

Carriage of Multidrug-Resistant Organisms Among Patients and Healthcare Workers in Haemodialysis Units in Public Hospitals in Yaounde, Cameroon

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ABSTRACT

Background: The emergence and spread of Multidrug-resistant organisms (MDRO) represent a major public health issue, particularly amongst patients in haemodialysis units. Haemodialysis units regularly receive patients with renal failure, yet almost all hospitals in Cameroon with haemodialysis units lack a resistance surveillance program. The lack of data on the epidemiology of MDRO in haemodialysis units led us to conduct this study, with the aim of determining the frequency of MDRO carriage among patients and healthcare workers in haemodialysis units in Cameroon.

Methods: Nasal and rectal swabs were collected from patients and healthcare staff in two referral hospitals of Yaounde, between November 2023 and April 2024. Bacteria isolated on selective media were identified by biochemical tests and mass spectrometry. Antibiotic resistance profiles and resistance phenotypes were detected according to EUCAST/CASFM 2023 recommendations.

Results: A total of 155 participants were selected, including 130 patients and 25 staff members. Several comorbidities were identified among the patients, including cardiovascular diseases and infections. From the 275 samples collected we isolated 59 (21.45%) MDRO with 54 (91.2%) from patients and 5 (8.48%) from healthcare personnel. Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), third-generation cephalosporinase-producing *Enterobacteriaceae* (3CPE) and extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E) were more represented among patients compared to healthcare personnel with no significant difference in the two hospitals. There was no significant difference between the age groups ($P=0.438$). The resistance profile of MDRO was similar in the two hospitals.

Conclusion: This study revealed a high prevalence of MDRO in the haemodialysis unit, highlighting the need for systematic screening of patients on arrival at the haemodialysis unit and for the strengthening of standard precautions.

KEYWORDS: Multidrug-Resistant Organisms, haemodialysis units, MRSA, VRE, 3CPE and ESBL-E

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INTRODUCTION

The emergence and spread of multidrug-resistant organisms represent a growing global public health threat. The World Health Organization (WHO) has established a list of priority pathogens requiring intensified efforts in research and development of new treatments. Among these priority MDRO are MRSA, ESBL-E, 3CPE, VRE, carbapenemase-producing *Enterobacteriaceae* (CRE), carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) and carbapenem-resistant *Acinetobacter baumannii* (CRAB)[1]. These bacteria, frequently isolated in hospital settings, are responsible for severe infections such as bacteremia and device-associated infections, thereby compromising the management of vulnerable patients [2][3]. A study conducted in an intensive care unit in Greece showed that *Acinetobacter baumannii* isolates recovered from hospital equipment and from the hands of healthcare workers were identical to those responsible for sepsis in patients [4]. Bacteraemia remains a major cause of mortality, accounting for approximately 23% of deaths [5].

Patients with chronic kidney disease (CKD) constitute a vulnerable population at increased risk of MDRO infections in hospital settings due to their frequent visits for haemodialysis. Globally, CKD affects approximately 697.5 million people [6]. In Cameroon, a study conducted in 2021 reported a CKD prevalence of 11% in the general population [7]. The infectious risk associated with haemodialysis units is linked to several factors, including the hospital environment and medical practices [8]. Because of limited therapeutic options for MDRO infections, mortality rates are increasing among the most vulnerable populations. In a study conducted in the intensive care unit of the Gabriel Touré University Hospital in Mali, Diop et al. reported a prevalence of healthcare-associated infections (HAI) of 17.5%, with a mortality rate of 57.1% among affected patients [9].

In Cameroon, studies have reported a concerning emergence of Multidrug-resistant bacteria (MDRB) in high-risk hospital units. At the Yaounde University Teaching Hospital (YUTH), a study on MRSA nasal carriage among healthcare workers and patients in medical, surgical, and intensive care units reported an overall prevalence of 41.3% [10]. In the intensive care unit of the Douala General Hospital, 64% of ESBL-E producing isolates and 12% of MRSA were identified in patients with post-operative sepsis [2]. However, data on the epidemiology of MDRO in haemodialysis units are scarce. It is therefore necessary to assess the distribution of MDRO among healthcare workers and patients in the haemodialysis units.

MATERIALS AND METHODS

Study design and participants

We conducted a cross-sectional study from November 2023 to April 2024 in University Teaching Hospital of Yaounde (UTHY) and General Hospital of Yaounde (GHY), Cameroon. The selection of the study sites was carried out by convenience sampling among the reference health facilities of Cameroon.

Hospitalized patients and healthcare workers from haemodialysis units were included in this study. The facilities receive a high frequency of patients with renal failure for treatment. Patients with renal failure and healthcare workers who provided informed consent were included in the study. Socio-demographic and clinical data were collected from each participant using a pre-tested questionnaire validated by the research team. At least one sample of rectal and nasal swab was obtained from each participant. Participants who did not give at least one sample were excluded. The collected samples were transported on ice to the bacteriology laboratory of the UTHY and stored at 4°C before microbiological analysis.

Isolation and identification of MDRO

The samples were inoculated into Brain–Heart Infusion (BHI) broth and incubated at 37°C for 24 hours. After incubation, smears were prepared from the BHI broth and Gram staining was performed. Samples that were positive on microscopy were sub-cultured onto selective media as appropriate. When Gram-positive cocci were observed, positive culture of BHI, previously inoculated with nasal swab, was inoculated on Chapman agar supplemented with 2 mg/L oxacillin. BHI with Gram-positive cocci previously inoculated with rectal swab was transferred to Columbia agar supplemented with 5% blood, 15 mg/L nalidixic acid, 10 mg/L colistin and 6 mg/L vancomycin. BHI with Gram-negative bacilli previously inoculated with rectal swab was inoculated on MacConkey agar supplemented with crystal violet without ceftazidime and another with 4 mg/L ceftazidime. Cultures were incubated at 37°C for 18–24 hours. *Staphylococcus aureus* was identified using standard biochemical tests, including mannitol fermentation, catalase, DNase, and coagulase tests. Gram-negative bacilli were identified using oxidase, catalase tests, API 20E and MALDI–TOF mass spectrometry. *Enterococcus* were identified using catalase test, bile esculin test, 6.5% Sodium chloride (NaCl) growth test and MALDI–TOF mass spectrometry.

Susceptibility tests

Antimicrobial susceptibility testing was performed using the disc diffusion method (Kirby–Bauer method) on Mueller–Hinton agar except for *Enterococcus* where Columbia agar supplemented with 5% blood was used, in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing and the Antibiogram Committee of the French Society of Microbiology [11].

Briefly, the bacterial inoculum was prepared from 24-hour pure culture and standardized to 0.5 McFarland. Mueller–Hinton agar or Columbia agar supplemented with 5% blood was inoculated and antibiotic discs were placed on the surface of the medium. Antibiotic discs were Ampicillin (AMP), Kanamycin (KA), Tobramycin (TOB), Tetracycline (TET), Levofloxacin (LEV), Gentamycin (GN), Minocycline (MIN), Norfloxacin (NOR), Erythromycin (E), Pristinamycin (T), Clindamycin (CC), Nitrofurantoin (F), Trimethoprim+sulfamethoxazole (SXT), Teicoplanin (TEC), Vancomycin (VA), Rifampicin (RD),

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Fusidic acid (FUS), Linezolid (L), Amoxicillin + Clavulanic Acid (AMC), Piperacillin (PIP), Piperacillin + Tazobactam (PTZ), Cefalexin (CL), Cefoxitin (FOX), Ceftazidime (CAZ), Cefotaxime (CTX), Ceftriaxone (CRO), Cefepime (FEP), Meropenem (MPM), Ertapenem (ETP), Nalidixic acid (NA), Ciprofloxacin (CIP), Fosfomycin (FOS), Aztreonam (ATM), Amikacin (AK). After 15 minutes at room temperature, this was incubated at 37°C for 18–24 hours and inhibition zones were measured and scored as susceptible, intermediate or resistant.

MRSA was identified based on resistance to cefoxitin. ESBL-E were screened using the double-disc synergy test, which consisted of placing third-generation cephalosporin discs 3 cm around amoxicillin/clavulanic acid. The appearance of heart-shaped clear zones that distort the zone of inhibition after 24h of incubation at 37°C indicates positive results. Enterobacteria with resistance to third-generation cephalosporins were confirmed as 3CPE. Carbapenemase was confirmed by imipenem susceptibility test, when the presence of an inhibition zone less than 28 mm around imipenem was observed. VRE was confirmed when resistance to vancomycin was detected on *Enterococcus*.

Data analysis

The data collected were analyzed using the software GraphPad, version 8.01 and Microsoft Office Excel, version 2016. Prevalence was expressed as a percentage. The chi-squared test was used to compare the frequencies of bacterial exposure, with a 95% confidence interval. P values < 0.05 were considered statistically significant.

RESULTS

Characteristics of the study population

The data were collected from 155 participants including 130 (83.87%) patients with kidney failure and 25 (16.13%) healthcare personnels. Eighty-two (53%) were men and 73 (47%) were women (Table I). The age of participants ranged from 8 to 89 years, with an average age of 43.69 years. Two hundred and seventy-five samples were collected from the participants, including 155 nasal and 120 rectal swabs. The healthcare personnel provided 25 nasal and 16 rectal swabs. The patients provided 130 nasal and 104 rectal swabs.

TABLE I: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS

	Number	Frequency (%)
Age (Years)		
[0 - 10]	02	1,3
[11 - 20]	12	7,7
[21 - 30]	22	14,2
[31 - 40]	33	21,3
[41 - 50]	35	22,6
[51 - 60]	22	14,2
[61 - 70]	23	14,8
[71 - 80]	05	3,2

[81 - 90]	01	0,6
Gender		
Female	73	47
Male	82	53
Types of participants		
Patients	130	83,87
Staff	25	16,13
Types of samples		
Rectal	120	77,4
Nasal	155	100

Distribution of comorbidities among patients

Figure 1 presents the distribution of comorbidities among the 130 patients with kidney failure. Several comorbidities were identified, including cardio-vascular diseases and infections. Most of the participants had high blood pressure (56.13%), followed by low blood pressure (16.13%), heart failure (16.13%) and diabetes (2.58%).

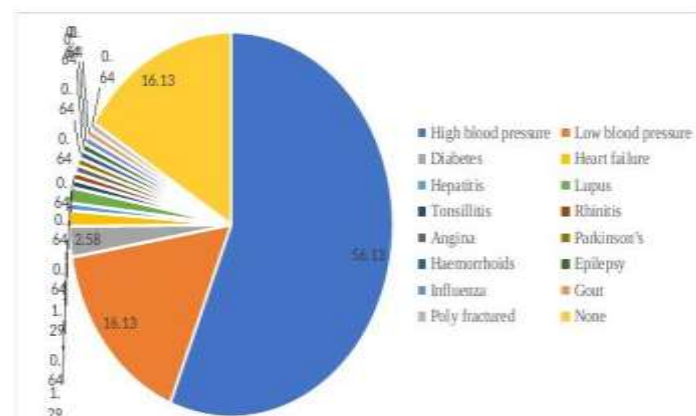


Figure 1: Distribution of Comorbidities among Participants

Frequency of multi-drug resistant bacteria

We identified 59 multidrug-resistant organisms (21.45%) from the 275 samples, with 54 (91.2%) from patients and 5 (8.48%) from healthcare personnel. Twenty VRE (16.66%) and 12 3CPE (10%) were isolated from the 120 rectal swabs and twenty-seven MRSA (17.41%) were isolated from the 155 nasal swabs.

The distribution of Multidrug-resistant bacteria (Table II) showed that MRSA, VRE, 3CPE and ESBL-E were more represented among patients compared to healthcare personnel with no significant difference in hospital A (P=0.57) and hospital B (P=0.65). The highest frequencies of MRSA were 94.12% and 90% in hospital A and B respectively. The highest frequencies of VRE were 75% in hospital A and 100% in hospital B whereas 3CPE were more represented with the frequencies of 88.88% and 100% in hospital A and B respectively. No ESBL-E was detected in one hospital, no CRPA and CRAB were detected in both hospitals.

The frequency of Multidrug-resistant bacteria was highest among participants of 31–50 years (37.29%), followed by participants with age ≥ 61 years (27.12%) and those of 11–30

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years (25.42%). There was no significant difference between the age groups (P=0.438).

TABLE II: DISTRIBUTION OF MULTIDRUG-RESISTANT BACTERIA

	MRSA n (%)	VRE n(%)	3CPE n (%)	ESBL-E (%)	n CRPA (%)	n CRAB (%)	n P-value
Healthcare facility A							
Staff (n=4)	1(5.88)	2(25)	1 (11.11)	0 (0)	0 (0)	0 (0)	0.57
Patients (n=31)	16(94.12)	6(75)	8 (88.88)	1 (100)	0 (0)	0 (0)	
Healthcare facility B							
Staff (n=1)	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.65
Patients (n=23)	9 (90)	12(100)	2 (100)	0 (0)	0 (0)	0 (0)	
Age groups (year)							
11 – 30	9(60)	3(20)	3 (20)	0 (0)	0 (0)	0 (0)	0.438
31 – 50	6 (27.27)	9(40.90)	6(27.27)	1 (4.54)	0 (0)	0 (0)	
51 – 60	4 (66.66)	1(16.66)	1 (16.66)	0 (0)	0 (0)	0 (0)	
≥ 61	8 (50)	7(43.75)	1(6.25)	0 (0)	0 (0)	0 (0)	

MDRB: Multidrug-resistant bacteria; **MRSA:** Methicillin resistant *Staphylococcus aureus*; **VRE:** Vancomycin-resistant enterococci; **3CPE:** Third-generation cephalosporinase-producing Enterobacteriaceae; **ESBL-E:** Extended-spectrum beta-lactamase-producing Enterobacteriaceae, **CRPA:** Carbapenem resistant *Pseudomonas aeruginosa* ; **CRAB :** carbapenem-resistant *Acinetobacter baumannii*

Resistance profile of multidrug-resistant bacteria

The resistance profiles of MRSA were similar in the two hospitals with 15 antibiotics ineffective in each hospital. The highest resistance frequencies were observed on AMP (100%), FOX (100%), LEV (100%), for MRSA. Concerning VRE, the resistance profile was similar in the two hospitals except resistance to levofloxacin which was present in hospital B but not in hospital A. The highest frequencies of resistance were observed on VA (100%) and SXT (95%). 3CPE presented the

broadest resistance profile with 22 and 21 ineffective antibiotics in hospitals A and B respectively. The resistance profile was similar in the two hospitals with highest frequencies on AMP (100%), AMC (100%), PIP (100%), PTZ (100%), CL (100%), CAZ (100%), FOX (100%), CTX (100%), CRO (100%).

TABLE III: FREQUENCIES AND RESISTANCE PROFILES OF MDRB

MRB	Resistance frequencies (%)	Resistance profile	Facilities
MRSA	AMP 100%, FOX 100%, LEV 100%, GN 70.37%, KA 92.59%, NOR 92.6%, TET 85.18%, TOB 62.96%, MIN 62.96%, E 85.2%, SXT 85.2%, PT 62.96%, CC 40.74%, FUS 48.15%, RD 22.22%	AMP-FOX-GN-KA-TOB-MIN-TET-LEV-NOR-CC-E-PT-FUS-RD-SXT	HCF A
		AMP-FOX-GN-KA-TOB-MIN-TET-LEV-NOR-CC-E-PT-FUS-RD-SXT	HCF B
VRE	VA 100%, SXT 95%, TEC 65%, E 50%, F 40%, AMP 35%, LEV 10%, L 10%	AMP-TEC-VA-E-L-F-SXT	HCF A
		AMP-TEC-VA-E-L-F-SXT-LEV	HCF B
3CPE	AMP 100%, AMC 100%, PIP 100%, PTZ 100%, CL 100%, CAZ 100%, FOX 100%, CTX 100%, CRO 100%, FEP 91.66%, MPM 83.33%, ETP 91.66%, NA 91.66%, CIP 50%, LEV 75%, GN 50%, TOB 50%, SXT 91.66%, ATM 41.67%, AK 25%, FOS 25%, F 33.33%	AMP-AMC-PIP-PTZ-CL-FOX-CAZ-CTX-CRO-FEP-ATM-MPM-ETP-NA-CIP-LEV-AK-GN-TOB-SXT-F-FOS	HCF A
		AMP-AMC-PIP-PTZ-CL-FOX-CAZ-CTX-CRO-FEP-ATM-MPM-ETP-NA-CIP-LEV-AK-GN-TOB-SXT-F	HCF B

ESBL-E	AMP 100%, AMC 100%, PIP 100%, PTZ 100%, CL 100%, CAZ 100%, FOX 100%, CTX 100%, CRO 100%, FEP 100%, MPM 100%, ETP 100%, NA 100%, CIP 100%, LEV 100%, GN 100%, TOB 100%, SXT 100%, ATM 100%, AK 100%, FOS 100%, F 0%	AMP-AMC-PIP-PTZ-CL-FOX-CAZ-CTX-CRO-FEP-ATM-MPM-ETP-NA-CIP-LEV-AK-GN-TOB-SXT-FOS	HCF A
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Legend: AMP: Ampicillin; KA: Kanamycin; TOB: Tobramycin; TET: Tetracycline; LEV: Levofloxacin; GN: Gentamycin; MIN: Minocycline; NOR: Norfloxacin; E: Erythromycin; PT: Pristinamycin; CC: Clindamycin; F: Nitrofurantoin; SXT: Trimethoprim+sulfamethoxazole; TEC: Teicoplanin; VA: Vancomycin; RD: Rifampicin; FUS: Fusidic acid; L: Linezolid; AMC: Amoxicillin + Clavulanic Acid; PIP: Piperacillin; PTZ: Piperacillin + Tazobactam; CL: Cefalexin; FOX: Cefoxitin; CAZ: Ceftazidime; CTX: Cefotaxime; CRO: Ceftriaxone; FEP: Cefepime; MPM: Meropenem; ETP: Ertapenem; NA: Nalidixic acid; CIP: Ciprofloxacin; FOS: Fosfomycin; ATM: Aztreonam; AK: Amikacin. MRSA: Methicillin-resistant *Staphylococcus aureus*; VRE: Vancomycin-resistant enterococci; 3CPE: Third-generation cephalosporinase-producing *Enterobacteriaceae*; MDRB: Multidrug-resistant bacteria; HCF A: Healthcare facility A; HCF B: Healthcare facility B.

DISCUSSION

This study was conducted to assess the prevalence and distribution of multidrug-resistant bacteria among patients and healthcare staff in a haemodialysis unit. In this study, several comorbidities were identified among the patients with kidney failure, with high blood pressure in first place (56.13%), followed by low blood pressure (16.13%), heart failure (16.13%) and diabetes (2.58%). Several studies have shown that chronic kidney disease is a high risk factor for cardiovascular complications such as heart failure and blood pressure, common causes of death in patients with kidney failure [12][13]. The study of Tobe et al. also revealed that kidney disease is the most common complication of diabetes, affecting half of all patients with diabetes [14]. In our study, we carried out selective cultures for MRSA, VRE and 3CPE by adding oxacillin, vancomycin and ceftazidime, respectively, to the culture media. Susceptibility testing identified resistance to other antibiotics in the selected isolates. All 59 (21.45%) isolates identified were multidrug-resistant. Fifty-four (91.2%) were from patients and 5(8.48%) from healthcare staff. A study conducted by Gonsu et al. in Yaoundé had revealed a lower prevalence of MDRB (18.06%) [10]. This lower prevalence may be explained by the fact that only healthcare workers were included in their study. The high prevalence observed in our study indicates a high circulation of multidrug-resistant bacteria in haemodialysis units, especially in patients with comorbidities such as cardiovascular disease, heart failure and diabetes. A meta-analysis conducted by Ye et al. revealed that diabetes and cardiovascular disease were risk factors for MDRO infection in patients [15]. The presence of these multidrug-resistant bacteria, combined with other cardiovascular complications, could be life-threatening for patients. The lower prevalence of multidrug-resistant bacteria among healthcare staff in the haemodialysis unit, compared with patients might explain the transmission of these bacteria to staff, exposing them to infections and other complications.

MRSA ranked first at Hospital A and second at Hospital B. VRE were predominant at Hospital B but ranked third at Hospital A, after 3CPE. A previous study conducted in China revealed that patients with impaired renal function have a higher risk of infections by multi-drug resistant bacteria, including *Staphylococcus aureus*, *Enterococcus spp.*, *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter spp.* [16]. In contrast to our study, previous studies have revealed that

enterobacteria were the main pathogens responsible for nosocomial infections due to their high prevalence in various hospital environments and their ability to develop resistance to antibiotics [2][9]. The high prevalence of MRSA observed in our study may be explained by the fact that MRSA is a pathogen mainly transmitted via hand contact and can persist when hand hygiene and the wearing of protective equipment are not strictly enforced. Studies conducted by Gonsu et al. in the intensive care unit of Yaoundé University Hospital had also revealed a high prevalence of MRSA among patients in the intensive care unit [17]. This may be due to a higher proportion of nasal samples compared with rectal samples, attributed to the difficulty many participants had in providing a rectal sample.

The frequency of MRSA, VRE, 3CPE and ESBL-E was higher among patients than healthcare staff, with no significant difference. In this study, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were not isolated, which contradicts the findings of Oweis et al. who reported *Acinetobacter baumannii* (76.8%) and *Pseudomonas aeruginosa* (14.9%) as the most common isolates in patients with renal failure [18]. This difference could be explained by the nature of the samples used in the study of Oweis et al. which did not include rectal and nasal swabs [18]. The frequency of ESBL-E in this study is significantly lower than that reported by Toudji et al. in 2017 in Togo, which was 22.44% [19]. This difference could be explained by the nature of the samples, which were mainly pus and urine in the study of Toudji et al.

With regard to 3CPE, the frequency observed in our study is significantly higher than the 2% reported by Gonsu et al. [10], which could reflect the gradual acquisition of resistance by these bacteria over time.

The resistance profile of MRSA was very broad and similar in both hospitals, with resistance observed for 15 antibiotics. Only one antibiotic distinguished the resistance profiles between the two hospitals for VRE (levofloxacin) and for 3CPE (fosfomycin). Our results demonstrate that the epidemiology of resistance is similar between the hospitals in the haemodialysis unit and affects the same classes of antibiotics. These results highlight the spread of resistance between hospitals but also between staff and patients.

Our findings highlight the need for systematic screening for multidrug-resistant organisms in patients admitted to the haemodialysis unit to limit transmission between patients and

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exposure of staff in this unit and also to prevent complications caused by MDR bacteria in patients with renal failure.

Our study has limitations. The proportion of rectal samples was small because some participants were uncomfortable to provide this sample. The low proportion of healthcare staff included compared with patients could influence the prevalence of MDRO observed amongst staff. The fact that data were collected from only two hospitals may question the generalizability of our findings. However, our results contribute to the epidemiology of resistance, given the lack of data on MDRO in haemodialysis units in Cameroon. It would be necessary to extend this study in hospitals with haemodialysis units in different countries and populations.

CONCLUSION

This study revealed a high prevalence of MDRO in the haemodialysis unit, affecting healthcare staff and patients with renal failure and comorbidities. This research highlights the need for a multidisciplinary approach to prevent MDRO, including systematic screening of resistance when patients are admitted to the haemodialysis unit and the strengthening of standard precautions.

COMPETING INTERESTS

The author(s) declare that they have no competing interests.

FUNDING

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ETHICAL APPROVAL

This study was approved by the Ethical Committee of the Delegation of Public Health for the Centre Region of Cameroon under the approval number: 2182/CRERSHC/2023. Administrative authorisations were obtained from the directors of the two hospitals. This evaluation was conducted according to the guidelines of the Declaration of Helsinki. Written informed consent was obtained from each participant, and confidentiality was strictly preserved by coding all the samples and other patient documents. All information obtained was confidential; participants' data were entered into the database and access to this database was protected with a password.

AVAILABILITY OF DATA AND MATERIALS

The datasets supporting the conclusions of this study are presented in this main paper.

AUTHOR'S CONTRIBUTIONS

GMN conceived and designed the intellectual content and wrote the original manuscript; EM, GE conducted the primary analysis, edited and reviewed the manuscript; MF and YLGN edited and reviewed the manuscript; CG, AC, WBA, AN, MM, MPN and AN prepared figures and tables; SGM, LCM, BFB, VN reviewed the manuscript; FKF and HGK conceived the

intellectual content, coordinated research activities and reviewed the manuscript. All authors have approved the final manuscript and agreed to be personally accountable for the author's own contributions.

ETHICAL CONSIDERATIONS

Ethical approval was obtained from the Regional Ethics Committee for Human Health Research at the Centre Region under reference number 2182/CRERSHC/2023. Administrative authorisations were obtained from the directors of the facilities. Written informed consent was obtained from each participant, and confidentiality was strictly maintained.

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