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Environmental Aerobic Bacterial Associated with Infectious and General Solid Waste: Screening at the "Université des Montagnes" Teaching Hospital Waste Accumulation Sites

Victoire Kom Fotso [a,b], O'Neal Dorsel Youté [a,b,c*], Anselme Michel Yawat Djogang [b,d], Steve Carole Ditchou Nganso [e], Esther Guladys Kougang [a,b,f], Blandine Pulchérie Tamatcho Kweyang [g], Julius Nwobegahay [c], Adolarice Nana Feukeu [a,b], Valere Igor Deumi Monthé [a,b], Vanessa Ornella Alactio Tangueu [a,b,h] and Pierre René Fotsing Kwetche [a,b,d,e,i]

[a] School of Biomedical Sciences, Higher Institute of Health Sciences, Université des Montagnes, Cameroon.
[b] Laboratory of Microbiology, Université des Montagnes Teaching Hospital, Cameroon.
[c] Military Health Research Center, Cameroon.
[d] School of Pharmacy, Higher Institute of Health Sciences, Université des Montagnes, Cameroon.
[e] Hospital Hygiene Unit, Université des Montagnes Teaching Hospital, Cameroon.
[f] National Veterinary Laboratory, Cameroon.
[g] Laboratory of Microbiology, Faculty of Science, University of Yaoundé I, Cameroon.
[h] Center for Research on Health and Priority Diseases, Institute of Medical Research and Medicinal Plants Studies, Ministry of Scientific Research and Innovation, Cameroon.
[i] School of Human Medicine, Higher Institute of Health Sciences, Université des Montagnes, Cameroon.

Authors' contributions

This work was carried out in collaboration among all authors. Authors PRFK and VKF conceptualized the study and performed methodology. Author PRFK did data validation and supervision. Author VKF did the project administration, investigation and data curation. Authors VKF and ODY did the formal analysis. Authors VKF and PRFK did the resources. Authors VKF and ODY did data visualization and wrote original draft. Authors VKF, ODY, AMYD, SCDN, EGK, BPTK, JN, ANF, VIDM, VOAT and PFFK wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript.

**Corresponding author: E-mail: yout.oneal2@gmail.com;*

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ABSTRACT

Background: Waste is a source of potentially dangerous microorganisms for users of healthcare facilities, and its accumulation is a risk factor for infectious agent dissemination.
Objective: The first aim of the present study was to identify and quantify aerobic bacteria around solid waste accumulation sites at the "Université des Montagnes" Teaching Hospital. The second one was to assess bacteria susceptibility to common conventional antibacterial agents.
Methods: Soil samples and airborne bacteria were collected at various distances from the target solid waste accumulation sites. Bacteria isolation and susceptibility tests were thereafter conducted according to standard protocols.
Results: Isolated organisms (123 bacterial isolates) included *Staphylococcus* spp (48%), Gram-positive rods (32%) and Gram-negative rods (20%). Polymorphism and bacterial loads were highest at the sampling locations closer to the accumulation sites and decreased with increasing distances from these sites. Overall findings revealed that variations of polymorphism and bacterial loads is likely associated with anthropogenic activities. In addition, susceptibility rates for *Staphylococcus* were high.
Conclusion: Together with the overall bacterial population distribution trends, the high susceptibility rates recorded deserve better understanding in future research initiatives for optimal hospital hygiene.

Keywords: Ambient air; antibiotics; bacteria; hospital; soil; waste.

1. INTRODUCTION

Hospital-acquired infections (HAIs) refer to infectious diseases (IDs) caused by microorganisms contracted during a stay in a healthcare facility [1]. HAIs are major public health issues for their frequencies, their severity and burden at all levels of the healthcare system pyramid [1,2]. The knowledge and control of the microbial flora in a healthcare facility appear therefore primordial for contextual orientation towards designing policies for IDs control and prevention [3]. One of the potential sources of microorganisms involved in IDs at the healthcare facility is waste where about 15% of waste are hazardous to human, animal and environmental welfare. Whether from biological or non-biological origin, waste includes various types of pollutants (chemical, radioactive, pharmaceutical, biological, and microbiological) and in most cases, is discarded without subsequent re-use policy. This danger to patients' health requires proper management to avoid any negative influence on both the patient's care and his rehabilitation processes [4]. When they are inadequately managed, waste serves as breeding ground and reservoirs for microbial dissemination. Assessing the microbial load in the air in the vicinity of some dump sites, Igborgbor *et al*. (2015) and Owhonka *et al*. (2024) reported a decreasing microbial load trend as distance increased from the dumps. These authors also identified potentially pathogenic airborne agents [5,6]. In their context in addition, these authors also highlighted the microbiological risks that was associated with waste in their environment where the investigation was conducted. Furthermore, waste can also be a source of drug-resistant microorganisms selected during healthcare

procedures and hospital environmental sanitation's. Drug-resistant microorganisms are etiologies of broad ranges of HAIs [3]. Also, best and commonly known associated etiologies are drug-resistant bacteria that may evolve as true or as opportunistic pathogens [1-3,7-9], most of which are environmentally ubiquitous organisms. Thus, in the waste management pathways, waste accumulation sites and the surrounding environments should be monitored in order to anticipate intervention in preventing HAIs. Acknowledging that the likelihood of opportunistic infections will rise with the increasing life expectancy and relates old ages, introduction and implementation of contextual policy is paramount for optimal caregiving performances in healthcare facilities.

The present investigation was conducted to identify and quantify aerobic bacteria in infectious and general solid waste accumulation sites at "Université des Montagnes" Teaching Hospital. The susceptibility profile of isolates to common antibacterial drugs was also investigated. Resulting pieces of information could provide reliable sources of data that would enable better knowledge on ubiquitous microbial population which is in part a foundation for the struggle that aims at mitigating the risk of infectious diseases (IDs) in general and HAIs in particular for better healthcare in the local context.

2. MATERIAL AND METHODS

2.1 Study Design

This descriptive cross-sectional study was conducted at "Université des Montagnes" Teaching Hospital (UdMTH) under research authorization N° 2021/0052/AED/UDM/CUM provided by the UdMTH's Head. Related activities were conducted over three months (January 1st through March 31st, 2021). In the environment, the work focused on air and soil. Specimen screening was performed at the UdMTH Laboratory of Microbiology.

2.2 Infectious and General Solid Waste (IGSW) Circuit and Accumulation Sites

Waste was handled by trained and equipped cleaning personnel. Infectious solid wastes (ISW) were either disposed in conventional safety boxes for sharps wastes, in hard-plastic containers, or in dustbins equipped with plastic garbage cans. Every day, ISW containers were manually collected and transported to the transitory storage site from where they were later on (once a week) carried with wheelbarrows to the final treatment site for incineration. Once on the final treatment site (the incinerator), the wastes were kept for two days average prior to incineration. General solid wastes (GSW) were disposed off in dustbins without garbage bags. Every morning, these containers that were often without cover were hand-carried to a collection site then, to a pit with wheelbarrows. Near the pit, the wastes were separated from non-biodegradable wastes. Non-biodegradable wastes were recovered by a recycling industry while the remaining was literally burned off onsite.

The pit was located between the hospital building and the incinerator. The distances between the IGSW accumulation sites and the hospital building are displayed in Table 1.

2.3 Sample Collection

Sampling was carried out in three locations (A, B and C) around each IGSW accumulation site. These locations were situated at 10 (location A), 20 (location B) and 30 (location C) meters from the GSW collection site, the ISW temporary storage site and the incinerator. The pit and the incinerator shared the same sampling locations, which were situated at less than 1 meter, at 10 and 20 meters from the pit.

Table 1. Distances between infectious and general solid waste accumulation sites and the hospital building

IGSW accumulation sites	Distances (meters)
GSW collection site	40
ISW temporary storage site	40
Pit	90
Incinerator	100

IGSW: infectious and general solid waste; GSW: general solid waste; ISW: infectious solid waste

The samplings processes were conducted over 4 days (1 specimen per sampling location). For soil, several grams of surface soil were collected in the morning with sterile spatulas and sterile pots. For air, airborne microorganisms were captured by passive direct contact (sedimentation) according to Fotsing Kwetché *et al*. [10] with slight modifications. Briefly, Petri dishes with culture media were opened and deposited at the sampling points in the morning, and recovered 6 hours later. Each sample was promptly conveyed to the laboratory for analytical steps.

2.4 Culture

In the laboratory, Petri dishes used to trap airborne bacteria were incubated aerobically at 37°C for 24 hours. For soil samples, a suspension was prepared by mixing 1 g of the specimen with 3 mL of sterile peptone water first. Second, 50 µL of the inoculum was spread over the entire surface of each culture medium with a sterile Pasteur pipette rake. All the inoculated culture media were incubated aerobically at 37°C for 24 hours. Namely, the culture media used included Mannitol Salt agar, McConkey agar and chocolate agar (trypticase soy agar supplemented with 5% of sheep blood). The culture step was conducted 6 times per type of culture medium.

2.5 Bacterial Morphological Identification and Enumeration

After incubation, bacterial colonies on the agar plates were identified and described based on their size, shape, color, opacity, colony's surface consistency, and edges. At the same time, they were enumerated in each plate as colony-forming units per Petri dish (CFU/Petri dish). Results from airborne bacteria were expressed in CFU/60 mm diameter Petri dish/6 hours. For soil samples, these values were reported in CFU/g of soil according to the following formula: $N = 60 \times n$ (where N is the number of CFU/g of soil, n the number of CFU/Petri dish, and 60 the ratio of suspension volume to inoculum volume).

2.6 Microscopic and Biochemical Identification of Bacteria

This step was performed according to previous protocol [11]. Subsequent to enumeration, microscopic examination of a Gram-stained smear was carried out. Subsequently, a catalase, free coagulase and mannitol fermentation tests were conducted for Gram-positive cocci (GPC). Identification of other groups was limited to microscopic traits.

2.7 Susceptibility Tests to Antibiotics

Susceptibility tests followed on *Staphylococcus*, and were performed by disk diffusion (Kirby-Bauer) with reference to the "Comité de l'Antibiogramme de la Société Française de Microbiologie, EUCAST" [12] standards. Fresh (24-h sub-culture) and pure bacterial populations were used for susceptibility tests. Antibiotics used belonged to the arsenal of conventional antibacterial drugs that are commonly used in Cameroon and in the setting. Namely they were Gentamicin (10 µg), Ofloxacin (5 µg), Oxacillin (1 µg), Penicillin (10 U), Tetracycline (30 µg), and Trimethoprim/Sulphamethoxazol (1.75/23.25 µg). For identification and susceptibility tests, *S. aureus* ATCC 29213 was used for quality control.

2.8 Data Analysis

Investigated variables comprised the number of bacterial isolates, bacterial loads and the clinical category (susceptible, intermediate, resistant) of recovered isolates. Data processing was eventually conducted with analytical tools provided by Microsoft Excel 2016 software.

3. RESULTS

3.1 Description of Identified Bacterial Population

Overall, 123 bacterial isolates were selectively recovered from the subjected specimens. Out of these, *Staphylococcus* spp. overwhelmed the isolation rates (48%) with 1/3 coagulase-positive and 2/3 coagulase-negative isolates. Other bacterial groups recovered were Gram-positive rods (32%) and Gram-negative rods (20%). More detailed distribution of isolates recovered and their bacterial loads in the environment around the waste accumulation sites is displayed as shown in Tables 2 and 3.

Analytically, data from Table 2 reveals that the majority of isolates were found at the first sampling site (location A) situated around the IGSW accumulation points. Further, the number of isolate's types decreased with increased distances from these sites. The same trend was also observed for bacterial polymorphism, since bacterial diversity was highest in the environment around the GSW collection site.

Table 2. Bacteriological profile in ambient air and soil around IGSW accumulation sites

Samples	IGSW accumulation sites	Sampling locations	GNR	GPR	*Staphylococcus* spp.
Airborne bacteria	**GSW collection site**	A	2	3	5
		B	1	2	3
		C	1	3	2
	ISW temporary storage site	A	3	3	4
		B	2	2	3
		C	0	2	1
	Incinerator/pit	A	1	2	4
		B	1	2	3
		C	1	1	0
Soil	**GSW collection site**	A	2	3	6
		B	2	2	4
		C	2	4	3
	ISW temporary storage site	A	3	2	4
		B	1	1	4
		C	0	1	3
	Incinerator/pit	A	2	3	6
		B	1	2	3
		C	0	1	1

IGSW: infectious and general solid waste; GSW: general solid waste; ISW: infectious solid waste; GNR: Gram-negative rods, GPR: Gram-positive rods

Table 3. Bacterial loads around IGSW accumulation sites

Samples	IGSW accumulation sites	Sampling locations	GNR	GPR	*Staphylococcus* spp.
Airborne bacteria (CFU/60 mm diameter Petri dish/6 hours)	**GSW collection site**	A	111 ± 9	122 ± 5	107 ± 7
		B	114 ± 6	124 ± 3	109 ± 9
		C	114 ± 6	121 ± 3	112 ± 8
	ISW temporary storage site	A	84 ± 4	93 ± 3	96 ± 6
		B	76 ± 3	79 ± 2	83 ± 5
		C	56 ± 4	65 ± 1	78 ± 4
	Incinerator/pit	A	21 ± 4	18 ± 4	19 ± 2
		B	20 ± 4	7 ± 3	9 ± 3
		C	8 ± 3	4 ± 2	5 ± 4
Soil (CFU/g of soil)	**GSW collection site**	A	$(3.7 \pm 0.004) \times 10^3$	$(2.7 \pm 0.006) \times 10^3$	$(3.5 \pm 0.005) \times 10^3$
		B	$(2.9 \pm 0.003) \times 10^3$	$(3.6 \pm 0.002) \times 10^3$	$(2.6 \pm 0.006) \times 10^3$
		C	$(1.8 \pm 0.003) \times 10^3$	$(3.5 \pm 0.003) \times 10^3$	$(2.5 \pm 0.005) \times 10^3$
	ISW temporary storage site	A	$(1.9 \pm 0.004) \times 10^3$	$(2.4 \pm 0.003) \times 10^3$	$(2.8 \pm 0.004) \times 10^3$
		B	$(1.7 \pm 0.006) \times 10^3$	$(2.3 \pm 0.003) \times 10^3$	$(2.7 \pm 0.005) \times 10^3$
		C	$(9.3 \pm 0.04) \times 10^2$	$(2.1 \pm 0.009) \times 10^3$	$(2.4 \pm 0.005) \times 10^3$
	Incinerator/pit	A	$(5.1 \pm 0.03) \times 10^2$	$(4.0 \pm 0.02) \times 10^2$	$(5.7 \pm 0.01) \times 10^2$
		B	$(2.4 \pm 0.06) \times 10^2$	$(2.1 \pm 0.03) \times 10^2$	$(2.4 \pm 0.01) \times 10^2$
		C	90 ± 4	$(1.2 \pm 0.03) \times 10^2$	90 ± 4

GSW: general solid waste; ISW: infectious solid waste; GNR: Gram-negative rods, GPR: Gram-positive rods; CFU: colony-forming units

Table 4. Antibiotic susceptibility profile of *Staphylococcus* isolates

Antibiotics	Clinical categories		
	%S	%I	%R
Gentamicin (10 µg)	100	0	0
Ofloxacin (5 µg)	85	0	15
Oxacillin (1 µg)	60	0	40
Penicillin (10 U)	60	0	40
Tetracycline (30 µg)	50	0	50
Trimethoprim/Sulphamethoxazol (1.75/23.25 µg)	90	5	5

%S: rate of susceptible isolate; %I: rate of moderate resistance isolate; %R: rate of resistance isolate

Data from Table 3 indicate that the overall loads of bacteria sedimenting from ambient air and those recovered from the soil surface are higher around the GSW collection site and the ISW temporary storage site than around the incinerator and the pit. Airborne bacteria loads remain similar at all sampling points situated around the GSW collection site, but decreased when the distance increased from the other waste accumulation sites. For soil, there was also a general decrease in soil bacterial loads when distances from the IGSW accumulation sites increased. The lowest values were recorded around the incinerator and the pit.

3.2 *Staphylococcus* Susceptibility to Antibiotics

The susceptibility test was performed on the 59 *Staphylococcus* isolates. Table 4 provides more details on their susceptibility trend to antibacterial drugs used in the procedures.

Relatively higher rates of antibiotic-susceptible *Staphylococcus* can be observed. Highest susceptibility rates were recorded with Gentamicin, Ofloxacin and Trimethoprim/Sulphamethoxazol; while intermediate phenotypes were relatively rare throughout the procedure.

4. DISCUSSION

The present piece of work aimed at identifying and quantifying aerobic bacteria in the environment around the infectious and general solid waste accumulation sites at the “Université des Montagnes” Teaching Hospital and then, highlighting the trend of bacteria dissemination from these sites. Soil and airborne non-stringent organisms were chosen to mark general populations sizes and dissemination likelihood. Antibiotics susceptibility profile was thereafter assessed for *Staphylococcus* isolates.

Overall, Gram-positive bacteria were most frequently recovered, literally overwhelmed by Gram-positive cocci while Gram-negative rods (GNR) were the least common. Other authors working with similar targets in health facilities’ environment also reported these bacterial populations in their findings [10,13,14]. Those authors justified their findings with reference to the affinity that the bacteria population had with molecular oxygen. In addition, the chemical composition of the bacterial cell envelop likely plays significant role in their distribution. In fact, the Gram-positive feature confers primary resistance to large numbers of environmental stresses. In some instances, members belonging to certain groups could encapsulate to further resist in larger numbers.

Gram-positive rods (GPR) were less frequent than *Staphylococcus*. Investigating the microbiological effects of hospital wastes on the environmental microbial flora in Nigeria, Oyeleke *et al*. (2009) reported the opposite trend in which GPR overwhelmed GPC [15]. One approach to explaining this difference could be the type of isolation media, which may have lacked specificity for bacterial growth. In other words, the use of other agars such as deMan Rogosa Sharpe (MRS) agar, nutrient agar, trypticase soy agar without supplementation, could generate different results, since Mannitol Salt and supplemented trypticase soy agar was used in the present one, while MRS preferentially allow the growth of some GPR. The inherent chemical composition of hosting soils could also affect the findings of Oyeleke *et al*. (2009) [15] and explain the results of these work because, in connection with the nutrients in environments, biochemical compounds might well vary in types and concentrations from one waste accumulation site to the other. Future work should adjust the overall procedures in order to agree or disagree with these hypotheses, through both approaches could work together. Otherwise, and technically, results are dependent upon culture media and deserve therefore appropriate interpretations for

optimal intervention in controlling environmental microbial flora.

The bacterial populations profile recovered revealed an almost similar distribution around the IGSW accumulation sites for each specimen category. This may be in connection with the fact that these sites are located on the same plot of land. The overall highest rate of bacterial isolation and polymorphism were recorded at the sampling point closest to the IGSW accumulation sites. Among the waste accumulation sites, the highest rate of bacterial isolation and polymorphism were recorded around the GSW collection site. In terms of bacterial loads, they were highest around the GSW collection site, then around the ISW temporary storage site and the incinerator or the pit. Also, except for airborne bacterial loads around GSW collection site, airborne and soil bacterial loads were observed to decrease with distances from accumulation points. Globally, a similar trend for airborne and soil bacterial loads was recorded. These findings on the bacterial distribution and loads could be justified by human activities. In fact, this view could reasonably be supported because the variations are low. This bacterial population variation trend which associates with human activity has previously been observed by other investigators [10,13] when they assessed the bacterial presence on surfaces and in the ambient air in health facility premises. Also, this trend is supported by Smith *et al*. [16] when, highlighting the association between air and surface bacterial loads, observed that passive air sampling provides quantitative data that could explain surface contamination.

With a glance on the decreased bacterial loads, Igborgbor *et al*. (2015) and Owhonka *et al*. (2024) also reported similar trend from dump sites in Nigeria [5,6]. These observations confirm and remind that the risk of contracting infectious agents increases as one moves closer to waste accumulation sites; consistent with the basic discarding principle which recommends that waste accumulation or storage site should be far away from the human living environments.

Airborne bacterial loads around the GSW collection site were globally constant from one sampling location to the other, consistent with the fact that GSW were not always kept in closed packages such as garbage bags like ISW. As it was not often covered, this absence of protection likely allows radial dissemination of microorganisms in the air during handling. Through their work, cleaning staff activities might evolve as a major engine for microorganisms' dissemination.

Antibiotic susceptibility tests revealed that the majority of *Staphylococcus* isolates were largely susceptible to subjected antibiotics, suggesting that environmental microbial flora have not selected resistance phenotypes as commonly anticipated in health facilities and particularly in waste. Very low susceptibility rates have previously been reported on surfaces and ambient air of the same healthcare facility by previous authors [10,13]. Both findings suggest that bacterial population found in hospital wards do not disseminate through other neighboring environments. These allegations could be supported by the above trend of dissemination at distances from the waste accumulation sites, in line and more interestingly with fact that selector drivers could not be common in the waste. However, it is not enough to understand why resistance rates in isolates of hospital wards' environment are relatively high compared to microbial flora of the external building environment at the UdMTH during this present investigation. As multi-resistant bacteria have been identified in the hospital [10,13,17], this high susceptibility rate suggests that bacteria that are kept in the garbage bags or GSW hardly select resistance traits or, if they are clinical isolates, they rapidly loss the selected genetic elements that confer resistance to antibiotics. Some mobiles of deselection may interfere and need to be mastered beyond the fact that the cleaning staff is ideally competent and that hospital hygiene is a permanent concern at the UdMTH. In future work and in addition to the environment around the waste accumulation sites, additional sampling sites into the waste accumulation environment, mainly the soil under the garbage and the waste in the garbage bags at different times during its accumulation could verify above hypotheses.

5. CONCLUSION

The first aim of this study was to identify and quantify aerobic bacteria in the environment around the infectious and general solid waste accumulation sites at the "Université des Montagnes" Teaching Hospital. Bacterial recovered were dominantly Gram-positive bacteria. Polymorphism and bacterial loads were high at the sampling location closest to the accumulation points, and decreased with increasing distances from these sites. Variations

in polymorphism and bacterial loads were thought to be due to the anthropogenic activities. Second, in the bacteria susceptible assessment, bacteria subjected were largely susceptible to antibacterial agents used. Together with the overall bacterial population distribution trends, the high susceptibility rates deserved better understanding in future research initiatives.

DATA AVAILABILITY

Data associated with this work were not deposited into a publicly available repository. All the data of this work are present in this paper.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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