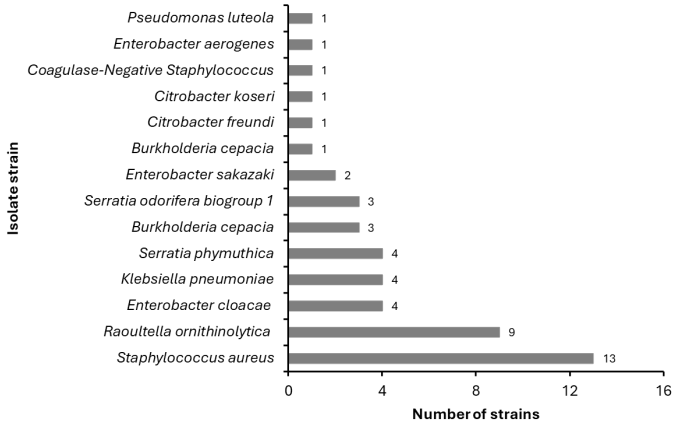

Figure 2: Patterns of isolated strains in the pediatric departments, Yaoundé, 2023 (n = 50).

Sensitivity profile to antimicrobials

Raoultella ornithinolytica showed 100% resistance to amoxicillin/clavulanic acid, amoxicillin, ceftazidime, cefotaxime, gentamicin, and chloramphenicol; no strains of *Klebsiella pneumoniae* were resistant to imipenem or amikacin.

All *Serratia* strains were 100% resistant to amoxicillin/clavulanic acid; only the *Serratia odorifera* strain showed resistance to imipenem (68%), gentamicin (33%), and amikacin (33%).

Discussion

Healthcare-associated infections (HAIs) represent a significant threat to patient safety. They occur in hospitalized patients, leading to serious complications, increased length of stay and hospital costs, and even higher mortality rates [10]. Medical equipment and surfaces are colonized by germs that can cause these infections. Such microorganisms may originate from healthcare staff, patients, air, or visitors. A bacteriological study of various samples taken from the healthcare environment allows for the identification of the different germs constituting the microbial reservoir at the root of these infections [6]. These reservoirs serve as a basis for evaluating the quality of hospital hygiene.

Microbial flora of the healthcare environment

The highest number of positive cultures was observed in the Cardiology-Pulmonology unit. This high positivity rate may be explained by observed failures in the cleaning system or by the interactions between healthcare staff, caregivers, and visitors, given the type of patients treated in this unit [8]. To reduce this positivity rate, emphasis must be placed on hygiene protocols

Table 2: Resistance profile of *Enterobacteriaceae* isolated in the healthcare environment of the pediatric department, Yaoundé, 2023.

Antibiotics tested	Enterobacteria n (%)									
	Raoultella ornithinolytica	***Klebsiella pneumoniae***	***Serratia phymuthica***	***Serratia. odorifera***	***Serratia marcescens***	***Enterobacter cloacae***	***Enterobacter sakazaki***	***Enterobacter aeorogens***	***Citrobacter freundi***	***Citro-bacter koseri***
Number of isolates	9 (100)	4 (100)	4 (100)	3 (100)	1 (100)	5 (100)	2 (100)	1 (100)	1 (100)	1 (100)
Penicillin										
Amoxicillin	9 (100)	2 (50)	2 (50)	3 (100)	1 (100)	4 (80)	1 (50)	1 (100)	0 (0)	1 (100)
Amoxicillin + clavulanic acid	9 (100)	4 (100)	4 (100)	3 (100)	1 (100)	5 (100)	2 (100)	1 (100)	1 (100)	1 (100)
Carbapenem										
Imipenem	5 (55)	0 (0)	0 (0)	2 (68)	0 (0)	3 (60)	0 (0)	0 (0)	0 (0)	0 (0)
Cephalosporins										
Ceftazidime	9 (100)	2 (50)	2 (50)	1 (33)	1 (100)	5 (100)	2 (100)	1 (100)	0 (0)	1 (100)
Cefotaxime	9 (100)	2 (50)	2 (50)	1 (33)	1 (100)	5 (100)	2 (100)	1 (100)	0 (0)	1 (100)
Aminoglycosides										
Gentamicin	9 (100)	0 (0)	0 (0)	1 (33)	0 (0)	3 (60)	1 (50)	1 (100)	0 (0)	1 (100)
Amikacin	5 (55)	0 (0)	0 (0)	1 (33)	0 (0)	2 (40)	1 (50)	1 (100)	0 (0)	0 (0)
Quinolone										
Levofloxacin	2 (22)	2 (50)	2 (50)	0 (0)	0 (0)	2 (40)	0 (0)	1 (100)	0 (0)	1 (100)
Ciprofloxacin	6 (67)	1 (25)	1 (25)	1 (33)	0 (0)	3 (60)	1 (50)	1 (100)	0 (0)	1 (100)
Ofloxacin	5 (55)	1 (25)	1 (25)	0 (0)	0 (0)	4 (80)	1 (50)	1 (100)	0 (0)	1 (100)
Phenicol										
Chloramphenicol	9 (100)	1 (25)	1 (25)	1 (33)	1 (100)	2 (40)	0 (0)	1 (100)	0 (0)	0 (0)

within the department and on the strict adherence to professional conduct and hygiene practices by patients, visitors, staff, and caregivers alike [11-13].

Beds were found to be the most contaminated surfaces, while trolleys were the most affected among medical equipment. Beds constitute a reservoir for germs because they are surfaces frequently neglected by cleaning teams. Indeed, in healthcare facilities, beds are often cleaned only when transitioning from one patient to another. This can lead to increased microbial growth during long hospitalizations, and improper cleaning makes the bed a vector for the spread of germs within the ward. Similarly, trolleys used to transport medication and nursing equipment can serve as transmission vectors from one patient to another if hand hygiene precautions—such as handwashing, wearing gloves, and using alcohol-based hand rub—are not systematically followed [14]. Our results corroborate earlier findings in major private and public referral hospitals in Cameroon, where handwashing stations and trolleys were the most contaminated sites [8,15]. The high prevalence of bacteria on most surfaces may be due to poor hygiene and the use of inappropriate cleaning products.

Staphylococcus aureus was the most frequently isolated microbial agent. It possesses a high capacity to adapt to both hospital environments and human hosts. *Staphylococcus aureus* is part of the commensal microbiota of the nasal mucosa in approximately 20% to 40% of the general population [16,17]. It is the species most commonly involved in healthcare-associated infections (HAIs). It affects patients with disrupted skin and mucosal barriers, such as individuals with chronic diseases or weakened immune systems, particularly newborns and premature infants [17,18]. An observation made at the Yaoundé University Hospital identified *S. aureus* as a common pathogen in healthcare-associated skin and soft tissue infections [19]. Our results are similar to those reported at the *Centre Médical le Jourdain, the gynecological department of a referral hospital* in Yaoundé and the Douala General Hospital, where *S. aureus* was the most isolated strain [8,15,20].

Among the Enterobacteriaceae, *R. ornithinolytica* and *E. cloacae* were the most frequently isolated in the care environment. *E. cloacae* can be associated with various infections, such as respiratory tract infections, enteric fever syndrome, and pancreatitis; the presence of these strains may result from environmental contamination by fecal flora, indicating a failure in environmental maintenance processes. *R. ornithinolytica* has been found in aquatic environments, soil, insects, fish, ticks, and termites [21,22]. This bacterium converts histidine into histamine, causing histamine poisoning accompanied by skin flushing, better known as under the name of scombroid syndrome associated with fish poisoning [23,24]. In addition to skin flushing, this syndrome can cause vomiting, diarrhea, headaches, or pruritus, depending on the amount of histamine ingested [25]. The incidence of human diseases associated with *R. ornithinolytica* is low, with few clinical infection cases requiring treatment reported to date. The low prevalence of *R. ornithinolytica* infections in literature could be explained by the difficulties encountered in correctly identifying this species using conventional methods.

Regarding non-fermenting Gram-negative bacilli, *B. cepacia* was the most frequently isolated. These bacteria most often cause pneumonia, ear, nose, and throat (ENT) infections, ocular infections, urinary tract infections, septicemia, and secondary

bacteremia linked to the contamination of catheters and injectable solutions [26-28]. Moreover, this germ has been as the leading cause of severe infections among immunocompromised patients in Burkina-Faso [29].

The germs found on surfaces also depend on air quality, as airborne particles eventually settle on surfaces. Therefore, surface sampling in a room reflects not only the quality of bio-cleaning but also the effectiveness or failure of the air treatment system. The contamination of the hospital environment is primarily characterized by the interaction between the bio-contamination of surfaces, hands, and airborne contamination. This interaction is facilitated within the environment by the sedimentation of airborne germs onto surfaces, floors, medical equipment, or bedding [30].

Among the non-fermenting Gram-negative bacilli, *Pseudomonas luteola* was resistant to most antibiotics tested, and *Burkholderia cepacia* was 100% resistant to amoxicillin/clavulanic acid (Table 3).

Table 3: Resistance profile of isolated non-fermenting Gram-negative bacilli in the pediatric departments, Yaoundé, 2023.

Tested antibiotics	Non-fermenting Gram-negatives n (%)	
	Burkholderia cepacia	***Pseudomonas luteola***
Number of isolates	**4 (100)**	**1 (100)**
Penicillins		
Amoxicillin	1 (25)	1 (100)
Amoxicillin + clavulanic acid	4 (100)	0 (0)
Carbapenems		
Imipenem	0 (0)	1 (100)
Cephalosporins		
Ceftazidime	2 (50)	1 (100)
Cefotaxime	0 (0)	0 (0)
Aminoglycosides		
Gentamicin	0 (0)	1 (100)
Amikacin	0 (0)	0 (0)
Quinolones		
Levofloxacin	1 (25)	1 (100)
Ciprofloxacin	0 (0)	1 (100)
Ofloxacin	4 (100)	1 (100)
Phenicols		
Chloramphenicol	0 (0)	1 (100)

Among the isolated Gram-positive Cocci, *Staphylococcus aureus* was resistant (100%) to Penicillin G, oxacillin, ceftriaxone, levofloxacin, and ciprofloxacin (Table 4).

Table 4: Resistance profile of isolated *Staphylococcus spp.* in the pediatric departments, Yaoundé, 2023.

Tested antibiotics	***Staphylococcus* spp. n (%)**	
	Staphylococcus aureus	**Coagulase-negative *Staphylococcus***
Number of isolates	**13 (100)**	**1 (100)**
Penicillins		
Penicillin G	13 (100)	1 (100)
Oxacillin	13 (100)	1 (100)
Cephalosporins		
Ceftriaxone	13 (100)	1 (100)
Aminoglycosides		
Gentamicin	4 (31)	0 (0)
Amikacin	10 (77)	0 (0)
Quinolones		
Levofloxacin	13 (100)	1 (100)
Ciprofloxacin	13 (100)	1 (100)
Macrolides		
Erythromycin	11 (85)	0 (0)
Azithromycin	12 (92)	0 (0)
Fusidanes		
Fusidic acid	11 (85)	0 (0)
Phenicols		
Chloramphenicol	5 (38)	1 (100)

Sensitivity profile of isolated strains

The *S. aureus* strains were 100% resistant to Penicillin G and Oxacillin. They were also 100% resistant to the macrolides and quinolones tested in our study. Our results are similar to those found by Ango et al. in their 2020 study in the intensive care units of Treichville, Côte d'Ivoire, which reported 100% resistance to Penicillin G, 78.5% to Oxacillin, and 92.8% to Erythromycin [31]. Our results also corroborate findings from a gynecological department of a referral hospital in Yaoundé [8]. This high resistance rate may be due to the inappropriate use of antibiotics by the population [32]. It is therefore crucial to implement strategies to combat penicillin-resistant staphylococci.

Among the non-fermenting Gram-negative bacilli, *B. cepacia* strains were 100% resistant to amoxicillin-clavulanic acid and 50% resistant to ceftazidime; they showed a low resistance rate (25%) to the other antibiotics tested, specifically amoxicillin and levofloxacin. *P. luteola*, isolated from the IV pole, was resistant to several molecules tested, with 100% resistance to imipenem, ceftazidime, gentamicin, and all tested quinolones.

The study of antibiotic resistance profiles shows that the same bacteria are circulating within the hospital environment. It is therefore urgent that measures be taken to improve hospital hygiene. These results highlight the potential danger these bacteria pose to the health of all patients, healthcare and administrative staff, and visitors. These resistances are the result of selection pressure, and their impact is significant as they risk contaminating patients, potentially leading to fatalities or even outbreaks.

Limitations

As study was conducted in a specific department, the results may not accurately reflect the situation in the entire health facility. In addition, the limited sample size may limit the generalizability of findings. These limitations highlight the need for a more comprehensive study with a larger sample size.

Conclusion

Several germs were isolated across the various pediatric

departments and handwashing stations, beds, and trolleys were the most contaminated sites. A high rate of antibiotic resistance was observed. Surfaces and medical devices serve as reservoirs for bacteria that cause healthcare-associated infections (HAIs) and their presence reflected insufficient observance of hospital hygiene measures. Controlling hospital bacterial ecology requires mandatory and sustained hospital hygiene protocols, alongside best practices in antibiotic therapy.

Acknowledgements

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Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1 and an ethical clearance issued (No.0408/UYI/FMSB/VDRC/DAASR/CD).

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