Original Research Article

Strengthening environmental hygiene for healthcare-associated infections prevention in maternity ward: Outstanding findings from a multisite survey in the Ndé Division, West Cameroon

ABSTRACT

Background: In health facilities, mothers and their children are amongst the most vulnerable groups likely to contract healthcare-associated infections.
Aim: The present investigation aimed at characterizing bacterial flora (profile, load, antibiotic susceptibility) in the maternity premises of the Bangangté District Hospital (BDH) and the "Université des Montagnes" Teaching Hospital (UdMTH).
Methode: From September 2nd through November 2nd, 2019, it was worked on surfaces and bacteria of air. Specimen collection was conducted by surface swabbing and passive adhesion of air-borne bacteria, respectively. Isolation, enumeration and identification and susceptibility tests were carried out according to standard bacteriological protocols.
Results: Out of the 126 specimens collected and screened, 98.4% resulted in positive cultures. A total of 168 isolates were then recovered, consisted of *Staphylococcus* spp. (68% and 51%), Gram-positive rods (30% and 35%) and Gram-negative rods (2% and 14%), in the UdMTH and BDH, respectively. Bacterial profile recorded were almost similar in all specimens subjected. Bacterial loads varied greatly (as low as < 10 CFU/cm² for surfaces or < 283 CFU/60 mm diameter Petri dish for air and as high as > 200 CFU/cm² for surfaces or > 848 CFU/60 mm diameter Petri dish for air). Bacterial loads appeared to be likely in connection with local activity density. Susceptibility tests revealed high resistance rates while Imipenem was most potent.
Conclusion: Overall findings are reliable clue that could guide advocacy for infections prevention through mitigation of contamination risks in health facilities.

Keywords: Healthcare Associated Infections, Bacteria, Maternity, Surface, Ambient air, Health environment

Comment [h1]: Arrange alphabetically

1. INTRODUCTION

Observed at the global scale, healthcare facilities are conducive ecosystems that promote selection and spread of etiologies of healthcare associated infections (HAIs) [1]. In this environment, HAIs are caused by infectious agents that easily disseminate through (but not limited to) hands, equipment, medical devices and air [2,3]. Also referred to as nosocomial infections, a healthcare associated infection are disorders caused by microorganisms contracted during a stay in a healthcare facility [4]. They have long been serious causes of concern for hospitalized patients in connection with their frequency, their severity and the related economic burdens. Moreover, and over time, patients with HAIs undergo progressive weakening of their immune system which eventually increase mortality and morbidity rates in susceptible hosts [5]. The risk of developing life-threatening hospital bacterial infections increases when the agents are resistant available and affordable drugs [3,6]. Globally, the resistance rates amongst potential etiologies of HAIs are high and include those recorded with methicillin-resistant *Staphylococcus*, multidrug resistance Gram-negative rods (*Klebsiella* spp., *Enterobacter* spp., *Acinetobacter* spp., *Pseudomonas* spp.), and vancomycin-resistant *Enterococcus* [7].

Among the barriers set up against HAIs in healthcare facilities nowadays the quality of patient care is a priority. This is otherwise, firmly associate with the management of healthcare environments in order to mitigate the selection and spread of potential infectious agents. These options are provided by the general policy in force in each site and serves as driver to the concept of "hospital hygiene", fundamental in controlling microbial populations in the patient's environments [4]. Another one relies on the surveillance of antibiotic resistance trends in the hospital environment. Although the role of antibiotics is proved to be very valuable in the fight against bacterial diseases in living systems, their use in healthcare environments, animal husbandry and crop production is regarded as engine for selection of resistance traits. Their use in prophylaxis and growth supplementation, for instance, are common practices in resource-limited communities since members can hardly afford biological evidences prior to drug administration. In turn, this practice further threatens the validity of available therapeutic arsenal [8] in a vicious cycle.

Mitigating HAIs is primordial in high-risk environments like maternity wards where potential hosts are typically vulnerable. In 2011, HAIs were the third leading cause of maternal mortality and the second leading cause of early neonatal mortality. They were estimated at 10.9% in Senegal, 12% in Côte d'Ivoire, 10% in Benin, 14% in Mali and 12% in Cameroon [9]. Mastering the environmental microbiological flora to which mothers and children are exposed during hospital stay appears paramount requirement for safer healthcare services. Within the framework aiming at monitoring the bacterial flora in the global strategy targeting mitigation of resistant HAIs, the present survey was initiated. It specially aimed at characterizing the bacterial populations in the maternity units and disclosing the antibiotic susceptibility/resistance profile of those that are potential etiologies of HAIs. This work is part of a broader program investigating tools likely to contribute to the strengthening of quality care at the "Université des Montagnes" teaching hospital and the District Hospital of Bangangté (Ndé division West-Cameroon) in which reservoirs of multi-resistant bacteria have consistently been reported [10,11].

2. MATERIAL AND METHODS

2.1 Study design

This cross-sectional descriptive study was conducted from September 2nd through November 2nd, 2019 in the maternity wards of the "Université des Montagnes" teaching hospital (UdMTH) and the Bangangté District Hospital (BDH), two health facilities in the Ndé division, West-Cameroon. Sampling was initiated when all administrative and ethical requirements were met. Namely they were the ethical clearance N°2019/031/UdM/PR/CIE provided by the institutional ethical board, the research authorization N°2019/059/AED/UDM/CUM obtained from the UdMTH head and the signed written agreement from the BDH director. Subsequent to data collection and sampling, bacteriological screening was performed at the Microbiology Laboratory of the UdMTH.

2.2Sampling and bacteria analysis

The specimens subjected were collected from the childbirth and hospital rooms. The targeted specimens and collection sites included the ambient air in the work-place and surfaces of some tools (flat surfaces of the childbirth tables, instrument tables and the scale). Prior to each sampling, various sets of related pieces of information were collected for tracing.

Specimen collection was performed by wet swabbing on flat surfaces and by passive direct contact (sedimentation) for bacteria of the ambient air according to Fotsing Kwetche et *al*. [12]. Sampling was conducted over three days; three times per day. On surfaces, the procedure was repeated at 9 a.m., 11 a.m. and 1 p.m. For bacteria of the ambient air, culture media in open Petri dishes were deposited at 9 a.m. and the three sampling times corresponded to time differences of 45 min (air sample 1), 1 hour (air sample 2) and 1 h 30 min (air sample 3) from the opening to the closing of dishes.

The analytic protocol followed according to Fotsing Kwetche et *al*. [12] with slight adjustments. After enumeration, bacterial loads were recorded and characterized. They were thus classified as low for loads below 10 CFU/cm² for surfaces or 283 CFU for 60 mm diameter Petri dish for air, high for loads above 30 CFU/cm² for surfaces or 848 CFU for 60 mm diameter of Petri dish for air, and moderate for the loads found between these two extremes for each type of specimen. In this work, Gram-positive rods (GPR) were not enumerable (slick growth). During the identification, the tests for nitrate reductase, indole and TDA were added for Gram-negative rods (GNR). For each type of isolate, three colonies were randomly selected, re-streaked on nutrient agar and for separate used in susceptibility tests. For each health facility, 30% of isolates were later reselected for susceptibility tests. The susceptibility tests were performed according to the recommendations of the “Comité de l'Antibiogramme de la Société Française de Microbiologie, CA-SFM, EUCAST, V2.0 May 2019”. A total of 12 antibacterial agents selected and used for this purpose. Namely, they were Amoxicillin (25 µg), Amoxicillin/Clavulanic Acid (20/10 µg), Cefoxitin (30 µg), Ceftriaxone (30 µg), Cefuroxime (30 µg), Gentamicin (15 µg), Imipenem (10 µg), Levofloxacin (05 µg), Nalidixic Acid (30 µg), Oxacillin (01 µg), Penicillin G (10U), and Tetracycline (30 µg).

2.3Data processing and expected outcomes

Expected pieces of information included bacterial isolation rates and loads; antibiotic susceptible/resistant isolates profiles. These variables were recorded and analyzed with statistical tools provided by Microsoft Excel 2013 software.

3. RESULTS

3.1 Observational findings

In the target health facilities, the maternity care activities were diversified. The decontamination protocols in force were conducted in two major steps at the UdMTHi.e.cleaning with tap water plus detergent, and disinfection with chlorinated water. In the BDH, three steps were observed and included in order cleaning with tap water plus detergent, disinfection with chlorinated water and disinfection with 70% alcohol. These protocols were not consistently followed due to unavailability of disinfectant and decontamination solutions in both settings.

3.1 Sampling and laboratory screening

Overall, 126 specimens were collected (81 at the UdMTH and 45 at the BDH). From these, 168 bacterial isolates were recovered (65 from the BDH and 103 from the UdMTH). Further details on the distribution of specimens and positive cultures are shown in table 1.

Table 1. Rates of positive cultures

Specimen and culture information	“Université des Montagnes” Teaching Hospital		Bangangté District Hospital		Total
	Childbirth room	Hospital room	Childbirth room	Hospital room	
Specimen number	54	27	42	3	126
Positive cultures (%)	98.1	96.3	100	100	-
Number of bacterial isolates	69	34	61	4	168

Overall, 98.4% of the cultures were positive. Identification resulted in diversified bacterial polymorphism in all settings. More detailed related pieces of information on the distribution of recovered isolates per sites are provided in figure 1 and table 2.

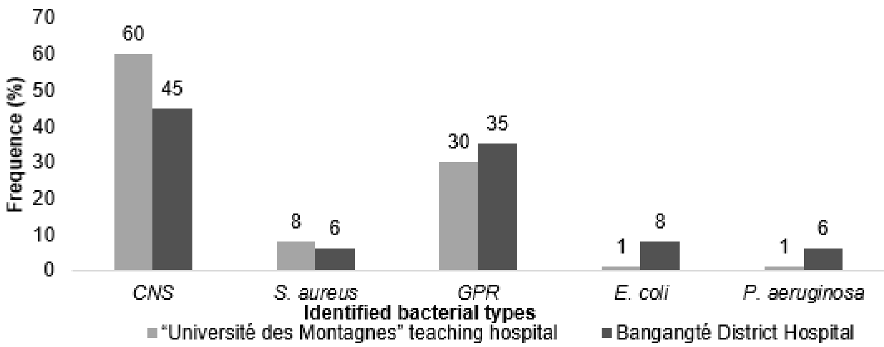

Fig. 1. Distribution of bacteria isolates per health facility
CNS: Coagulase negative *Staphylococcus*; GPR: Gram-positive rods

Bacterial diversity was higher at the BDH. In addition, most bacterial populations were coagulase-negative staphylococci, they were followed by Gram-negative rods, dominated by *E. coli* and *P. aeruginosa*.

Table 2. Bacterial population per sampling sites

Health facility	Collection site	Specimens	bacteria type				
			GPR	*E. coli*	*P. aeruginosa*	*S. aureus*	CNS
UdMTH	**CR**	Air	04	00	00	00	07
		S	09	00	00	00	06
		CT 1	01	00	00	03	05
		CT 2	05	00	00	01	06
		CT 3	04	00	00	02	06
		IT	02	00	00	00	08
	HR	Air	02	00	00	00	09
		IT 1	01	01	01	01	08
		IT 2	03	00	00	00	08
BDH	**CR**	Air	04	00	00	00	08
		S	07	02	00	01	05
		CT 1	03	00	03	01	04
		CT 2	04	02	00	01	05
		IT	04	01	01	01	04
	HR	IT	01	00	00	00	03

UdMTH: "Université des Montagnes" teaching hospital; BDH: Bangangté District Hospital; CR: Childbirth room; HR: Hospital room; S: Scale; CT: Childbirth table; IT: Instrument table; CNS: Coagulase negative *Staphylococcus*; GPR: Gram-positive bacillus rods

Globally, similar profile was observed for from one site to the other. Also, in-site variation trends were similar from one location to the other. Otherwise, the ambient air and the work surfaces of each room were colonized by slightly similar bacterial populations. From one hospital the other, this similarity was observed between specimen collection sites. The highest bacterial diversity were recorded on the tools table 1 at the UdMTH hospital childbirth room and the tools table at the BDH childbirth room. The CNS isolate numbers predominated isolation rates on the tools, except the scales where GPR were more recovered.

Although the composition according to the bacterial population profile was approximately similar, the composition in connection with bacterial load was very diverse. Tables 3 and 4 provide more details on these loads.

Table 3. Load of enumerable bacteria from the ambient air

Air sample	UdMTH		BDH
	CR	HR	CR
	CFU of CNS/Petri dish 60 mm diameter		

Day 1	**Air specimen 1**	1000	53	16
	Air specimen 2	1000	54	1000
	Air specimen 3	1000	56	1000
Day 2	**Air specimen 1**	-	4	3
	Air specimen 2	-	25	1000
	Air specimen 3	1000	40	-
Day 3	**Air specimen 1**	24	30	1000
	Air specimen 2	02	37	1000
	Air specimen 3	1000	33	04

UdMTH: "Université des Montagnes" teaching hospital; BDH: Bangangté District Hospital; CR: Childbirth room; HR: Hospitalization room; CNS: Coagulase negative *Staphylococcus*; CFU: colony-forming unit

The overall bacterial loads varied significantly (67%) or remain similar (33%) between the ambient air samples from each room during the collection days at UdMTH. The bacterial density of CNS was, in more than half of the cases (5 out of 9) very high in the childbirth rooms. In the UdMTH, these loads were 18 to 33 times higher than the findings recorded in the hospital wards.

Table 4 displays the bacterial loads recorded on the work surfaces at the "Université des Montagnes" teaching hospital and the Bangangté District Hospital.

Table 4. Bacterial loads (CFU/cm²) of enumerable isolates on the surfaces per sites

Hospitals	**Sites**	**Work surfaces**	**Enumerated bacterial types**	**Collecting period**								
				Day 1			**Day 2**			**Day 3**		
				9 a.m.	**11 a.m.**	**1 p.m.**	**9 a.m.**	**11 a.m.**	**1 p.m.**	**9 a.m.**	**11 a.m.**	**1 p.m.**
UdMTH	**CR**	**S**	**CNS**	-	-	-	400	400	400	400	400	400
		CT 1	**CNS**	-	-	-	35	5	42	23	400	-
			S. aureus	8	400	40	-	-	-	-	-	-
		CT 2	**CNS**	-	-	-	17	5	5	2	2	1
			S. aureus	-	68	-	-	-	-	-	-	-
		CT 3	**CNS**	-	-	-	51	11	108	400	400	14
			S. aureus	38	120	-	-	-	-	-	-	-
		IT	**CNS**	-	360	11	99	86	16	4	400	400
	HR	**IT 1**	**CNS**	-	42	12	99	30	16	20	400	400
			S. aureus	58	-	-	-	-	-	-	-	-
			P. aeruginosa	-	-	-	-	-	-	-	400	-
			E. coli	-	-	-	-	-	-	-	-	10
		IT 2	**CNS**	400	400	400	400	7	400	-	400	400
BDH	**CR**	**S**	**CNS**	-	-	-	170	-	400	400	2	10
			S. aureus	-	-	-	-	400	-	-	-	-
			E. coli	-	-	-	-	-	-	320	400	-
		CT 1	**CNS**	-	-	400	-	-	-	400	400	400
			S. aureus	-	-	-	256	-	-	-	-	-
			P. aeruginosa	400	400	400	-	-	-	-	-	-
		CT 2	**CNS**	-	400	-	400	-	400	2	400	-
			S. aureus	-	-	-	-	400	-	-	-	-
			E. coli	-	-	400	-	-	-	-	-	400

	IT	**CNS**	No sampling			2	-	400	2	400	-
		S. aureus				-	43	-	-	-	-
		P. aeruginosa				-	-	-	-	-	6
		E. coli				-	-	-	-	18	-
HR	**IT**	**CNS**	400	400	3	No sampling					

UdMTH: "Université des Montagnes" teaching hospital; BDH: Bangangté District Hospital; CR: Childbirth room; HR: Hospital room; S: Scale; CT: Childbirth table; IT: Instrument table; CNS: Coagulase negative *Staphylococcus*.

Overall, work surfaces were colonized by a fairly high bacterial load (31 - 100 CFU/cm²), at least once. In addition, at the UdMTH, except the childbirth table 2, work surfaces were often colonized by very high bacterial load (> 300 CFU/cm²). At the BDH, bacterial loads were very high in the majority of cases. Per sampling day, bacterial loads often remained relatively constant (5.1% of cases).

3.2Bacteria susceptibility profile

Susceptibility tests revealed high rates of drug resistance and multidrug-resistant isolates; most of which were potential etiologies of healthcare associated infections. Table 5 provides further detailed pieces of findings on the isolate's susceptibility/resistance profile. Overall, 50 isolates were subjected per health facility.

Table 5. Bacterial clinical categories (%) at the UdMTH and BDH

Antibiotics	**UdMTH (n=31)**			**BDH (n=19)**		
	%S	**%I**	**%R**	**%S**	**%I**	**%R**
Amoxicillin (30 µg)	13	43	43	13	47	40
Amx/Clav (20/10 µg)	27	37	37	13	33	53
Cefoxitin (30 µg)	13	20	67	00	13	87
Ceftriaxone (30 µg)	13	07	80	07	27	67
Cefuroxime (30 µg)	10	23	67	00	53	47
Gentamicin (15 µg)	23	70	07	07	87	07
Imipenem (10 µg)	77	13	10	58	42	00
Levofloxacin (05 µg)	19	68	13	21	58	21
Nalidixic Acid (30 µg)	00	00	100	00	22	78
Oxacillin (01 µg)	45	55	00	20	60	20
Penicillin G (10 U)	28	66	7	40	50	10
Tetracycline (30 µg)	00	26	74	00	37	63

R: Resistant; S: Susceptible; I: Intermediate; NT: Not tested; CNS: Coagulase negative *Staphylococcus*; GPR: Gram-positive rods; Amox/Clav: Amoxicillin/Clavulanic Acid

It appears that some bacterial isolates were resistant to several antibacterial drugs families. Overall, the lowest resistance rates were recorded with Imipenem while the majority of the antibiotics used have a better effectiveness at the UdMTH.

4. DISCUSSION

The present investigation aimed at characterizing the bacterial flora in the maternity wards of two health facilities in the Ndé Division, appreciating their variation with time and determining the antibiotic susceptibility/resistance profile of isolates that are potential agents of healthcare-associated infections. In each of the health facilities, Gram-positive cocci predominated, followed by Gram-positive rods. Similar tends were reported by previous research in the same facilities a few years ago, [10-12]. Consistent with the previous authors, colonization by these bacterial populations could actually be anticipated, based on their non-stringent feature which allows their easy growth in diverse environmental constraints. Nothing was planned to appreciate the hygiene follow-up but, although it is known to have improved meanwhile, developing monitoring policies to serve as a routine process emerge as a health priority. This is feasible in this area which is endowed with multiple assets like human resources which are listed amongst the best capacitated in the West region of Cameroon.

This predominance could be justified by intrinsic characteristics of these bacteria. These include (but not limited to) the chemical composition of the cell envelope, their affinity with molecular oxygen, non-stringency in connection with nutritional demands and other environmental stresses like dry and hot environments. Moreover, some GPR from the genus *Bacillus*, can develop spores that further promote resistance to environmental stresses imposed by detergents, for instance, that bare commonly substances used in routine cleaning [10-12]. Strains from this group are common hosts of surfaces in healthcare settings. Based on the low and intermediate virulence of most species, bacteria belonging to the genus *Bacillus* are often regarded as deserving little or no importance in IDs [13]. With advances in medicine that associate with increased life expectancy and the number of vulnerable hosts in elderly, their potential role as etiologies of resistant opportunistic infections in healthcare environment became obvious [14]. Previous authors suggested that Gram-positive bacteria like *Staphylococcus* spp., could effectively serve to monitor the density of bacterial population in work environments [12]. Also, and consistent with above development, the higher rates of Gram-negative rods recorded at the BDH could be, at least partially, justified by the fact that the BDH work surfaces more frequently humidified with plain water during specimen collection. This aligns with the hypothesis on wet environments which favor survival and growth of Gram-negative rods [2,15] including *P. aeruginosa* in the hospital environment [15], as well as the sample collection protocol effectiveness which optimizes removal of bacteria from target surfaces [16-19]. In quality control, detection of Gram-negative rods (coliforms, more specifically) typically indicates recent adulteration of the collection site. Otherwise, their presence would reflect weaknesses in preserving proper environment for safer healthcare; a key priority to be addressed. During childbirth, feces, and the residues of the feces can contaminate work surfaces and equipment, as well as the hands of care providers; underlining the necessity for sustainable running water availability. Their presence, like as that of bacteria types such as *S. aureus* and *P. aeruginosa*, is a risk for healthcare-associated infections in at-risk individuals.

The similarity primarily observed in bacteria profile indicates a bacteria population's homogeneity. The minor differences recorded between the profiles these bacterial populations could be explained by the intrinsic variability associated with each activity, the healthcare workers aptitude, the type of healthcare services offered, and constrains due to resource availability. Moreover, the loads of bacteria on the work surfaces in childbirth rooms were high, in line with developments from previous authors [20]. Air adulteration may explain the contamination of some unused surfaces such as the childbirth table 2 at the UdMTH. Smith et *al*. [21] and Fotsing Kwetche et *al*. [12] reporting association between air and surface bacterial loads asserted that passive air sampling effectively provides quantitative data likely to assess surface contamination. However, it appears primordial to include large numbers of sampling points when ambient air loads are investigated through. On the surfaces, wide variations in bacterial loads (from low to very-high) and population heterogeneity were observed with time during the present survey. It is recognized that humans' presence and activities have great impacts on the microbial population on ward's surfaces (> 30 CFU/cm²). The high load increases the risk of HAIs and represent indirect evidence of weaknesses in the healthcare hygiene policies in force. Such bacterial loads highlight the importance of decontaminating the care environment which is one of the key objectives of hospital hygiene. This impact is also recalled and defended by several authors [1,22]. Various data suggest basic cheaper and available tools that could be used in strategizing policies aiming effective decontamination in instances of resource limitation [23].

In the context of the present investigation, the high resistance rates recorded is a predictor of therapeutic failure in case identified organisms become involved in infections. The resistance rates recorded could at first glance be attributed to the use of antimicrobial drug as it is known to take place in healthcare settings. Consistently however, research findings indicate that community-selected resistance shouldn't be overlooked [24,25]. In fact, the potential of healthcare facilities to screen microbial susceptibility/resistance profiles prior to drug administration is few in the Ndé division. Otherwise, in most cases drug administration empirically oriented, favored by the low purchasing power of the general populations on one hand and the human resources at both clinical and biological levels. This gape should be address for effective and sustainable antimicrobial resistance stewardship that is at its early steps in Cameroon.

A glance on this susceptibility trends as introduced above reveals low rates. The low susceptibility has been reported by authors in the same health a few years ago [11-13], also associated with likely cross- or co-selection. This may be in connection with the routine use of antibiotics and sometimes inappropriate use in the hospital setting as developed above [26]. Moreover, it could also result from the dissemination of resistance genes carried by mobile genetic elements from other resistance-selection engines as animal husbandry and agriculture. Animal husbandry and agriculture in the Ndé division should therefore, be regarded as drivers on which AMR should focus, beyond healthcare settings. These consistently high resistance rates are additional paramount indications that advocate holistic and sustainable stewardship policies.

Still from these findings, it can be anticipated that targeting a few sites in the hospital would provide an overall idea of the microbial flora of this environment, although specific pieces of information will be required for a better infection prevention initiative from one place to the other [14,27]. Current findings globally point out the necessity for infection control policies revision that will encompass the flexible local hospital environments which host several variants of susceptible hosts (newborns and elderly). They ultimately provide a few reliable tools that will be useful in the AMR stewardship in Cameroon. Further pieces of information and reliable indicators likely to attest bacteria spread from one site to the other will be key targets in future investigations.

5. CONCLUSION

In the maternity units of the Bangangté District Hospital and at the “Université des Montagnes” teaching hospital, the bacterial populations on work surfaces and in the ambient air of the work premises were mainly *Staphylococcus* spp., found with invariably high loads. These findings were likely in connection with local activity density. The susceptibility/resistance rates also fluctuated in the recovered isolates. Overall, these results highlighted the need for strengthening work-environment hygiene for safer mothers-and-child.

DATA AVAILABILITY

All the data of this work are present in this paper.

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